FAMÉ 2023

Mátraháza
7–9 June 2023

Programme Oral and Poster Abstracts

MCMXXXI
SOCIETAS
PHYSIOLOGICA
HUNGARICA
M.É.T

M M V B T

HUPHAR
Hungarian Society for Experimental and Clinical Pharmacology

Itt élünk.
Itt kutatunk.
Itt fejlesztünk.

www.gedeonrichter.com

RICHTER GEDEON
Az egészség a küldetésünk

LZD: 2023.05.17. PR/45/2023

WELCOME

Dear Colleagues,

On behalf of the Organizing Committee, we welcome you warmly to the conference of the Hungarian Society of Experimental and Clinical Pharmacology (HUPHAR), Hungarian Society of Physiology and Hungarian Society of Microcirculation with the contribution of the Hungarian Society of Anatomy, which is organized between 7th-9th June 2023 in Mátraháza. Based on our tradition, the main goal of the conference is to understand physiological regulatory mechanisms and pathophysiological processes, to explore the cellular and molecular mechanisms of diseases, to identify novel diagnostic and therapeutic biomarkers, as well as drug developmental perspectives. The main focus of this conference is on molecular biological research tools for omics approach and precision medicine.
We put great emphasis on providing opportunities for young colleagues to present their results in form of oral or poster presentations with extensive discussions and poster competitions, besides organizing social programs as well.

We sincerely hope to see you in June 2023 for a high quality, memorable and not least enjoyable meeting.

ORGANISERS

Organiser of the CONFERENCE:

HUNGARIAN SOCIETY FOR EXPERIMENTAL AND CLINICAL PHARMACOLOGY
HUNGARIAN PHYSIOLOGICAL SOCIETY
HUNGARIAN SOCIETY FOR MICROCIRCULATION AND VASCULAR BIOLOGY

President of the Conference:

Zsuzsanna Helyes
MFT and MÉT Board Member, University of Pécs

Organizing Committee

Péter Ferdinandy (President of the MFT, Semmelweis University)
Katalin Monostory (Natural Sciences Research Centre)
Ferenc Bari (President of MÉT, University of Szeged)
Kata Csekő (University of Pécs)
Éva Szőke (University of Pécs)
Gabriella Kékesi (Secretary of MÉT, University of Szeged)

Venue of the Conference
Mátraháza
HOTEL ÓZON & LUXURY VILLAS**** superior

Technical Organiser of the Event
Altagra Business Services and Travel Agency Ltd.
www.altagra.hu

SPONSORS

The Organising Committee expresses its grateful thanks to the Conference's sponsors and exhibitors.

Abbvie Kft.

Acéllabor Kft.

Akronom Kft.

Animalab Hungary Kft.

Auro-Science Consulting Kft.

BioCenter Kft.

Bio-Kasztel Kft.

Biomedica Hungária Kft.

Central European Biosystems Kft.

Circulos Kft./BioTech Hungary Kft.

SPONSORS

The Organising Committee expresses its grateful thanks to the Conference's sponsors and exhibitors.

Dialab Kft.

Greiner Bio-One Hungary Kft.

Innovo Kft.

Merck Life Science Kft.

Nanosurf GmbH, Joó Katalin Kft.

PER-FORM Hungária Kft.

Richter Gedeon Nyrt.

Sarstedt Kft.

UNICAM Magyarország Kft.

World Courier Hungary Kft.

TABLE OF CONTENTS

iBioTech innovative

www.ibiotech.hu

info@ibiotech.hu

Az immunoassay eljárás új evolúciója

1000x érzékenyebb, mint a hagyományos ELISA módszer

Új innovatív lehetőségeket kínál a gyógyszerfejlesztés és monitorozás területén

Alacsony mennyiségű biomarkerek rutin szerű detektálása vérszérumból és vérplazmából

Quanterix™

A lényeg mindig a jéghegy csúcsa alatt van

Per-Form Hungária Kft.

„Analitikai és élettudományi műszerek szakértő támogatással”

https://per-form.hu
https://perforum.hu
perform@per-form.hu
https://www.facebook.com/performhungariakft/
+36 1 251-1116

PerkinElmer

ANALITIKA:

- Ultraibolya-látható és közeli infravörös spektrométerek (UV/VIS/NIR)
- Fourier transzformációs infravörös, közeli és távoli infravörös spektrométerek (FT-IR/NIR/FIR) és mikroszkópok, automatikus FT-IR gázelemző rendszerek
- Fluoreszcens spektrométerek
- Gázkromatográfia (GC) és Gázkromatográfia-tömegspektrometria (GC/MS)
- Gázkromatográfiás mintaelőkészítés, gőztéranalízis (headspace, HS) és termikus deszorpció (ATD)
- Folyadékkromatográfia (HPLC, UHPLC)
- Atomabszorpciós spektrométerek (AAS)
- Induktív csatolású plazma optikai emissziós spektrométerek (ICP-OES)
- Induktív csatolású plazma tömegspektrométerek (ICP-MS)
- Higanyanalizátorok
- Termikus analizátorok (DMA, DSC, STA, TGA, TMA)
- Mikrohullámú roncsolók

ÉLELMISZER- ÉS TAKARMÁNYANALITIKA:

- Asztali és folyamatba integrálható monokromátoros és diódasoros NIR spektrométerek
- Reológiai műszerek (esésszámmérők, sikérmosó, textúra vizsgáló, lézeres térfogatmérő készülék, rotációs viszkoziméterek, vízabszorpciót meghatározó farinográf típusú készülék)
- Boranalizátor

Revvity

- NGS reagensek
- Mikrofluidikai készülékek
- Robotizált munkaállomások és folyadékkezelő robotok
- Digitális patológia készülékek
- High Content Screening készülékek
- Sejtes és szöveti képalkotó készülékek
- Kisállat in vivo képalkotó készülékek (microCT és biolumi/fluo)
- Plate reader készülékek
- Radiometria detektor készülékek (béta, alfa, gamma)
- AlphaScreen/AlphaLisa, Delfia/Lance módszerek
- Nukleinsav izoláló készülékek
- Automata sejtszámláló készülékek
- Citometriai képalkotó készülékek

SCIEX

- Folyadékkromatográfia (HPLC, UHPLC, Microflow LC, NanoLC)
- Hármas kvadrupól tömegspektrométerek (QqQ-MS/MS)
- Hibrid hármas kvadrupól – lineáris ioncsapda tömegspektrométerek (QTRAP-MS)
- Hibrid kvadrupól – repülési idő tömegspektrométerek (QTOF-MS)

BICO Life Sciences: Cellink, Cytena

- 3D bioprinterek: extrúziós, digitális fény- és lézer alapú bionyomtató készülékek
- Egysejt-diszpenzerek, élősejt-analizátorok, bioreaktorok
- Biotinták, hidrogélek, bionyomtatás fogyóeszközök és alapanyagok, sejtek és médiumok

PEAK

- Nitrogén, hidrogén és zéró levegő generátorok

BIOCENTER

SARTORIUS

IncuCyte – ÉLŐSEJT-ANALÍZIS KÖZVETLENÜL AZ INKUBÁTORBAN!

Az IncuCyte előnyei

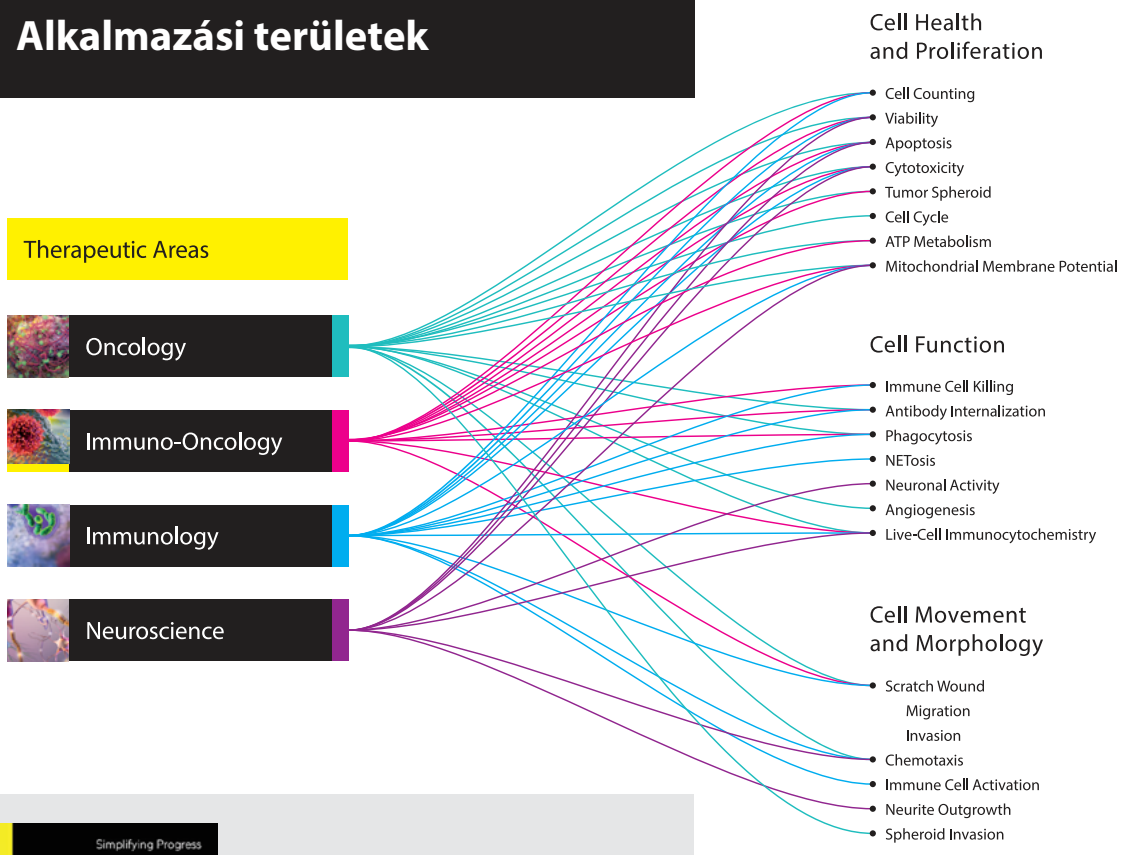
Egyszerű és összetett kérdések megválaszolása egyetlen rugalmas platformmal. Élősejtvizsgálat HD fáziskontraszt és a akár öt fluoreszcens csatorna használatával.

Vizsgálat közben a sejtek háborítatlanul maradnak az inkubátorban. Jelölésmentes vizsgálatok és speciális összetételű reagensek használatával megőrizzük a sejtek életképességét.

Valósidejű élősejt-analízis: A sejtek folyamatos monitorozásával nem marad ki adatpont. Sejttípus-specifikus és időfüggő biológiai aktivitásmérés.

Hatékonyságnövelés: egyszerre több felhasználó által indított kísérletek, és akár hat különböző plate vizsgálata egyidejűleg. Élőben követhető kísérletek a hálózatra kapcsolt számítógépekről, korlátlan számú felhasználóval.

Alkalmazási területek



Az IncuCyte készüléket bemutató film letölthető:
https://bit.ly/sartoriusvideo

Bővebb információ a gyártó honlapján:
https://bit.ly/sartoriusinfo

Érdeklődjön: **info@biocenter.hu**

GENERAL INFORMATION

Registration

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza – Hotel Lobby

7. June 2023, from 10 am

The organisers kindly ask participants to please come to the registration desk first. They are also kindly requested to wear their name badges until the end of the conference.

Presentations

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Kékes Room

Participants wishing to present a lecture are requested to prepare their presentation in MS PowerPoint format in English and hand it to the technician in the lobby of the auditorium at the latest during the last coffee break before the presentation. Presentations cannot be modified at this point. The presentations will be copied onto the computer by the technician colleague who will be available to the presenters during their presentation. The language of the presentation can be either English or Hungarian, depending on the needs of the audience and the presenter. Technicians will provide a control monitor, a wireless presenter including a laser pointer and a countdown timer monitor each room.

Poster session

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, at the corridors of the Conference Hall and Tramini Wine Bar.

Posters should be exhibited until 12.00 on Wednesday 7th June and kept outside until 5:00pm on Friday 9th June, the end of the Conference. Organisers will provide poster stands and all necessary equipment to hang the posters on it. Poster size: 90 cm (width) 150 cm (height).

The poster discussion will take place on Thursday afternoon 8th June 2023 and Poster presenters are kindly requested to be present in person during the Poster Session to answer questions from interested parties.

The award for the Best youth poster presentations awarded by the Scientific Committee. The awards ceremony will take place together with the closing of the conference.

Exhibition, coffee break

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Hall of the Kékes Room

Lunches and Dinner

HOTEL ÓZON & LUXURY VILLAS**** superior, Mátraháza, Mátra Restaurant

Breakfast

All guests will be able to have breakfast in their own accommodation

Social programme

Morning refreshment run with the **Greiner Bio-One team** on 8th and 9th June at 7:00 am, meeting at the reception of HOTEL ÓZON & LUXURY VILLAS****.

Thursday, 8th June 2023, 7:00 pm, Mátra Restaurant: **Gala dinner and dance**

Saturday, 10th June 2023, **Organised excursions in the Mátra Mountains.**

Arrival, departure

To *check in* at the hotels, you must present your ID and address card. This rule applies to all accommodation in Hungary. Please make sure you are aware about your accommodation *check in* and *check out* time. These are available on the webpage. Check-out after the official check out time will incur an additional charge. Hotels will provide luggage room on both days.

Program code explanation

First letter of the name of days: W-Wednesday, T-Thursday, F-Friday

Second letter is the venue of the performance:

KA- **Kékes A** Room

KB- **Kékes B** Room

G Galya Room

Numbers:

1st number: number of the section within a day

2nd number: serial number within a section

Mi, akik az Altagra csapatát alkotjuk, mindig kreatív és személyre szabott megoldásra törekszünk, legyen az akár hibrid, online vagy hagyományos konferencia. Hiszünk abban, hogy minden munkánkkal értéket teremtünk, élményt nyújtunk, törődést adunk. Ezért válnak a megrendelőink visszatérő partnerekké.

Altagra Szervező és Utazási Iroda Kft.

Szakértő partner a konferenciaszervezésben közel 25 éve

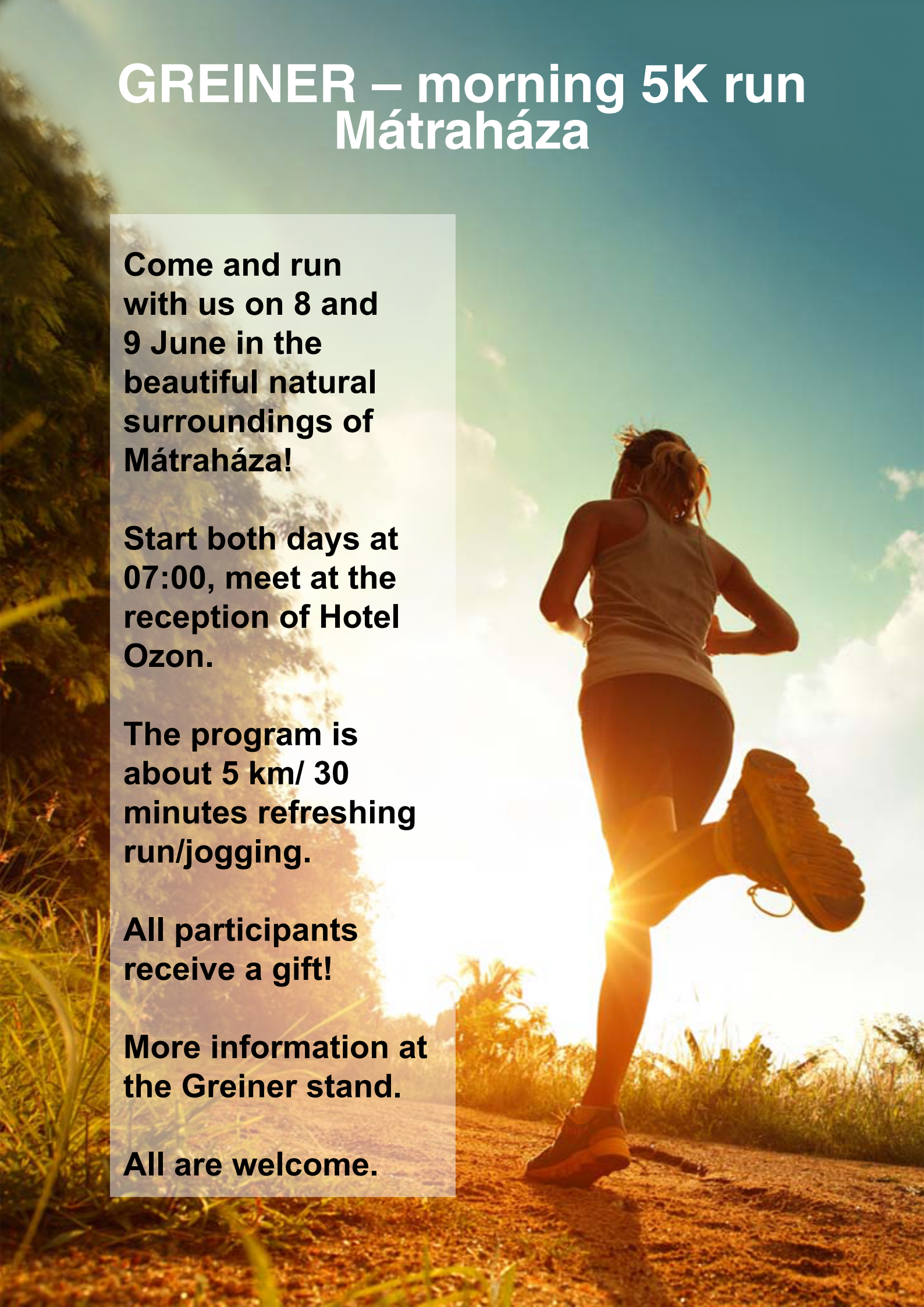
ALTAGRA

ALTAGRA SZERVEZŐ ÉS UTAZÁSI IRODA KFT.

H-1118 Budapest, Csukló utca 6/a.

altagra@altagra.hu • www.altagra.hu

GREINER – morning 5K run Mátraháza



Come and run with us on 8 and 9 June in the beautiful natural surroundings of Mátraháza!

Start both days at 07:00, meet at the reception of Hotel Ozon.

The program is about 5 km/ 30 minutes refreshing run/jogging.

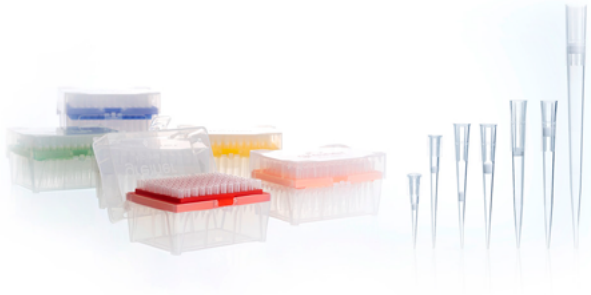
All participants receive a gift!

More information at the Greiner stand.

All are welcome.

PIPETTE LIKE A PRO

SAPPHIRE pipettahegyek



Maximális precizitás és folyadék visszanyerés a Greiner Bio-One termékeivel.

- Dobozos, ömlesztett és utántöltős kiszerelésekben.
- Filteres és filter nélküli kivitelek.
- „Low retention”-kis visszatartású pipettahegyek.
- Univerzális illeszkedés: a pipettahegyek kompatibilisek minden általánosan használt egy- és többcsatornás pipettával.
- Hosszított pipettahegy forma: csökkenti a kontaminációk kockázatát pipettázás során.
- Tökéletesített pipettahegy kialakítás: a sima felszínek csökkentik a folyadék visszatartást, ezáltal minimalizálják a költséges reagensek és minták veszteségeit.
- Kitűnő átlátszóságú orvosi minőségű polipropilénből készül a minták tökéletes láthatósága érdekében.
- Egyszerűen olvasható beosztás a minta térfogat gyors ellenőrzésére.
- Vékonyított fal kialakítás: tökéletes illeszkedés és pontosság változatlan teljesítmény mellett, csökkentett műanyag felhasználással.
- Rugalmas pipettahegy és gallér kialakítás: minimalizált felszívási és kienegedési erő és optimális illeszkedés.
- **We take your rack back:** visszavesszük és újrahasznosítjuk használt pipettahegyes dobozait.

- / DNáz, Rnáz, humán DNS és PCR inhibítor mentes
- / Nem pirogén és nem citotoxikus
- / Kioldódó anyagokra tesztelt
- / Sterilitási biztonsági szint SAL 10-6
- / EU-ban gyártott és a nemzetközi szabványoknak megfelelően tesztelt
- / Teljesen automtizált csomagolás a részecske-szennyezések megelőzése érdekében
- / Teljes nyomonkövethetőség

További információért és ingyenes mintákért keresse kollégáinkat a Greiner standon vagy az alábbi elérhetőségeken:

Andras.Jancso@gbo.com
Laszlo.Merva@gbo.com

Global Technology.
Local **Solutions.**

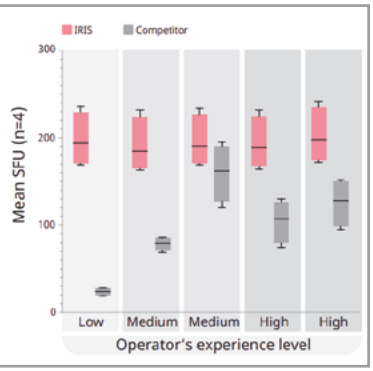
BIOMEDICA

Spot your next discovery with Biomedica & Mabtech

- Increase your understanding of immune responses
- FluoroSpot and ELISpot technology is used to quantify the secretion profile under physiological conditions
- Cells in suspension are added to the wells of a plate and the secreted proteins of each individual cell are captured by specific antibodies immediately after secretion and throughout the stimulation process.

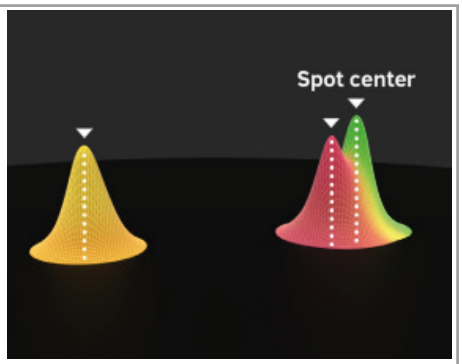
Fluorospot and ELISpot assays are best experienced with the Mabtech IRIS™ and Mabtech ASTOR™ readers

- Both readers are robust, have high throughput capabilities and are manufactured in Sweden
- Plug and play: Automated reader configuration and default analysis settings minimize user-defined subjective input and thus reduce bias of the operator
- User-friendly software: Easy to operate and train new users
- Multiplex ready: Up to 4 plex with Fluorospot
- CFR21 part 11 compliance



The secretion profile of every single cell

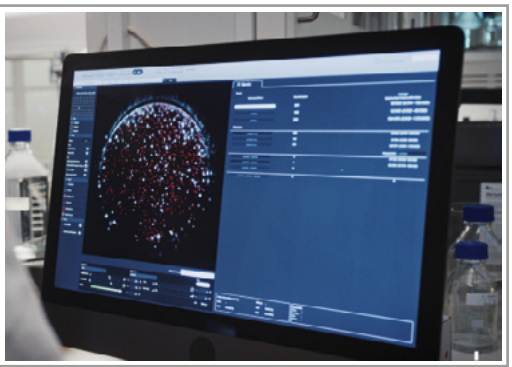
- By far the most striking feature of the readers is their novel spot-counting algorithm, RAWspot, which can identify spot centers with unseen accuracy
- The 3-dimensional model allows conclusions about the volume of each spot and provides a measurement for the amount of secreted analyte per cell, the relative spot volume (RSV).



Read once, adjust later

- Change count settings and experiment without affecting the originally captured RAW image data
- All signals from the spots are already recorded, so adjustments can be done off-line and you never have to re-read a plate

A new era of almost-too-easy!



Biomedica Hungària Kft.
Hungária krt. 128-132.• 1143 Budapest • Hungary
T +36 1 225 38 50 • F +36 1 201 26 84 • office@bmgrp.hu • www.bmgrp.hu

Exhibitor game for the participants on FAMÉ 2023 Congress Mátraháza • 7–9 June 2023

Dear Participant,

Three high-value gift packages wait for their owners, which will be raffled at the Gala dinner on 8th June, among the participants who participate in the announced prize draw. The prize draw takes place as follows.

When visiting the exhibitor stands, you can collect a stamp from the representative of the company at each stand. Anyone who obtains the necessary stamp at all stands and, based on the information gathered during the stand visit, correctly answers the questions concerning the exhibitors and then hands in the paper with their name on it by 5:30 p.m. on Thursday at the Conference registration, will participate in the draw.

Those who participate in the draw in person are entitled to the prize.

Thank you for your participation and we wish you a meaningful time!

The organizers

Millipore®
Preparation, Separation,
Filtration & Monitoring Products

MERCK

A Labeling System Built for the Laboratory

MilliSentials™ Lab Labeling System

For a complete laboratory labeling solution with labels that can withstand anything your lab throws at them, look no further than the MilliSentials™ Lab Labeling System. Label with ease with adaptable labels that can withstand different solvents and temperatures. Connect throughout the lab with a compact WIFI capable printer and custom-designed laboratory labeling software.

MilliSentials™ Lab Printer

- **Easy to use**
- **Small footprint –**
  10.5 in. × 8.0 in. × 7.5 in. (LWH)
- **WIFI capable**

MilliSentials™ Lab Software

- **Pre-defined templates** – Templates built for laboratory vessels
- **Network Friendly** – Software can be accessed from mobile devices allowing for on the go printing
- **Template Saving** – Build common label formats and save them as templates

MilliSentials™ Lab Adaptable Labels

- Laboratory grade labels that provide
  - **Temperature resistance from** -196°C to 100°C
  - **Chemical resistance** to Water, Ethanol, Xylene, Bleach, DMSO
- **Adaptability** with custom perforations allow one label size to fit plates, 0.5-0.6 mL tubes, 1.0-2.0 mL tubes, Petri dishes, slides, cryovials, Falcon tubes

For more information, visit
SigmaAldrich.com/MilliSentialsLabLabelingSystem

Elérhetőségünk:
Merck Life Science Kft.
Október huszonharmadika utca 6-10., 1117 Budapest
rendeles@merckgroup.com, +36 1 463 8100

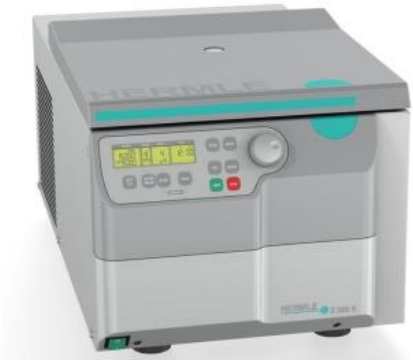
DIALAB KFT. MŰSZER AJÁNLATA

Biohazard fülkék

-86 C-os fagyasztók

Laboratóriumi hűtők

CO_2 inkubátorok



Centrifugák

Mérlegek

Liofilizálók

Fotométerek, küvetták

Autoklávok

Mikroszkópok

Pipetták, adagolók

Keverők, rázók

Alapítva: 1990

+ 36 30 961 44 68
+ 36 1 212 25 05
info@dialab.hu
www.dialab.hu

FAMÉ 2023

Mátraháza
7–9 June 2023

Programme

MCMXXXI
SOCIETAS
PHYSIOLOGICA
HUNGARICA
M.É.T

M M V B T

HUPHAR
Hungarian Society for Experimental
and Clinical Pharmacology

June 7, 2023 Wednesday

10:00		**Registration – Hotel Lobby**
10:30-10:40		**MÉT General Assembly (1st date) – Kékes Room**
10:40-12:40		**MÉT Board and Management Meeting**
12:00-13:30		**Lunch- Mátra Restaurant**
13:00-14:30	W-K	**Opening of the Conference** **Péter Ferdinandy** Semmelweis University, Institute of Pharmacology and Pharmacotherapy, Budapest **Ferenc Bari** University of Szeged, ÁOK Orvosi Fizikai és Orvosi Informatikai Intézet, Szeged **Katalin Monostory** Research Centre for Natural Sciences, Budapest **Issekutz Presentation and Award Ceremony** **Lendvai Balázs** Richter Gedeon Nyrt. *Novel strategies of industrial drug research with CNS focus* **MFT és MÉT Award Ceremony**
14:30-15:15	W-K1	**Ralf Baron** Division of Neurological Pain Research and -therapy, Department of Neurology, University Hospital, Kiel, Germany *Stratifying neuropathic pain patients in pharmacological trials: the way forward*
15:15-15:45		**Coffee break**
15:45-17:15	W-KA2	***Novel targets for the treatment of chronic pain and related co-morbidites*** Chairs: Zsuzsanna Helyes, Éva Borbély
	W-KA2-1	**Lilla Gunkl-Tóth[1,2]**, Gergely Orsi[3,4], Krisztina Csókási[5], Gábor Sütő[6], Gábor Kumánovics[7], Noémi Császár-Nagy[8,9], Szabolcs Takács[10], Eszter Szigedi[9], Zsófia Nagy[9], Zsolt Hodoványi[9], Lili Duzsik[9], Zoltán Vidnyánszky[11], József Kun[1,12], Péter Urbán[12], Attila Gyenesei[12], György Nagy[13,14,15], Zsuzsanna Helyes[1,2] **15'+5'** [1] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, [2]ELKH-PTE Chronic Pain Research Group, Eötvös Loránd Research Network (ELKH), Pécs,[3]MTA-PTE Clinical Neuroscience MR Research Group, Eötvös Loránd Research Network (ELKH), Pécs,[4]Department of Neurology, Medical School, University of Pécs, Pécs,[5]Institute of Psychology, Faculty of

Humanities and Social Sciences, University of Pécs, Pécs,[6]Second Department of Medicine and Nephrology-Diabetes Centre, University of Pécs, Pécs,[7]Department of Rheumatology and Immunology, Medical School, University of Pécs, Pécs,[8]National University of Public Services; Budapest ,[9]Psychosomatic Outpatient Clinics, Budapest ,[10]Department of Psychology, Karoly Gaspar University; Budapest,[11]Brain Imaging Centre, Research Centre for Natural Sciences, Budapest,[12]Szentágothai Research Centre, Bioinformatics Research Group, Genomics and Bioinformatics Core Facility, University of Pécs, Pécs,[13]Department of Rheumatology and Clinical Immunology, Department of Internal Medicine and Oncology, Semmelweis University, Budapest,[14]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest,[15]Heart and Vascular Centre, Semmelweis University, Budapest

The Comprehesive analysis of pain and inflammantion in difficult to treat rheumatoid arthritis with a complex clinical, psychological, multiomic and fMRI evaluation

W-KA2-2 **Ádám István Horváth[1,2,3]**, Nikolett Szentes[1,2,3], Valéria Tékus[1,2], Tamás Kálai[4], Szilárd Pál[5], Péter Mátyus[6], Zsuzsanna Helyes[1,2,3,7,8] **15'+5'**

[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs; [2]National Laboratory for Drug Research and Development, Budapest; [3]Eötvös Loránd Research Network, ELKH-PTE Chronic Pain Research Group, Pécs; [4]Institute of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Pécs, Pécs; [5]Institute of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, University of Pécs, Pécs; [6]E-Group ICT Software Zrt., Budapest; [7]PharmInVivo Ltd., Pécs, [8]ALGONIST Biotechnologies GmBH, Vienna

Analgesic effect of the novel multi-target drug candidate SzV-1287 in models of neuropathic and inflammatory pain

W-KA2-3 **David Á. Karádi[1]**, Anna R. Galambos[1], Péter P. Lakatos[2], Joost Apenberg[1], Sarah K. Abbood[1], Mihály Balogh[1,3], Kornél Király[1], Pál Riba[1], Nariman Essmat[1], Edina Szűcs[4], Sándor Benyhe[4], Zoltán Varga[1], Éva Szökő[2], Tamás Tábi[2], Mahmoud Al-Khrasani1[1] **10'+5'**

[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Nagyvá-rad tér 4, H-1089, Budapest, Hungary, [2]Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Nagyvárad tér 4, H-1089 Budapest, Hungary, [3]Pharmaceutical Analysis, Groningen Research Institute of Pharmacy, University of Groningen, 9700 AD, Groningen, The Netherlands, [4]Institute of Biochemistry, Biological Research Center, Temesvári krt. 62, H-6726 Szeged, Hungary

Effect of concomitant administration of telmisartan and morphine on mononeuropathic pain and opioid antiniciceptive tolerance in rats

W-KA2-4 **Éva Borbély[1],** Eszter Kepe[1], Angéla Kecskés[1], Valéria Tékus[1], Zsófia Hajna[1], Zsuzsanna Helyes[1,2,3] **10'+5'**

[1]Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Hungary, [2]National Laboratory for Drug Research and Development, Budapest, Hungary, [3]Eötvös Loránd Research Network, Chronic Pain Research Group, University of Pécs, Hungary

Role of the tachykinin hemokinin-1 in mouse models if chronic pain, anxiety and depression

W-KA2-5 **Zoltán Jakus[1]**, Gabor Kovács[1], Kornél Molnár[1], Stella Sági[1], Éva Kemecsei[1], Zoltán Lipinszki[2], Raghu P Kataru[3], Babak J Mehrara[3], Norbert Pardi[4] **15'+5'**

[1]Semmelweis University, Budapest, Hungary [2]Lendulet Laboratory of Cell Cycle Regulation, Institute of Biochemistry, Biological Research Centre, Budapest, Hungary [3]Department of Surgery, Division of Plastic and Reconstructive Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Characterization of the role of lymphatics in nucleoside-modified mRNA-LNP vaccine induced immune response

15:45-17:15 W-KB2 ***New test methods of cardiotoxicity and potential therapeutic modalities***

Chair: István Baczkó

W-KB2-1 **Anikó Görbe[1]**, Zoltán Giricz[1], Péter Ferdinandy[1,2] **15'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary, [2]Pharmahungary Group, Szeged, Hungary

Hidden cardiotoxicity - cardiac safety testing in ischemic and comorbid conditions: development of preclinical test platforms

W-KB2-2 **Attila Kiss[1,9],**Dostal[1], L P. Szabo[1], R. Steiner [1], P. Pokreisz[1], T. D. Hofreither[2],T. Tamara[2], S. Baydar[1], J Reiner[1], D Abraham[3], K Zins[3], B.Hack[4], T Kovács[5], Zs., Onodi[5], E Lilliu[5], , Z Varga[6], Birner-Gruenberger R[2]. N.Pilat[1,6], HM. Gong[7], J.A. Kirk[7], L Bakiri[8], X Koenig[4] , E.F. Wagner[8,1]BK. Podesser[1,9] **15'+3'**

[1]Center for Biomedical Research and Translational Surgery, Medical University of Vienna; [2] Institute of Chemical Technologies and Analytics, Faculty of Technical Chemistry, Technical University of Vienna, [3]Center for Anatomy and Cell Biology, Medical University of Vienna, [4]Department of Neurophysiology and Neuropharmacology, Medical University of Vienna, [5]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary. HCEMM-SU Cardiometabolic Immunology Research Group, Semmelweis University, Budapest, Hungary. MTA-SE Momentum Cardio-Oncology and Cardioimmunology Research Group, Semmelweis University, Budapest, Hungary, [6]Department of Cardiac Surgery, [7] Department of Cell and Molecular Physiology, Loyola University Stritch School of Medicine, Maywood, IL, USA. [8]Genes and Disease Group, Medical University of Vienna, [9]Ludwig Boltzmann Institute for Cardiovascular Research

Evidence for cardiomyocyte dysfunction in cancer-induced cachexia in mice

W-KB2-3 **Péter Bencsik[1,2]**, Kamilla Gömöri[1,3], Bence Pósa[1], Éva Kenyeres[1], Tamara Szabados[1,2], Bence Ágg[2,4], Barnabás Váradi[4], Viktória Tóth[4], Gergely Ágoston[5], István Leprán[1], Nazha Hamdani[3], Anikó Görbe[1,2,4], Péter Ferdinandy[2,4] **15'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary, [2]Pharmahungary Group, Szeged, Hungary, [3]HCEMM-Cardiovascular Research Group, Department of Pharmacology and Pharmacotherapy, University of Budapest, Budapest, Hungary and Institut für Forschung und Lehre (IFL), Molecular and Experimental Cardiology, Ruhr University Bochum, Bochum, Germany, [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [5]Institute of Family Medicine, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Microrna-Mrna Bioinformatics Target Prediction In A Rat Model Of Volume Overload-Induced Left Ventricular Hypertrophy

W-KB2-4 **Balázs Horváth[1]**, Tamás Hézső[1], Zsigmond Kovács[1], Csaba Dienes[1], József Óvári[1], János Magyar[1], Tamás Bányász[1], Norbert Szentandrássy[1], István Baczkó[2], András Varró[2], Péter P Nánási[1] **15'+3'**

[1]Department of Physiology, Faculty of Medicine, University of Debrecen;[2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged

Dynamics of the Late Na+ current in human, canine and guinea pig ventricular myocardium

W-KB2-5 **György László Nadasy [1]**, M Szekeres[2], A Fees[3], X Chang [1], László Hunyady [1], LP Szabo [4], BK Podesser[4], A Kiss[4]. **15'+3'**

[1]Physiology Dept, Semmelweis University, Budapest; [2]Dept of Morphology and Physiology, Health Sciences Faculty, Semmelweis University Budapest; [3]Kansas State University, Manhattan, KS, US; [4]Center for Biomedical Research, Medical University of Vienna.

Diabetic remodeling processes of the intramural coronary resistance arteries of streptozotocin treated mice show characteristic segmental specificity. Potential role of the connective tissue organizator protein Tenascinc

17:40-18:40 **MÉT General Assembly (2nd date)**

19:00-20:00 **Dinner- Mátra Restaurant**

20:00-21:30 W-K3 **Youth Lecture**

Chair: Balázs Enyedi, Éva Szőke

W-K3-1 **Ámos Gajda** [1], Noémi Vida[1], Gyöngyvér Vigyikán [1], Ádám Hodoniczki [1], Zoltán Varga[1], Attila Rutai[1], Vivien Móró[2], Zoltán Hantos[2], Gabriella Varga[1], Dániel Érces[1] **13'+5'**

[1]Institute of Surgical Research, University of Szeged [2]Department of Anaesthesiology and Intensive Therapy, Semmelweis University, Budapest

Investigation of acute respiratory failure syndrome and its renal complications in a new large animal model

W-K3-2 **Diána Kaszás**[1,2,3], Anna Török[1,3], Benoit Roux[1,3], Fabian Dehne[1,3], László Fazekas[1,2,3], Szimonetta Tamás[1,2,3], Klaudia Vágó-Kiss[1,2,3], Enyedi Balázs[1,2,3] **13'+5'**

[1]Department of Physiology, Semmelweis University, Budapest,[2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, [3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest

Decyphering tissue damage induced purinergic and calcium signaling pathways in zebrafish

W-K3-3 **Lina Hudhud** [1,2], Krisztina Pohoczky [1,2,4], Katalin Rozmer [1,2], Angéla Kecskés [1,2], Éva Szőke [1,2,3], Zsuzsanna Helyes [1,2,3] **13'+5'**

[1] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs H-7624 Pécs, Hungary,[2] National Laboratory for Drug Research and Development, H-1117 Budapest, Hungary,[3] Eötvös Lorand Research Network, Chronic Pain Research Group, University of Pécs, H-7624, Pécs, Hungary,[4] Department of Pharmacology, Faculty of Pharmacy, University of Pécs, H-7624 Pécs, Hungary

TRPA1 but not TRPV1 ion channel is expressed on the K7M2 mouse osteosarcoma cells and its activation reduces viability

W-K3-4 **Vera Tarjányi** [1], Vietrov Tymur[1], Anna Szilágyi [1], Réka Szekeres[1], Barbara Takács[1], Dániel Priksz[1], György Trencsényi [2], Juhász Béla[1], Zoltán Szilvássy [1], Mariann Bombicz [1] **13'+5'**

[1]Department of Pharmacology and Pharmacotherapy, University of Debrecen, [2]Medical Imaging Clinic, Nuclear Medicine Department, University of Debrecen

Effects of a Janus kinase Inhibitor on comorbidities observed in a rheumatoid arthritis model of ZSF1 rats

W-K3-5 **Zsigmond Kovács**[1], Csaba Dienes[1], József Óvári[1], János Magyar[2], Tamás Bányász[1], Péter Pál Nánási[3], Balázs Horváth [1], Ádám Fehér[4], Zoltán Varga[4], Norbert Szentandrássy[5] **13'+5'**

[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2] Division of Sport Physiology, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,[3] Department of Dental Physiology and Pharmacology, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary,[4] Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,[5] Department of Basic Medical Sciences, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary

The effects of ABT-333 (dasabuvir) on the electrophysiology of the canine left ventricular cardiomyocytes and expressed hERG channels

June 8, 2023 Thursday

8:00 **Registration- Hotel Lobby**

9:00-9:45 T-K1 **Clive Page - Plenary Lecture**

Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London

Novel anti-inflammatory drugs for the treatment of respiratory diseases

9:45-10:15 **Coffee break**

10:15-10:45 T-KA2 **Andreas Papapetropoulus - Keynote Lecture**

Laboratory of Pharmacology, Department of Pharmacy, National and Kapodistrian University of Athens

Roles of hydrogen sulfide in metabolic health and obesity

10:15-10:45 T-KB2 **Ines Drenjančević - Keynote Lecture**

Faculty of Medicine Osijek University Josip Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, Croatia, Scientific Center of Excellence for Personalized Health Care University of Osijek, Croatia

High salt diet affects the cerebral vascular reactivity – comparative studies in animals and humans

10:45-12:15 T-KA3 ***Possibilities and limitations of nanomedicine***

Chairs: János Szebeni, Ferenc Bari

T-KA3-1 **Rita Frank[1,2],** Orsolya M. Tóth[3], Ákos Menyhárt[1,2], Ferenc Bari[3], Eszter Farkas[1,2] **10'+2'**

[1] HCEMM-USZ Group of Cerebral Blood Flow and Metabolism, [2]Department of Cell Biology and Molecular Medicine, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Hungary[3]Department of Medical Physics and Informatics, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Hungary

Nimodipine in solution or associated to pH-sensitive nanoparticles attenuates ischemic injury

T-KA3-2 **László Dézsi[1,2],** Gergely Kozma[1,2,] Tamás Mészáros[1,2], Tamás Bakos[1], László Rosivall[1,2,] Gábor Szénási[1] and János Szebeni[1,2] **10'+2'**

[1]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary; [2]SeroScience LTD., Budapest, Hungary

The significance of porcine model predicting hypersensitivity reactions to nanoparticles

T-KA3-3 **Gábor Szénási[1]**, Tamás Bakos[1], Erik Őrfi[1], Tamás Mészáros[1,2], László Hricisák[2], László Rosivall[1,2], Péter Hamar[2], Zoltán Benyó[2], János Szebeni[1,2] and László Dézsi[1,2] **10'+2'**

[1]Institute of Translational Medicine, Semmelweis University, Budapest, [2]SeroScience Ltd., Budapest, Hungary

Comparison of liposomal drug-induced blood pressure changes in mice and rats

T-KA3-4 **Gábor Katona[1],** Ildikó Csóka[1] **10'+2'**

[1]Institute of Pharmaceutical Technology and Regulatory Affairs, Szeged

Novel perpesctives in the utilization of nanomedicines for brain targeting via intranasal route

T-KA3-5 **Nóra Igaz[1]**, Krisztina Szőke[1], Csenge Bocz[1], Dávid Kovács[1], Andrea Rónavári[2], Rita Szabó[3], Róbert Polanek[3], Andrea Buhala[4], Csaba Vizler[4], László Tiszlavicz[5], Zsolt Rázga[5], Katalin Hideghéty[3], Zoltán Kónya[2], Mónika Kiricsi[1] **10'+2'**

[1]Department of Biochemistry and Molecular Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary [2]Department of Applied and Environmental Chemistry, University of Szeged, Rerrich Béla tér 1, H-6720 Szeged, Hungary [3]ELI-ALPS, ELI-HU Non-Profit Ltd., Szeged, Hungary [4]Institute of Biochemistry, Biological Research Centre, Szeged, Temesvári krt. 62, H-6726 Szeged, Hungary [5]Department of Pathology, University of Szeged, Állomás utca 2, H-6720 Szeged, Hungary

Radiosensitizing effect of metal nanoparticles in combination with histone deacetylase inhibitors

T-KA3-6 **Szilvia Veszelka**, Anikó Szecskó, Gergő Porkoláb, Emese K. Páli, Mária Mészáros, Mária A. Deli **10'+2'**

Biological Barriers Research Group, Institute of Biophysics, , Biological Research Centre, Szeged

Targeted drug delivery across the blood-brain barrier by nanoparticles

T-KA3-7 **Csaba Bankó 15'**

Agilent Technologies, Inc. 5301 Stevens Creek Blvd. Santa Clara, CA 95051, United States

Multiplexing live-cell imaging and real-time impedance readout with RTCA eSight System

10:45-12:15 T-KB3 **New findings on the mechanisms of depression**

Chair: Dobolyi Árpád

T-KB3-1 **Attila Tóth 15'+5'**

Electrophysiology Research Group, Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University

Antidepressive effect of NMDA receptor antagonists via the normalization of the sleep

T-KB3-2 **Anita Kovács[1]**, Evelin Szabó[1], Kristóf László[1], László Lénárd[1], Zsuzsanna Tóth[2], Dóra Zelena[1] **10'+5'**

[1] Institute of Physiology, University of Pécs, Faculty of Medicine, [2] Laboratory of Neuroendocrinology and In Situ Hybridization, Institute of Anatomy, Tissue and Developmental Biology, Semmelweis University, Faculty of Medicine

The role of prolactin-releasing peptide (PrRP) in the development of depressive-like symptoms in rats

T-KB3-3 **Márta Balaskó,** Dóra Krisztina Kovács **10'+5'**

Institute for Translational Medicine, Medical School, Unoiversity of Pecs, Hungary

How to prevent obesity: the potential of urocortin 2 – based on animal experiments

T-KB3-4 **Fanni Dóra[1,2]**, Tamara Hajdu[2,3], Éva Renner[1], Krisztina Paál[5], Alán Alpár[1,4], Miklós Palkovits[1], Christos Chinopoulos[5], Árpád Dobolyi[2,3] **10'+5'**

[1]Human Brain Tissue Bank, Semmelweis University, Budapest, [2]Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, [3]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, [4]Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, [5]Department of Biochemistry and Molecular Biology, Semmelweis University, Budapest

Selective induction of Krebs cycle enzyme subunits in the parahippocampal cortex of suicide victims

T-KB3-5 **Vivien Csikós[1]**, Rashmi Kumari[1], Fanni Dóra[2], Arpád Dobolyi[1] **10'+3'**

[1]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary[2]Human Brain Tissue Bank and Microdissection Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University, 1094 Budapest, Hungary.

Effects of adult social isolation on the behavior and brain genomics of rats

T-KB3-6 **Katalin Eszter Ibos[1],** Éva Bodnár[1], Szakács Júlia[1], Zsolt Bagosi[1], Zsolt Bozsó[2], Krisztina Csabafi[1] **10'+2'**

[1]University of Szeged, Albert Szent-Györgyi Medical School, Department of Pathophysiology, Szeged
[2]University of Szeged, Albert Szent-Györgyi Medical School, Department of Medical Chemisrty, Szeged

Kisspeptin-8 suppresses locomotion, induces a transient hyperthermia and alters gene expression in the ventral tegmental area – nucleus accumbens circuit in rat

12:15-13:15 **Lunch- Mátra Restaurant**

13:15-13:45 T-KA4 **Thomas Voets - Keynote Lecture**

Laboratory of Ion Channel Research, VIB-KU Leuven Center for Brain & Disease Research &Dept. of Cellular and Molecular Medicine, KU Leuven, B-3000 Leuven, Belgium

TRPM3 – from the periphery to the brain

13:15-13:45 T-KB4 **Calkovska Andrea[1]**, Hanusrichterova Juliana[2], Kolomaznik Maros[2], Uhrikova Daniela[3] **Keynote Lecture**

[1]Department of Physiology and [2]Biomedical Centre Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia; [3]Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Physiological role of pulmonary surfactant: Much more than low surface tension

13:45-15:15 T-KA5 **"Sensational" ion channels- physiology and pharmacology at the forefront of temperature and pain sensation**

Chairs: András Garami, Péter Sántha, Balázs István Tóth

T-KA5-1 **Jan-Erik Siemens 18'+3'**

University of Heidelberg, Heidelberg, Germany

A heat acclimation-induced neuronal pacemaker that drives heat tolerance

T-KA5-2 **László Csanády[1]**, Ádám Bartók[1] 18**'+3'**

[1]Semmelweis University, Budapest

Molecular strategies for supersensitive central heat detection by TRPM2 channels

T-KA5-3 **István Nagy 18'+3'**

Imperial College, London, UK

RPS6KA5 (MSK1), in primary sensory neurons, is an essential regulator for the development and persistence of hypersensitivity to heat in inflammation

T-KA5-4 **Éva Szőke[1]**, Ádám Horváth[1], Maja Payrits[1], Andrea Nehr-Majoros[1], Gábor Berenkei[1], János Erostyák[2], Zsuzsanna Helyes[1] **12'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs; Hungary, [2]Department of Experimental Physics, University of Pécs, Pécs; Hungary

Analgesia via lipid rafts - Inhibition of activation of Transient Receptor Potential ion channels

T-KA5-5 **György Panyi[1]**, Muhammad Umair Naseem[1] 12**'+3'**

[1]Department of Biophysics and Cell Biology, University of Debrecen

The Kv1.3K+ channel is a therapeutic target in the treatment of autoimmune diseases

13:45-15:20 T-KB5 **Antimicrobial drug-development - In silico design, synthesis, and antiviral, antibacterial and antifungal evaluation**

Chairs: Anikó Borbás, Gyula Batta

T-KB5-1 **György G. Ferenczy,** Levente Mihalovits, György M. Keserű **10'+5'**
Research Centre for Natural Sciences,Budapest
Computational design of covalent inhibitors

T-KB5-2 **Jan Weber 15'+5'**
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences
New scaffolds for targeting HIV-1 provirus reactivation and silencing

T-KB5-3 **Ilona Bereczki[1,2,3],** Eszter Boglárka Lőrincz[1,4,] Pál Herczegh[1] and Anikó Borbás [1,2] **10'+5'**

[1] Department of Pharmaceutical Chemistry, University of Debrecen, Hungary, [2] National Laboratory of Virology, Szentágothai Research Centre, Pécs, Hungary, [3] ELKH-DE Pharmamodul Research Team, Debrecen, Hungary, 4 Doctoral School of Pharmaceutical Sciences, University of Debrecen, Hungary
Synhesis of antibacterial and antiviral glycopeptide antibiotic derivatives

T-KB5-4 **Henrietta Papp[1]**, Anett Kuczmog[2], Zoltán László Kopasz[1], Krisztina Rebeka Leiner[1], Mónika Madai[1], Ferenc Jakab[1,2] **10'+5'**

[1]National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, [2]Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, [3]Doctoral School of Biology and Sportbiology, University of Pécs, Pécs
In vitro testing of antiviral compounds in the National Laboratory of Virology

T-KB5-5 **Gyula Batta[1]**, András Czajlik[1], Gai Jiawei[1], Réka Erdei[1], László Izsépi[1], Pál Herczegh[2] and Ilona Bakai-Bereczki[2] **10'+5'**

[1]Institute of Chemistry, UD, Debrecen, [2]Institute of Pharmaceutical Chemistry, UD, Debrecen

Vancomycin Antibiotics and Antifungal Proteins as Antiviral Agents? The Use of NMR Spectroscopy and Artificial Intelligence.

T-KB5-6 **James Berwick 10'+5'**
Collaboration between: EPFL, ETHZ, ZHAW, Lund University and Nanosurf AG
Topographical, mechanical and mass characterization of biological samples with AFM

15:15-15:35 **Coffee break**

15:35-17:15 T-KA6 **Modern methods in education**

Chairs: Zoltán Varga, Dóra Zelena

T-KA6-1 **Pál Riba[1], Zoltán Varga[1] 13'+3'**

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Core concepts based education of pharmacology

T-KA6-2 **Anikó Görbe[1]**, Zsófia Onódi[1], Nabil V. Sayour[1], Pál Riba[1], Zoltán V. Varga[1] **13'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Introducing flipped classroom approach in pharmacology for medical students – a pilot from Semmelweis University

T-KA6-3 **Levente Kiss[1,2] 13'+3'**

[1]Department of Physiology, [2]Center of Educational Development, Methodology and Organization

Educational development programs at Semmelweis University: Past-Present-Future

T-KA6-4 **Gyula Sáry 13'+3'**

University of Szeged

Quantity or relevance?

T-KA6-5 **Rita Gálosi 13'+3'**

Physiology Institute, Medical School of University Pécs

Challenges in teaching physiology: Pros and Cons of frontal lectures and the opportunities of blended learning

T-KA6-6 **Dávid Karádi[1], Zoltán Varga[1] 13'+3'**

[1]Semmelweis University, Budapest

Teaching pharmacology on Instagram - two years of experience at Semmelweis University

15:35-17:15 T-KB6 **Novel mechanisms of gene expression regulation**

Chairs: Katalin Monostory, Tamás Orbán

T-KB6-1 **Ágnes Tantos 15'+5'**

Research Centre for Natural Sciences,Budapest

Regulatory roles of RNA binding in histone methyltransferases

T-KB6-2 **Lóránt Székvölgyi[1] 15'+5'**

[1]Lendület Genome Architecture and Recombination Research Group, Faculty of Pharmacy, University of Debrecen

Molecular investigation of chromosomal R-loops in neurodegenerative disease

T-KB6-3 **Dalma Müller[1]**, Balázs Győrffy[1,2] **15'+5'**

[1]Semmelweis University, Department of Bioinformatics, Budapest, Hungary,[2] TTK Oncology Biomarker Research Group, Budapest, Hungary

		Coupling epigenetic and clinical data of adenocarcinoma samples through a web application
	T-KB6-4	**Tamás I. Orbán[1]** Ábel Fóthi[1] **15'+5'** [1]Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary *Regulating the regulators: maturation and stability of microRNAs*
	T-KB6-5	**Elen Gócza[1]**, Nikolett Tokodyné Szabadi [1], Roland Tóth [1]; Maria Teresa Salinas Aponte[1], Bence Lázár [1,2]; Eszter Várkonyi [2]; Krisztina Liptói [2] **15'+5'** [1]Agribiotechnology and Precision Breeding for Food Security National Laboratory, Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology, Department of Animal Biotechnology, Gödöllő, Hungary [2]National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary *Exploration of the effect of heat treatment on epigenetic alteration in chicken primordial germ cells*
15:35-17:15	T-G6	**Pharmaceutical Medicine Section** *Chair: Sándor Kerpel-Fronius, Kata Mazalin*
	T-G6-1	**Róbert Pórszász[1,2] 20'+5'** [1]Department of Pharmacology and Pharmacotherapy, Faculty of General Medicine, University of Debrecen, Debrecen, Hungary, [2]National Institute of Pharmacy and Nutrition, Budapest, Hungary *The role of the committee for medicinal products for human use (CHMP) in the centralised authorisation of medicines*
	T-G6-2	**Judit Tarnai 20'+5'** Hungarian National Health Research Agency *The role,tasks and achievements of the Hungarian National Health Research Agency in clinical trials in Hungary*
	T-G6-3	**Kata Mazalin 20'+5'** Novartis Hungária Kft. *The status of investigator initiated trials in Hungary*
	T-G6-4	**Alex Ali Sayour[1]**, Loretta Kiss[1], Zsolt Bagyura[1], Béla Merkely[1] **20'+5'** [1]Heart and Vascular Center, Semmelweis University, Budapest *The Semmelweis LIpid Center for High–RisK Patients (SLICK) Trial*
17:15-18:45	T7	***Poster Section- Wine bar and Lobby***
17:15-17:30		**Board Meeting of the Hungarian Society for Microcirculation and Vascular Biology- Galya Room**
17:30-18:00		**General Assembly of the Hungarian Society for Microcirculation and Vascular Biology - Galya Room**
18:30-19:00		**Board Meeting of the Hungarian Society of Physiology - Galya Room**
19:30		**Dinner- Mátra Restaurant**

June 9, 2023 Friday

8:00 **Registration- Hotel Lobby**

08:45-9:15 F-KA1 **Evgeniya Tsedik-Keynote Lecture**
Thermo Fisher Scientific, Eindhoven, the Netherlands
Rational Drug Design with State-of-the-art Cryo-EM

8:45-9:05 F-KB1-1 **Helena Lenasi-Keynote Lecture**
Institute of Physiology, faculty of Medicine, University of Ljubljana,
Skin microcirculation during exercise and in the recovery: an insight into potential mechanisms behind

9:05-9:30 F-KB1-2 **Nina Vardjan[1,2]**, Anemari Horvat[1,2], Helena H. Chowdhury[1,2], Marko Kreft[1,2,3], Robert Zorec[1,2] - **Keynote Lecture**

[1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Slovenia, [2]Laboratory of Cell Engineering, Celica Biomedical, Ljubljana, Slovenia, [3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia
Metabolic excitability of astrocytes

9:15-11:00 F-KA2 ***Development of gene and cell therapy products in Hungary and the path to clinical introduction***
Chairs: András Dinnyés, Péter Ferdinandy

F-KA2-1 **Sándor Kerpel-Fronius[1]**
[1]Semmelwis University
Scientific, regulatory and ethical challenges developing Advanced Therapy Medicinal Products (ATMP)

F-KA2-2 **Katalin Lengyel 10'+5'**
National Institute of Pharmacy and Nutrition, Budapest
Regulatory considerations for clinical trials of advanced therapy medicinal products

F-KA2-3 **Gergely Kriván 10'+5'**
Central Hospital of Southern-Pest - National Institute of Hematology and Infectology - Department of Pediatric Hematology and Stem Cell Transplantation
Clinical application of advanced therapeutic preparations in pediatric hematopoietic stem cell transplantation

F-KA2-4 **Zoltán Veréb [1,2]** Balázs Bende[1], János Varga[1], Melinda Guba [2], Diána Szűcs [2], Tamás Monostori [2], Lajos Kemény [1,2,3] **10'+5'**
[1]Department of Dermatology and Allergology, University of Szeged-SZAOK, Szeged [2]Regenerative Medicine and Cellular Pharmacology Laboratory, IKIKK, Szeged [3]HCEMM-Skin Research Group SZTE, HCEMM, University of Szeged, Szeged
SVF cell therapy for the treatment of non-healing limb ulcers

F-KA2-5 **Judit E Pongrácz[1]** Judit Bóvári-Biri[1,2], Alexandra Steinerbrunnerné-Nagy[1,2], Péter Bakó[3], Márton Kovács[3], Péter Maróti[4], Alexandra Mihócs[5], András Vereczkei[5] **10'+5'**

[1]Institute of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs, Pécs, [2]Szentágothai Research Center, University of Pécs, [3]Department of Otorhinolaryngology, Clinical Centre, University of Pécs, [4]3D Printing and Visualization Center, University of Pécs, Pécs, Hungary, [5]Department of Surgery, University of Pécs, Pécs

Challenges in bioprinting tissues for clinical application - stapes

F-KA2-6 Anita Fehér[1], Andrea Schnúr[1], Suchitra Muenthaisong[1], Tamás Bellák[1,2], Ferhan Ayaydin[3,4], György Várady[5], Kinga Molnár[6] **András Dinnyés [1,7,8,9] 10'+5'**

[1]BioTalentum Ltd., Gödöllő, [2]Department of Anatomy, Histology and Embryology, Albert Szent-Györgyi Medical School, USZ, [3]Functional Cell Biology and Immunology Advanced Core Facility, HCEMM-USZ, Szeged, [4]Laboratory of Cellular Imaging, Biological Research Centre, ELKH, Szeged, [5]Research Centre for Natural Sciences, Institute of Enzymology, Budapest, [6]Department of Anatomy, Cell and Developmental Biology, Faculty of Science, ELTE, Budapest, [7]Stem Cell Research Group, HCEMM-USZ, Szeged, [8]Department of Cell Biology and Molecular Medicine, USZ, [9]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, MATE, Gödöllő

Generation of transplantable, functional human beta-cells from IRFP720-expressing induced pluripotent stem cells (iPSCs) for in vivo imaging

9:30-11:00 F-KB2 ***Drug- and disease associated changes in the gut microbiota***

Chair: Zoltán Zádori

F-KB2-1 **Dóra Zelena 12'+3'**

University of Pécs, Medical School, Institute of Physiology

Posttraumatic stress disorder from the viewpoint of the gut

F-KB2-2 **Gabriella Kékesi[1],** Szonja B. Plesz[1], Leatitia G. Adlan[1], Gyöngyi Horváth[1], Kálmán F. Szűcs[2], Róbert Gáspár[2], Balázs Ligeti[3], Zoltán S. Zádori[4] **12'+3'**

[1]Department of Physiology, [2]Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Szeged; [3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest; [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Gut microbiome composition and Gastrointestinal activity in the Wisket rat model of schizophrenia

F-KB2-3 **Zoltán Zádori[1],** Arezoo Haghighi[1], András S. Tóth[1], Bernadette Lázár[1], Szilvia B. László[1], Barbara Hutka[1,2], Mahmoud Al-Khrasani[1], Bence Ágg[1], Balázs Ligeti[3], Nóra Makra[4], Eszter Ostorházi[4], Dóra Szabó[4], Klára Gyires1 **12'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary;2 Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary (current address), 3Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary; 4Department of Medical Microbiology, Semmelweis University, Budapest, Hungary

The effect of conventional nonsteroidal anti-inflammatory drugs and selective COX-2 inhibitors on gut microbiota composition

F-KB2-4 **Dóra Szabó 12'+3'**

Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

The influence of antibiotics on transitory resistome during gut colonization with multiresistant Klebsiella pneumoniae

F-KB2-5 **Aitak Farzi[1],** Eva Tatzl[1,2], Slave Trajanoski[3], Michael K. Herbert[2,] Peter Holzer[1] **12'+3'**

[1]Division of Pharmacology, Otto Loewi Research Centre, Medical University of Graz, Graz, Austria, [2]Department of Anaesthesiology and Intensive Care, Medical University of Graz, Graz, Austria, [3]Core Facility Computational Bioscience, Centre for Medical Research, Medical University of Graz, Graz, Austria

Antibiotic-induced microbiota depletion in GUINEA PIG intestine reduces expression of α2b-adrenoceptors and their activity in peristaltic motor inhibition

F-KB2-6 **Lóránd Kiss[1]**, Erik Márk Orján[1], Eszter Sára Kormányos[1], Gabriella Mihalekné Fűr[1], Ágnes Dombi[2], Emese Réka Bálint[1], Zsolt Balla[1], Beáta Adél Balog[1], Ágnes Dágó[1], Ahmad Totonji[1], Zoárd István Bátai[2], Eszter Petra Jurányi[3], Ammar Al-Omari[2], Gábor Pozsgai[2], Viktória Kormos[2], Péter Nagy[3], Erika Pintér[2], Zoltán Rakonczay Jr[1], **12'+3'**

[1] Department of Pathophysiology, University of Szeged, Szeged, Hungary, [2] Department of Pharmacology and Pharmacotherapy, University of Pécs, Pécs, Hungary, [3] Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary.

A sulfide donor, dimethyl trisulfide, alleviates experimental acute pancreatitis

11:00-11:20 **Coffee break**

11:20-12:50 F-KA3 ***TDK Section***

Chairs: Gabriella Kékesi, Ferenc Bari

F-KA3-1 **Stefánia Csicsely[1,2],** Boldizsár Jójárt[1,2,3], Viktória Szabó[1,2,3], József Maléth[1,2,3] **10'+5'**

[1]Department of Medicine, Szent-Györgyi Albert Medical School, University of Szeged, Szeged, [2]HAS-USz Momentum Epithelial Cell Signaling and Secretion Research Group, Szeged, [3]HCEMM-USz Molecular Gastroenterology Research Group, Szeged

Development of in vitro fibrosis model for the evaluation of potential anti-fibrotic drug candidates

F-KA3-2 **Bíbor Gárdos[1]**, András Garami[1], Eszter Pákai[1] **10'+5'**

[1]Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pécs, Hungary

Investigation of the protective effect of human milk oligosaccharides (HMO) in bacterial endotoxin-induced fever

F-KA3-3 **Balázs Kaszás-Szenci[1]**, Norman Noel Tanner[1], Zoltán Márton Köhler[1], Kitti Szabó[1], György Trencsényi[2], Anikó Keller-Pintér[1] **10'+5'**

[1] Department of Biochemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary [2]Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The metabolic effects of the BMP-Inducer tilorone in in vitro and in vivo models

F-KA3-4 **Franciska Benyó [1]**, Bálint Király[1], Vivien Pillár[1], Anna Velencei[1] & Balázs Hangya[1] **10'+5'**

[1]Institute of experimental medicine, Budapest

The role of neuromodulatory systems in implicit learning

F-KA3-5 **Boldizsár Vámosi[1],** Szimonetta Tamás[1,2,3], Benoit Roux[1,3], Balázs Enyedi[1,2,3] **10'+5'**

[1]Department of Physiology, Semmelweis University, Budapest, [2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, [3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest

Investigating the production and role of Leukotriene B4 in neutrophils

F-KA3-6 **Fruzsina Maácz[1,2]**, Flóra Gölöncsér[1], Beáta Sperlágh[1] **10'+5'**

[1]Institute of Experimental Medicine, Budapest, Hungary, [2]Eötvös Lóránd University, Budapest, Hungary

Effects of sumatriptan on P2X7 purinergic receptor-mediated signaling in an amphetamine-induced acute mania mouse model

11:20-12:50 F-KB3 ***Tissue regeneration from inflammatory bases in skeletal muscle***

Chairs: Ernő Zádor, Zsolt Czimmerer

F-KB3-1 **Zsolt Czimmerer[1,2,12, *]**, Laszlo Halasz[3,12], Bence Daniel[3,11,12], Zsofia Varga[2], Krisztian Bene[1], Apolka Domokos[1,4], Marten Hoeksema[5], Zeyang Shen[5,6], Wilhelm K. Berger[3], Timea Cseh[1], Karoly Jambrovics[1], Zsuzsanna Kolostyak[1,4,] Ferenc Fenyvesi[7], Judit Varadi[7], Szilard Poliska[1], Gyorgy Hajas[8,9], Istvan Szatmari[1], Christopher K. Glass[5,10], Attila Bacsi[8,9] and Laszlo Nagy[1,3, *] **25'+5'**

[1] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2] Institute of Genetics, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary, [3] Departments of Medicine and Biological Chemistry, Johns Hopkins University School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA, [4] Molecular Cell and Immunobiology Doctoral School, Faculty of Medicine, University of Debrecen, H-4032, Debrecen, Hungary, [5] Department of Cellular and Molecular Medicine, School of Medicine, University of California, San Diego, La Jolla, CA, USA [6] Department of Bioengineering, Jacobs School of Engineering, University of California, San Diego, La Jolla, CA, USA, [7] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [8] Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [9] ELKH-DE Allergology Research Group, Debrecen, Hungary, [10] Department of Medicine, School of Medicine, University of California, San Diego, La Jolla, CA, USA, [11] Present address: Stanford University School of Medicine, Department of Pathology, Stanford, CA, USA, [12] These authors contributed equally to this work.,*Correspondence: lnagy@jhmi.edu, czimmerer.zsolt@brc.hu

The epigenetic bases of unique inflammatory responsiveness in the alternatively polarized macrophages

F-KB3-2 **Krisztián Bene[1],** Petros Tzerpos[1], Dóra Bojcsuk[1], Noemi Caballero Sanchez[1,2], Mohamed Osama[1], László Halász[3], Andreas Patsalos[3], László Nagy[1,3] **16' + 4'**

[1]Nuclear Receptor Research Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen. Hungary [2]Doctoral School of Molecular Biology and Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary [3]Departments of Medicine and Biological Chemistry, Johns Hopkins University School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, Florida, USA

The transcription factor BACH1 regulates macrophage development and inflammatory function

F-KB3-3 **Noemí Caballero-Sánchez [1,2]**, Petros Tzerpos [1], Krisztian Bene[1], Laszlo Halasz [3], Andreas Patsalos[3], Darby Oleksak[3], Dóra Bojcsuk[1], Gergely Nagy [1], Laszlo Nagy [1, 3] **16' + 4'**

[1]Department of Biochemistry and Molecular Biology, Nuclear Receptor Research Laboratory, Faculty of Medicine, University of Debrecen, Hungary [2] Doctoral School of Molecular Cell and Immunobiology, Faculty of Medicine, University of Debrecen, Hungary [3] Institute For Fundamental Biomedical Research, John Hopkins All Children's Hospital, St Petersburg, Fl, United States

Single-cell RNA profiling of immune cells uncovers the critical role of BACH1 during macrophage polarization in muscle repair

F-KB3-4 **Lulu Alsheikh Hussein,** Ernő Zádor **8' + 2'**

Institute of Biochemistry, Albert Szent-Györgyi Faculty of Medicine, University of Szeged

The Effect of Transfected Small Membrane Peptides In Muscle Regeneration

F-KB3-5 **Ernő Zádor 8' + 2'**

Institute of Biochemistry, Albert Szent-Györgyi Faculty of Medicine, University of Szeged

Regulins, calcium metabolism and muscle regeneration

12:50-13:50 **Lunch- Mátra Restaurant**

13:50-15:20 F-KA4 ***Drug development and innovation opportunities in Hungary***

Chairs: Viktor Román

F-KA4-1 **Krisztián Tárnok[1],** Krisztina Bauer[1], Ben Mahmoud Maissa[1], Anikó Rátkai [1], Attila Szücs[1], Katalin Schlett[1] **15'+3'**

[1] Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

OptoGiN (optically guided induced neuron): A new optogenetic approach to the guided development of human neurons, in vitro

F-KA4-2 **Thomas Brevig 15'+3'**

Richter Gedeon Plc., Budapest

Unmet treatment needs in schizophrenia and new drug development

F-KA4-3 **Beáta Sperlágh[1,2]** Dorottya Szabó[1,2], Paula Mut Arbona[1,2], Pál Tod[1], Flóra Gölöncsér[1] **15'+3'**

[1]Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Budapest, Hungary, [2]János Szentágothai Doctoral School, Semmelweis University, Budapest, Hungary

From perinatal infections to synaptic dysfuction: novel therapeutic targets for the treatment of autism spectrum disorder

F-KA4-4 **Ádám Dénes 15'+3'**
Institute of Experimental Medicine
Microglial dysfunction as a possible driver of COVID-19-related neuropathologies

F-KA4-5 **Balázs Pál**, Andrea Csemer, Adrienn Kovács, Baneen Maamrah, Krisztina Pocsai, Kristóf Korpás, Álmos Klekner, Péter Szücs, Péter P. Nánási **15'+3'**
University of Debrecen
Astrocyte- and NMDA receptor-dependent slow inward currents differently contribute to synaptic plasticity in an age dependent manner in mouse and human neocortex

13:50-15:20 F-KB4 ***Renaissance of the stress regulatory CRH family***
Chair: Dóra Zelena

F-KB4-1 **Ludmilla Filaretova 18'+2'**
Pavlov Institute of Physiology of Russian Academy of Sciences
CRH as a regulator of gastric mucosal sensitivity to ulcerogenic stimuli

F-KB4-2 **Alán Alpár 18'+2'**
Department of Anatomy, Histology and Embryology, Semmelweis University, Faculty of Medicine
Role of hypothalamic CRH neurones in different stress mechanism

F-KB4-3 **Viktória Kormos** [1], Ammar Al-Omari [1], Miklós Kecskés [2], Balázs Gaszner[3], Erika Pintér[1], **18'+2'**
[1]University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy [2]University of Pécs, Medical School, Department of Physiology [3]University of Pécs, Medical School, Department of Anatomy
Urocortinergic neurons of the Edinger-Westphal nucleus in acute alcohol exposure

F-KB4-4 **Dóra Zelena 18'+2'**
University of Pécs
Role of brainstem CRH in stress regulation

F-KB4-5 **Éva Bodnár[1]**, Katalin Eszter Ibos[1], Hoa Dinh Thi Thanh[2], Zsolt Galla[3], Péter Monostori[3], Júlia Szakács[1], Márta Sárközy[2#], Krisztina Csabafi[1] **8'+2'**
[1]University of Szeged, Albert Szent-Györgyi Medical School, Department of Pathophysiology, Szeged [2]University of Szeged, Albert Szent-Györgyi Medical School, Department of Biochemisrty, Szeged 3University of Szeged, Albert Szent-Györgyi Health Centre, Department of Pediatrics and Pedriatic Health Center, Szeged
Moderate chronic kidney disease induces anxiety in rats

15:20-15:40 **Coffee break**

15:40-17:10 F-KA5 ***Role of pattern recognition and autoinflammation in the mechanisms of common disorders***

Chairs: Ádám Dénes, Szilvia Benkő

F-KA5-1 **Szilvia Benkő 12'+3'**

Inflammationphysiology Research Group, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The role of pattern recognition receptors in physiological and patophysiological processes

F-KA5-2 **Krisztina Futosi[1,2,]** Tamás Németh[1,3,4,5,] Ádám Horváth[6,7,] Zsuzsanna Helyes[6,7,8] Attila Mocsai[1,2] **12'+3'**

[1]Semmelweis University, Budapest

Tyrosine kinase pathways in monosodium urate crystal-induced inflammatory processes

F-KA5-3 **Mihály Józsi[1],** József Kardos[1] **12'+3'**

[1]ELTE, Budapest

The soluble pattern recognition molecules pentraxins, complement and their collaboration in health and disease

F-KA5-4 **Zsofia Varga[1]**, Eszter Varadi[1,2], Marta Toth[3], Anett Mazlo[3], Attila Bacsi[3,4], Gergely Nagy[5], Laszlo Nagy[5,6], Gabor Szebeni[7], Balint Csorgo[8], Bence Daniel[9], Zsolt Czimmerer[1,10] **12'+3'**

[1]Institute of Genetics, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [2] Doctoral School in Biology, University of Szeged, Szeged, Hungary, [3] Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen,Hungary, [4] ELKH-DE Allergology Research Group, Debrecen, Hungary, [5] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [6] Departments of Medicine and Biological Chemistry, Johns Hopkins University, School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA, [7] Laboratory of Functional Genomics, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [8] Institute of Biochemistry, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [9] Department of Pathology, Stanford University, Stanford, CA 94305, USA, [10] Corresponding author: czimmerer.zsolt@brc.hu

The epigenetic bases of the distinct immunomodulatory factors and pathogen-derived molecules-regulated PD-L1 and PD-L2 expression in murine and human macrophages

F-KA5-5 **Zoltán Varga[1,2,3],** Tamás Gergely[1,2,3] **12'+3'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary [2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary [3]MTA-SE Momentum Cardio-Oncology and Cardioimmunology Research Group, Budapest, Hungary

Cardiotoxic effects of immune checkpoint inhibitors

F-KA5-6 **Gábor Juhász[1,2]**, Áron Szabó[1], Virág Vincze[1], Aishwarya Chhatre[1] **12'+3'**

[1]Biological Research Centre, Szeged, [2]Eötvös Loránd University, Budapest

Drosophila models of neuroinflammation and inflammatory bowel disease

15:40-16:20 F-KB5 ***Perspectives of Extracellular vesicles in medicine***

Chair: Giricz Zoltán

F-KB5-1 **Krisztina Buzas[1,12,13,] ***, Matyas Bukva[1,2, a,b], Edina Sebestyen-Gyukity[1,a], Timea Boroczky[1,2,12], Yasmin Ranjous[3], Maria Harmati[1], Gabriella Dobra[1,2], Adrienn Jenei[4], Laszlo Szivos[5], Katalin Hideghety[6,7], Krisztina Budai[8], Judit Olah[7], Peter Horvath[1], Gyorgy Lazar[9], Tamas Biro[10,11], Zoltan Konya[3], Pal Barzo[5], Almos Klekner[4] **15'+5'**

[1]Laboratory of Microscopic Image Analysis and Machine Learning, Institute of Biochemistry, Biological Research Centre, Szeged, Hungary, [2]Doctoral School of Interdisciplinary Medicine, University of Szeged, Szeged, Hungary, [3]Department of Applied and Environmental Chemistry, University of Szeged, Szeged, Hungary, [4]Department of Neurosurgery, Clinical Centre, University of Debrecen, Debrecen, Hungary, [5]Department of Neurosurgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [6]ELI-ALPS, ELI-HU Non-Profit Ltd., Szeged, Hungary, [7]University of Szeged, Faculty of Medicine, Department of Oncotherapy, Szeged, Hungary, [8]Department of Surgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [9]Department of Surgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [10]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [11]Monasterium Laboratory, Münster, Germany, [12]Department of Immunology, Faculty of Medicine, University of Szeged, Szeged, Hungary, [13]Department of Immunology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary, a: co-first authors, b:presenting author, *: corresponding author

Raman spectral signatures of plasma-derived extracellular vesicle-enriched isolates support the diagnosis of different cancerous diseases

F-KB5-2 **Brachyahu Meir Kestecher[1, 2,3]**, Sayam Ghosal[1, 2], Bernadett Réka Bodnár[1, 2], Adrienn Szabó[1, 2], Nabil Sayour[4], Tamás Gergely[4], Krisztina Németh[2,3], Zoltán Giricz[4], Zoltán Varga[4], Péter Ferdinandy[4], Edit I Buzás[1, 2,3], Xabier Osteikoetxea[1, 2] **15'+5'**

[1] HCEMM-SU Extracellular Vesicles Research Group, Budapest, Hungary Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary [2] Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary [3]ELKH-SE translational Extracellular Vesicle Research Group [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Circulating extracellular vesicle levels are inversely correlated to cholesterol levels in hypercholesterolemia mouse models

16:20-17:10 **Healthy aging: Role of catecolaminerg enhancers**

Chairs: Ildikó Miklya, Dóra Zelena

F-KB5-3 **Ildikó Miklya 5'**

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

The Role of the Enhancer Regulation

F-KB5-4 **László G. Hársing Jr.**, Júlia Timár, Ildikó Miklya **10'**

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

The Enhancer Compound (-)BPAP Facilitates Dopaminergic Neurotransmission Via Activation of TAAR1 Signaling

F-KB5-5 **Júlia Timár**, László G. Hársing, Jr., Ildikó Miklya **10'**

Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Combination of Enhancer Compounds (-)Deprenyl and BPAP with MAO-B Inhibitor Rasagiline which Fails to Show Enhancer Activity

F-KB5-6 **Aliz Judit Ernyey**, Ferenc Kassai, Kata Kozma, Imola Plangár, Zsuzsa Somfai, Ildikó Miklya, István Gyertyán **10'**

Department of Pharmacology and Pharmacotherapy, Semmelweis University Budapest

Effect of the enhancer compound (-)BPAP on the age-related decline of various cognitive functions

F-KB5-7 **Dóra Zelena 10'**

University of Pécs, Medical School, Institute of Physiology

Enhancer Compounds and Stress Conditions

Discussion 5'

17:10-17:40 F-KA **Conference closing, Poster awards**

abberior INSTRUMENTS

Superresolution STED
microscopy from its inventors

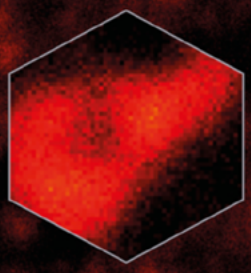
Facility Line

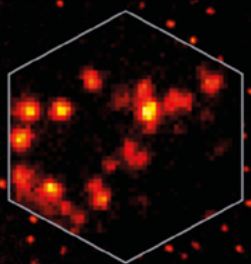
fastest time to result

Compact Line STEDYCON

expands any microscope to confocal & STED

„There are many reasons why your standard fluorescence microscope ...





... should be a STED“.

Stefan W. Hell, Nobel Laureate in Chemistry 2014

1 µm

UNICAM

1144 Budapest, Kőszeg utca 29.
Telefon: +36 1 221 5536
E-mail: unicam@unicam.hu
Web: www.unicam.hu

CEBIO
Central European Biosystems

18 csatornás Odyssey M képalkotó rendszer

LI-COR

Near-Infrared Fluorescent Western Blot
Visible Fluorescent Western Blot
Chemiluminescent Western Blot
Colorimetric Western Blot
In-Cell Western™ Assay
On-Cell Western Assay
Cell Health Assay
ELISA
Fluorescent Protein Gel
Colorimetric Protein Gel
Nucleic Acid Gel
EMSA/Gel Shift Assay
Fluorescent Tissue Section
Histological Staining
Protein Array

Optical System

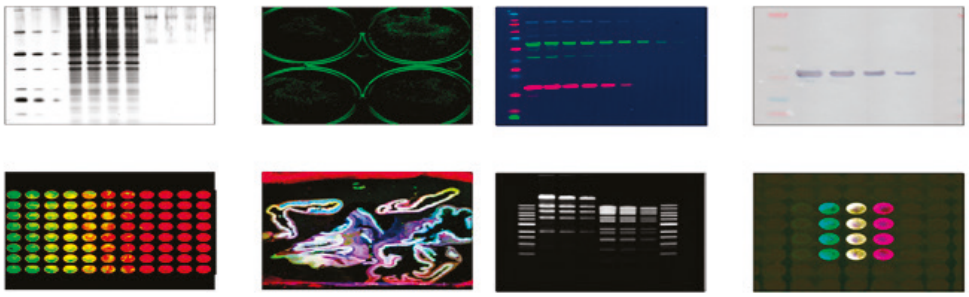
Image Field Size
25 cm W × 18 cm D (9.8” W × 7.1” D)
Scan Area of Chemi Region
15 cm W × 11 cm D (5.9” W × 4.3” D)

Resolution
5, 10, 20, 50, or 100 µm

Dynamic Range
>6 logs for chemiluminescence (optional) and fluorescence

Detectors
sCMOS image sensor
CCD Sensor for chemiluminescence

Light Sources
RGB LED (trans-illumination)
RGB LED (reflective illumination)
Solid-state diode laser at 488 nm
Solid-state diode laser at 520 nm
Solid-state diode laser at 685 nm
Solid-state diode laser at 785 nm



www.cebiosys.hu

MEGÚJULT A WEBOLDALUNK

T/ +36-1-226-5256 F/ /+36-1-226-5257
E/ cebiosys@cebiosys.com W/ www.cebiosys.hu
1044 Budapest, Óradna utca 4.

AKRONOM Kft.
1149 Budapest, Angol u.34.
Tel.: +36 1 799-9212
E-mail: info@ akronom.hu

Az **AKRONOM Kft.** a magyar állatkísérleti kutatási piac egyik meghatározó szereplője. Az általunk üzembe helyezett termékek szervizét garancia időn belül és azon túl is saját, akkreditált, szakképzett szervizünk végzi.

Termékköreink:

1. ***Laborállat tartó eszközök és berendezések***
   - komplett állattartó ketrec rendszerek, aktivitás ketrecek laborállatok számára
   - komplett „Aquatic" állattartó rendszer (zebra danio, afrikai karmosbéka)
   - szellőztetett szekrények szűrőtetős ketrecek számára (HEPA- és szénszűrővel)
   - egyedi szellőztetett (IVC) ketrec rendszerek, transzport és tárolós kivitelben is
   - hermetikusan záródó egyedi szellőztetett (DCC, ISOcage) ketrec rendszerek BSL2 BSL3 és BSL4 felhasználók számára speciális biztonsági munkavédelmi szekrénnyel
   - diuresis és metabolikus ketrecek, tartóállványokkal, hűtőtárolóval
   - állatcserélő biztonsági munkavédelmi szekrények (LAF box)
   - alomürítő berendezések; központi alom elszívó és töltő rendszer
   - laborállat tápok, almok és környezetgazdagító eszközök (fészekanyagok, papírcsövek)

2. ***Laborállat tartó eszközök mosásával és fertőtlenítésével* foglalkozó *berendezések***
   - palackmosó, ketrecmosó, állványmosó, műszermosó berendezés, mosóalagút
   - palacktöltő rendszer a fél-automatától a teljesen automatizált ürítő-mosó-töltő rendszerig
   - rozsdamentes acél átadó- és fertőtlenítő kabin a barrier sávhoz
   - autoklávok (25 litertől 8.712 literig), szárítószekrények (417 litertől 5.040 literig), gőzgenerátorok (16 kg/h-tól 1.450 kg/h-ig - pirogénmentes nagytisztaságú gőzt előállító is)
   - H_2O_2 generátorok és fertőtlenítő kamra

3. ***Laborállat forgalmazás***
   - ENVIGO laborállatok forgalmazása

4. ***Állatorvos- és labortechnológiai berendezések***
   - altatógépek (gáz vagy folyadék alapú), lélegeztető gépek, eutanázia berendezések
   - légzuhanyok

5. ***Laboratóriumi és tisztatéri bútorok és mobíliák***
   - teljes műtői és vizsgálói rozsdamentes bútor és mobília választék, átadóablakokkal
   - laborbútor rendszer fém SPF falrendszerrel, nyílászárókkal

6. ***Laboratóriumi és tisztatéri fal- és padló rendszer***
   - tisztatéri fal rendszer (minden szükséges kiegészítővel, pl. reteszelt nyílászárókkal)
   - tisztatéri padló rendszer (fal és lefolyó illesztéssel, 5 rétegben, különböző igényekre)

7. ***Tisztító, mosó- és fertőtlenítőszerek forgalmazása***

FAMÉ 2023

Mátraháza
7–9 June 2023

Oral abstracts

MCMXXXI
SOCIETAS
PHYSIOLOGICA
HUNGARICA
M.É.T

M M V B T

HUPHAR
Hungarian Society for Experimental
and Clinical Pharmacology

WEDNESDAY
7 JUNE 2023

W-K1 - STRATIFYING NEUROPATHIC PAIN PATIENTS IN PHARMACOLOGICAL TRIALS: THE WAY FORWARD

Prof. Dr. Ralf Baron
Division of Neurological Pain Research and -therapy, Department of Neurology, University Hospital, Kiel, Germany

Painful neuropathy represents a major medical problem and treatment has been unsatisfactory. A hypothetical concept was proposed in which pain is analysed on the basis of underlying mechanisms and sensory abnormalities. If a systematic clinical examination of the patient and a precise phenotypic characterization is combined with a selection of drugs acting at those particular mechanisms, it should ultimately be possible to design optimal treatments for the individual patient.

Several international consortia (German Research Network on Neuropathic Pain, IMIEuropain, Neuropain) established a large data-base that includes epidemiological and clinical data as well as standardized symptom questionnaires and quantitative sensory testing. More than 2000 patients with different neuropathic pain states have been examined.

Using a subgroup analysis, subgroups with different somatosensory profiles and pain generating mechanisms could be identified. Several recent clinical trials using this sensory profile- and mechanism-based classification could identify a differential treatment effect in subgroups of patients. Consequently, cohorts in clinical trials should be stratified and potentially enriched with patients who likely respond to the study drug based on mechanisms and sensory profiles. This approach has the potential to minimize pathophysiological heterogeneity within the groups under study and to increase the power to detect a positive treatment result. In clinical proof-of-concept trials the study population can be enriched prospectively on the basis of “a priori” defined entry criteria. This enrichment with patients who potentially require a specific treatment will increase the likelihood for positive trial outcomes.

W-KA2-1 - THE COMPREHESIVE ANALYSIS OF PAIN AND INFLAMMATION IN DIFFICULT-TO-TREAT RHEUMATOID ARTHRITIS WITH A COMPLEX CLINICAL, PSYCHOLOGICAL, MULTIOMIC AND fMRI EVALUATION

Lilla Gunkl-Tóth[1,2], Gergely Orsi[3,4], Krisztina Csókási[5], Gábor Sütő[6], Gábor Kumánovics[7], Noémi Császár-Nagy[8,9], Szabolcs Takács[10], Eszter Szigedi[9], Zsófia Nagy[9], Zsolt Hodovány[9], Lili Duzsik[9], Zoltán Vidnyánszky[11], József Kun[1,12], Péter Urbán[12], Attila Gyenesei[12], György Nagy[13,14,15], Zsuzsanna Helyes[1,2]

[1] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, [2]ELKH-PTE Chronic Pain Research Group, Eötvös Loránd Research Network (ELKH), Pécs,[3]MTA-PTE Clinical Neuroscience MR Research Group, Eötvös Loránd Research Network (ELKH), Pécs,[4]Department of Neurology, Medical School, University of Pécs, Pécs,[5]Institute of Psychology, Faculty of Humanities and Social Sciences, University of Pécs, Pécs,[6]Second Department of Medicine and Nephrology-Diabetes Centre, University of Pécs, Pécs,[7]Department of Rheumatology and Immunology, Medical School, University of Pécs, Pécs,[8]National University of Public Services; Budapest ,[9]Psychosomatic Outpatient Clinics, Budapest ,[10]Department of Psychology, Karoly Gaspar University; Budapest,[11]Brain Imaging Centre, Research Centre for Natural Sciences, Budapest,[12]Szentágothai Research Centre, Bioinformatics Research Group, Genomics and Bioinformatics Core Facility, University of Pécs, Pécs,[13]Department of Rheumatology and Clinical Immunology, Department of Internal Medicine and Oncology, Semmelweis University, Budapest,[14]Department of Genetics, Cell and Immunobiology, Semmelweis University, Budapest,[15]Heart and Vascular Centre, Semmelweis University, Budapest

Despite the therapeutic advances of rheumatoid arthritis (RA), optimal disease control cannot be obtained in the difficult-to-treat RA (D2T) population, leading to the persistence of symptoms, especially pain. Several factors can contribute to the pain in D2T RA, such as inflammatory activity, disturbances of pain processing, structural damage, and physical and psycho-social comorbidities, however the exact pathomechanisms are still unclear. We aimed to examine the background processes of RA pain to reveal the differences of the interactions among pain, inflammation, psychological and social factors.

11 healthy controls (HC) and 14 D2T RA patients were included. Clinical examination, psychological analysis (personal clinical interview, Rorschach test and validated surveys) and functional MRI (fMRI) with standardized pain stimulation was performed. Peripheral blood mononuclear cells (PBMC) were separated, and total RNA was isolated and prepared for transcriptomic analysis.

Psychological analysis revealed distinctive psychological features between D2T RA and healthy populations, as well as suggested differences among subgroups determined by the severity of inflammation and pain intensity. General health is the lowest and the reaction to painful stimuli is the highest in the population with both high inflammation and high pain. The presence of high pain alone is connected to the higher rate of depressed mood. Patients with high inflammatory parameters, independent of pain, showed a higher fear of motion and activity. Inflammation also seems to play an important role in dependence and incompetence, vulnerability to harm or illness and social isolation and alienation. In our fMRI experiments the connectivity strength between different brain areas involved in pain processing showed significant differences between D2T RA and healthy populations. In HCs painful heat stimulation significantly reduced functional connectivity strength (FCS) between the

prefrontal and posterior cingulate cortices, within the default mode network (DMN) and between the DM and frontoparietal networks, as well as in several connections of the medial prefrontal cortex (MPFC). Moreover, the left MFG showed a distinct, pain-related FCS pattern including several brain areas in D2T RA patients. D2T RA patients showed several differently expressed genes compared to HCs, mainly involved in immune cell migration and activation, cytokine- and chemokine-mediated signaling and neuronal regulation. There were 35 upregulated genes e.g. interleukin 15 and chemokine receptor 2 playing roles in maintaining inflammation, and Sortilin1 contributing to neuropathic pain. Meanwhile, 28 genes were downregulated such as tumor necrosis factor alpha-induced protein 3 controlling inflammatory responses.

Our research revealed several abnormalities in the pain processing pathways, pain matrix activation in the brain and psychological profile in D2T RA patients compared to healthy controls.

W-KA2-2 - ANALGESIC EFFECT OF THE NOVEL MULTI-TARGET DRUG CANDIDATE SZV-1287 IN MODELS OF NEUROPATHIC AND INFLAMMATORY PAIN

Ádám István Horváth[1,2,3], Nikolett Szentes[1,2,3], Valéria Tékus[1,2], Tamás Kálai[4], Szilárd Pál[5], Péter Mátyus[6], Zsuzsanna Helyes[1,2,3,7,8]
[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs; [2]National Laboratory for Drug Research and Development, Budapest; [3]Eötvös Loránd Research Network, ELKH-PTE Chronic Pain Research Group, Pécs; [4]Institute of Organic and Medicinal Chemistry, Faculty of Pharmacy, University of Pécs, Pécs; [5]Institute of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, University of Pécs, Pécs; [6]E-Group ICT Software Zrt., Budapest; [7]PharmInVivo Ltd., Pécs, [8]ALGONIST Biotechnologies GmBH, Vienna

SZV-1287 (3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime) is a novel multi-target drug, which irreversibly inhibits semicarbazide-sensitive amine oxidase (SSAO) metabolizing primary amines to irritants like methyglyoxal, hydrogen-peroxide and formaldehyde activating the transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) receptors. Furthermore, it also directly antagonizes these non-selective cation channels located on primary sensory neurons, immune cells and several central nervous system regions, and its main metabolite, oxaprozin, is a clinically used cyclooxygenase (COX) inhibitor. All these mechanisms inhibit pain processing and inflammation, therefore, SZV-1287 represents promising drug developmental perspectives. We summarize the discovery, preclinical pharmacodynamic, pharmacokinetic and toxicology data, as well as first in human safety and kinetic results of the novel analgesic candidate.

We provided proof-of-concept for the ability of single SZV-1287 administration (5-20 mg/kg, i.p.) to significantly reduce acute and chronic pain behaviors including sciatic nerve injury-induced neuropathic hyperalgesia in mice via inhibiting TRPA1 and TRPV1 activation. Repeated SZV-1287 injections attenuated chronic arthritis (edema, myeloperoxidase activity, histopathological changes) and related hyperalgesia, L4-L6 spinal dorsal horn neuroinflammation (microgliosis) even in the late phase of the model when neuropathic mechanisms are important. Since mainly under acidic conditions, SZV-1287 is transformed to the COX inhibitor oxaprozin, which is ineffective for neuropathy, enterosolvent capsule was formulated and tested during the preclinical development. SZV-1287 is quickly absorbed, its plasma concentration is stable for 2 h, and enters the brain. Although SZV-1287 significantly decreased the proton-induced TRPV1-mediated calcium-influx potentially leading to hyperthermia, it did not alter deep body temperature. It did not show any considerable toxicity either in rodents or in dogs. After the approval of the preclinical dossier by the Hungarian authority, the phase I trial is currently going on. The single ascending dosing: 75, 150, 300, 450 mg) has successfully been completed without any considerable side effects.

Based on the preclinical efficacy for chronic pain with special emphasis on neuropathic mechanisms and the preliminary human safety data, this analgesic candidate with a unique mechanism of action can progress to phase II studies in patients. This project is a good example for academic drug discovery and successful early development of a novel compound together with industrial partners.

Acknowledgements: ÚNKP-22-4, OTKA K-138046, RRF-2.3.1-21-2022-00015, TKP2021-EGA-16, Eotvos Lorand Research Network, National Brain Research Program 3.0

W-KA2-3 - EFFECT OF CONCOMITANT ADMINISTRATION OF TELMISARTAN AND MORPHINE ON MONONEUROPATHIC PAIN AND OPIOID ANTINICICEPTIVE TOLERANCE IN RATS

David Á. Karádi[1], Anna R. Galambos[1], Péter P. Lakatos[2], Joost Apenberg[1], Sarah K. Abbood[1], Mihály Balogh[1,3], Kornél Király[1], Pál Riba[1], Nariman Essmat[1], Edina Szűcs[4], Sándor Benyhe[4], Zoltán Varga[1], Éva Szökő[2], Tamás Tábi[2], Mahmoud Al-Khrasani1[1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Nagyvá-rad tér 4, H-1089, Budapest, Hungary, [2]Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Nagyvárad tér 4, H-1089 Budapest, Hungary, [3]Pharmaceutical Analysis, Groningen Research Institute of Pharmacy, University of Groningen, 9700 AD, Groningen, The Netherlands, [4]Institute of Biochemistry, Biological Research Center, Temesvári krt. 62, H-6726 Szeged, Hungary

Based on preclinical studies carried out in the last decades, angiotensin receptor type 1 (AT1) has been proposed as a potential pharmacological target in the treatment of neuropathic pain (NP). In addition, the connections between the renin-angiotensin system (RAS) and opioid system regarding analgesia have been described. Here we present the first evidence for an enhanced antiallodynic effect and a delay in opioid analgesic tolerance following co-administration of an angiotensin receptor blocker (ARB) and morphine.

Male Wistar rats (170-250 g) were used for both mononeuropathic (Seltzer ligation) and morphine analgesic tolerance models. Animals received acute or chronic oral treatment with ARBs losartan (50, 100 or 150 µmol/kg) or telmisartan (20, 40 or 80 µmol/kg) alone or in combination with subcutaneous morphine (10 µmol/kg). The antiallodynic effect was determined by dynamic plantar aesthesiometer (DPA). To assess opioid antinociceptive tolerance, in a separate experimental setting, the effect of morphine (31,08 µmol/kg), telmisartan (20 µmol/kg) or their combination was determined in the rat tail-flick assay following acute and chronic repeated treatments on days 1, 4 and 10. Area under the curve (AUC) values of the antinociceptive time-effect course were individually calculated for each animal. Morphine-stimulated [35S]-GTPγS binding assays were performed on spinal cord samples obtained from neuropathic animas. Finally, spinal cord and dorsal root ganglion tissue samples were obtained from naive rats and used for RNA Scope® in-situ hybridization, to determine OPRM1, AGTR1A, CALCA, and VGLUT1 mRNA presence.

Oral telmisartan (40 or 80 µmol/kg) or losartan (100 µmol/kg) produced acute antiallodynic effect. Upon chronic treatment of mononeuropathic animals the combination of subanalgesic doses of telmisartan and morphine ameliorated allodynia and resulted in a significant leftward shift in the dose-response curve of morphine in [35S]GTPγS binding assay, indicating restoration of morphine potency. Telmisartan delayed morphine analgesic tolerance development, seen as a significant difference between the AUC values obtained from the treatment groups on day 10. Colocalization of angiotensin and opioid receptor mRNAs were found at sites of particular importance for pain transmission.

Blockade of AT1 produced acute antiallodynia and in rat mononeuropathic pain. Chronic treatment with telmisartan enhanced the antiallodynic effect of morphine and delayed antinociceptive tolerance. Despite the reported decrease in opioid receptors in NP and morphine tolerance, telmisartan restored morphine potency on a spinal level. To fully elucidate the molecular mechanism, future studies are needed. These findings may provide the preclinical basis for exploiting AT1 blockade as a pharmacological target in pain conditions with opioid impairment and raise the possibility of repurposing ARBs in NP or opioid analgesic tolerance.

W-KA2-4 - ROLE OF THE TACHYKININ HEMOKININ-1 IN MOUSE MODELS OF CHRONIC PAIN, ANXIETY AND DEPRESSION

Éva Borbély[1], Eszter Kepe[1], Angéla Kecskés[1], Valéria Tékus[1], Zsófia Hajna[1], Zsuzsanna Helyes[1,2,3]
[1]Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Hungary, [2]National Laboratory for Drug Research and Development, Budapest, Hungary, [3]Eötvös Loránd Research Network, Chronic Pain Research Group, University of Pécs, Hungary
eva.borbely@aok.pte.hu

Substance P (SP) mediates pain via the NK1 tachykinin receptor, but NK1 antagonists have failed in clinical trials. Hemokinin-1 (HK-1) shares several (structural and immunological) similarities with SP, it is present in stress- and pain-related brain regions as well as in hypothalamic-pituitary-adrenal axis. It activates the NK1 receptor, but several other targets and mechanisms have also been proposed. We investigated its involvement in acute and chronic stress and pain conditions with C57Bl/6 wildtype (WT), HK-1 and NK1 receptor deficient mice.

Pain was investigated in acute (writhing test, resiniferatoxin-induced hyperalgesia, mast cell tryptase/complete Freund's adjuvant (CFA)-induced monoarthritis) and chronic/disease (traumatic mononeuropathy, K/BxN serum-transfer and CFA-induced arthritis, restraint and variable mild stress-induced) models in mice. Mechanosensitivity was measured by aesthesiometry, cold sensitivity by withdrawal latency from icy water, thermosensitivity by increasing temperature hot plate. Anxiety and depression-like behavior was assessed by light-dark box, open field, elevated plus maze, tail suspension and forced swim tests.

Mechanical and thermal hyperalgesia were significantly smaller in HK-1-deficient mice compared the WTs both in acute and chronic nociceptive tests as well as the restraint stress model. Variable stress-induced mechanical pain was greater in $Tac4^{-/-}$, but abscent in WT mice. Interestingly, NK1 receptor deletion did not influence pain behavior in either the acute or the chronic arthritis and neuropathy models. Furthermore, the lack of HK-1 results in depressive phenotype and increased susceptibility to stress.

These data suggest pro-nociceptive functions of HK-1, which is NK1 receptor-mediated in certain pain conditions, but not in all models, depending on the mechanisms. Furthermore, HK-1 also plays a role in the regulation of acute and chronic stress reactions. Identification HK-1 targets and signalling mechanisms might open novel directions in analgesic research.

Funding: Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, National Brain Research Program 3.0, National Research, Development and Innovation Office - OTKA K138046 and OTKA FK137951, TKP2021-EGA-16, János Bolyai Research Scholarship of the Hungarian Academy of Sciences, and ÚNKP-21-5 new National Excellence Program of the Ministry for Innovation and Technology, Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.

W-KA2-5 - CHARACTERIZATION OF THE ROLE OF LYMPHATICS IN NUCLEOSIDE-MODIFIED MRNA-LNP VACCINE INDUCED IMMUNE RESPONSE

Gabor Kovács[1], Kornél Molnár[1], Stella Sági[1], Éva Kemecsei[1], Zoltán Lipinszki[2], Raghu P Kataru[3], Babak J Mehrara[3], Norbert Pardi[4], Zoltan Jakus1
[1]Semmelweis University, Budapest, Hungary, [2]Lendulet Laboratory of Cell Cycle Regulation, Institute of Biochemistry, Biological Research Centre, Budapest, Hungary, [3]Department of Surgery, Division of Plastic and Reconstructive Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA., [4]Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Nucleoside-modified mRNA vaccines encapsulated into lipid nanoparticle (mRNA-LNPs) gained major scientific interest, but the molecular and cellular mechanisms involved in the induced immune response are still not fully understood. The lymphatics are involved in various immune processes, yet its role in the mRNA-LNP vaccine induced immune responses is still not known. Our aim was to investigate the role of the lymphatics in the immune response induced by mRNALNP vaccines. The expression of GFP mRNA-LNPs was monitored in wild-type and in a mouse model with local deletion of lymphatics. Mice with local lymphatic deficiency or with normal lymphatics were vaccinated with influenza hemagglutinin encoding mRNA-LNPs and specific antibody titers were determined. We characterized the cell types that uptake and express the mRNA-LNPs. We detected fluorescent signal in the regional lymph nodes 30 minutes after the injection of labelled LNPs. 4 hours after the injection of GFP mRNALNPs, GFP signal was detected in the regional lymph nodes, the signal peaked at 24-hour. Local deletion of lymphatics inhibited GFP protein expression in the regional lymph node. Mice vaccinated in lymphatic deficient region presented significantly lower hemagglutinin specific antibody titers compared to controls. Our results suggest that the lymphatics are involved in the uptake, transport and expression of nucleoside-modified mRNALNPs and are required for the induction of adequate immune response by mRNA-LNP vaccination. These results may provide better understanding of the lymphatics-dependent mechanism of action of mRNA-LNP-based platforms. Our results could contribute to the optimization of mRNA-LNP vaccines and reducing potential side effects

W-KB2-1 - HIDDEN CARDIOTOXICITY - CARDIAC SAFETY TESTING IN ISCHEMIC AND COMORBID CONDITIONS: DEVELOPMENT OF PRECLINICAL TEST PLATFORMS

Anikó Görbe[1], Zoltán Giricz[1], Péter Ferdinandy[1,2]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Hungary,
[2]Pharmahungary Group, Szeged, Hungary.

Unexpected cardiac adverse events are one of the leading causes of interruption of clinical trials and drug withdrawals. It has been shown that cardiovascular risk factors and comorbidities (such as aging, metabolic diseases, etc) and their medications (e.g. nitrates, antidiabetic drugs, statins, etc) may interfere with cardiac ischemic tolerance and molecular signaling of endogenous cardioprotection. Indeed certain drugs may exert adverse events on the diseased heart that is hidden in the healthy myocardium. Hidden cardiotoxic effects of drugs may occur due to (i) enhancement of unwanted signaling due to ischemia/reperfusion injury and/or the presence of risk factors and/or (ii) inhibition of cardioprotective signaling pathways, both of which may lead to ischemia-related cell death and pro-arrhythmic events. This led to novel concept of “hidden cardiotoxicity”, i.e. cardiotoxity seen only in the diseased heart, i.e. ischemia/reperfusion injury and/or its major comorbidities (Ferdinandy et al, Eur Heart J, 2018). Hidden cardiotoxicity cannot be revealed by the routinely used cardiac safety testing methods in “healthy” test systems, moreover, the mechanism of hidden cardiotoxocity is largely unknown. Therefore, we aimed to develop a preclinical in vivo and vitro platform and test already withdrawn drugs with hidden cardiotoxic properties (Brenner et al, Cells, 2020; Weber et al, Pharmaceuticals, 2022, Gergely et al, 2023) and new drugs with potential cardiotoxic properties. Here we summarize the current knowledge on hidden cardiotoxicity and urge the need for development of novel cardiac safety testing platforms for early detection of yet “hidden” cardiotoxicity.

W-KB2-2 - EVIDENCE FOR CARDIOMYOCYTE DYSFUNCTION IN CANCER-INDUCED CACHEXIA IN MICE

Christopher Dostal[1], L P. Szabo[1], Romy Steiner[1], Peter Pokreisz[1], T. D. Hofreither[2], Tomin Tamara[2], S. Baydar Simge[1], J Reiner[1], Abraham Dietmar[3], Karin Zins[3], Benjamin Hack[4], Tamás Kovács[5], Zsófia Onodi[5], Elena Liliu[5], Zoltán Varga[6], Birner-Gruenberger Ruth[2], Nina Pilat[1,6], Ham Gong[7], Jonathan Kirk[7], Latifa Bakari[8], Xavér König[4], Erwin F. Wagner[8,1], Bruno Podesser[1,9], Attila Kiss[1,9]
Affiliations: [1]Center for Biomedical Research and Translational Surgery, Medical University of Vienna, [2] Institute of Chemical Technologies and Analytics, Faculty of Technical Chemistry, Technical University of Vienna, [3]Center for Anatomy and Cell Biology, Medical University of Vienna, [4]Department of Neurophysiology and Neuropharmacology, Medical University of Vienna, [5]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary. HCEMM-SU Cardiometabolic Immunology Research Group, Semmelweis University, Budapest, Hungary. MTA-SE Momentum Cardio-Oncology and Cardioimmunology Research Group, Semmelweis University, Budapest, Hungary, [6]Department of Cardiac Surgery, [7] Department of Cell and Molecular Physiology, Loyola University Stritch School of Medicine, Maywood, IL, USA. [8]Genes and Disease Group, Medical University of Vienna, [9]Ludwig Boltzmann Institute for Cardiovascular Research

Background
Cachexia is a systemic wasting syndrome that associated with systemic inflammation as well as increases cancer-associated mortality. There is growing evidence that solid cancer itself can initiate a specific cardiomyopathy. It was previously demonstrated that cachexia or cancer progressively and differentially impacts on heart weight but the functional alterations are still largely unknown.

Methods
Colon-26 adenocarcinoma (C26; n=30) or shIL-6 (C26 shIL-6; n=30) cells were inoculated subcutaneously into the flank of syngeneic adult male BALB/c (9-11 weeks old) mice, meanwhile control mice were injected with PBS (n=25). Twenty days after the cells injection, cardiac function was assessed using transthoracic echocardiography, invasive hemodynamic and *ex vivo* isolated working heart methods. In addition, intracellular Ca^{2+} transient and force-calcium relationships were assessed in isolated single ventricular cardiomyocytes (CMs). Cancer or cancer-cachexia associated inflammation in the myocardium was assessed by quantitative analysis using flow cytometry. Cardiac fibrosis and capillary density in the myocardium were also evaluated using immunohistochemistry. Finally, label-free LC-MS/MS proteomics and redox proteomics analysis were performed in left ventricular (LV) tissue.

Results
Despite that tumor size was comparable between the cancer groups, C26 group showed a significant loss of subcutaneous fat and skeletal muscle ($p<0.05$, respectively), confirming a cachectic phenotype. We found that both of tumor-bearing mice groups have a LV systolic dysfunction but diastolic dysfunction was more prominent in cachectic mice. In addition, sarcomere dysfunction, including significantly reduced maximum calcium-activated tension (Tmax) and increased calcium sensitivity (decreased EC_{50}) was found in tumor-bearing mice in compared to controls ($p<0.05$, respectively). Interestingly, intracellular Ca^{2+} transient was markedly and exclusively increased in CM isolated from cachectic mice (over 80 cells/group, $p<0.01$ vs control and shIl-6 group; respectively). There were no difference between the

control and tumor-bearing mice regarding cardiac inflammation and fibrosis but capillary density was significantly increased in tumor-bearing mice compared to controls ($p<0.001$, respectively). Furthermore, comprehensive analysis of the cardiac proteome showed a significant difference between controls and cancer mice (over 50 proteins up- or downregulated), and redox proteomics showed differences between the two cancer groups ($p<0.05$).

Discussion

Taken together, these result indicate that LV dysfunction in cancer mice is mainly associated with sarcomere dysfunction and abnormal intracellular Ca^{2+} handling is present in mice with cachectic phenotype. These functional alterations are independent from changes in myocardial fibrosis and inflammation. These data provide new insights into how cancer and cancer-cachexia impacts the cardiovascular system.

W-KB2-3 - MICRORNA-MRNA BIOINFORMATICS TARGET PREDICTION IN A RAT MODEL OF VOLUME OVERLOAD-INDUCED LEFT VENTRICULAR HYPERTROPHY

Bencsik Péter[1,2], Gömöri Kamilla[1,3], Pósa Bence[1], Kenyeres Éva[1], Szabados Tamara[1,2], Ágg Bence[2,4], Váradi Barnabás[4], Tóth Viktória[4], Ágoston Gergely[5], Leprán István[1], Hamdani Nazha[3], Görbe Anikó[1,2,4], Ferdinandy Péter[2,4]
[1]Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary, [2]Pharmahungary Group, Szeged, Hungary, [3]HCEMM-Cardiovascular Research Group, Department of Pharmacology and Pharmacotherapy, University of Budapest, Budapest, Hungary and Institut für Forschung und Lehre (IFL), Molecular and Experimental Cardiology, Ruhr University Bochum, Bochum, Germany, [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [5]Institute of Family Medicine, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary

Background: Several etiologies accompany with eccentric cardiac hypertrophy due to volume overload (VO) such as mitral and aortic valve regurgitation and due to ischemia-induced remodeling. There remains no direct myocardial therapy to decelerate or reverse the progression of hypertrophy, so far. Therefore, our aim was to identify novel molecular targets involved in the development of VO-induced hypertrophy using transcriptomics and bioinformatics for target prediction.

Methods: In our experiments, volume overload (VO) was induced in 2-month-old male Wistar rats with aorto-caval fistula, using age-matched sham-operated animals (SO) as controls. Functional parameters were measured by echocardiography at the end of 4 and 8 months of follow-up. Total RNA, and then microRNA was isolated from left ventricular samples for deep sequencing analysis. Based on the available literature on microRNAs' expression profile, microRNA-mRNA target prediction was performed using online databases. Messenger RNAs related to at least 4 microRNAs of significantly altered expression were selected for biological validation at mRNA and protein levels.

Results: The development of eccentric hypertrophy was confirmed as left ventricular mass and heart weight/body weight ratio were increased in the VO groups, however, no functional changes were detected when compared to the age-matched SO groups. Out of the 752 microRNAs detected in left ventricular samples, 22 were down- and 12 were up-regulated in the VO group at 8 months as compared to the age-matched SO group. Bioinformatic target prediction identified 3 mRNA targets (Btg2, Rock2, and Nova1) with 5 microRNAs showing expression changes and an additional 12 mRNAs showing association with 4 microRNAs with altered expression. Biological validation has confirmed a significant down-regulation of Btg2 in the VO group after 8 months of follow-up at both mRNA and protein level as compared to the age-matched SO animals.

These results highlight the need for unbiased omics approach followed by bioinformatic target prediction and biological validation of the results to reveal underlying pathomechanism and identify novel potential pharmacological targets in cardiovascular diseases.

Keywords: volume overload; cardiac hypertrophy; miRNA profile; Btg2, bioinformatic target prediction

W-KB2-4 - DYNAMICS OF THE LATE NA+ CURRENT IN HUMAN, CANINE AND GUINEA PIG VENTRICULAR MYOCARDIUM

Balázs Horváth[1], Tamás Hézső[1], Zsigmond Kovács[1], Csaba Dienes[1], József Óvári[1], János Magyar[1], Tamás Bányász[1], Norbert Szentandrássy[1], István Baczkó[2], András Varró[2], Péter P Nánási[1]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen; [2]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged

Late sodium current ($I_{Na,late}$) flows under the plateau phase of mammalian cardiac action potentials. Upon upregulation, it can contribute to arrhythmogenesis, and therefore considered as possible antiarrhythmic drug target. However, many aspects of this current are still poorly understood.
The true profile of $I_{Na,late}$ in canine and guinea pig ventricular cells were studied and compared to $I_{Na,late}$ recorded in undiseased human hearts. $I_{Na\text{-}late}$ was defined as a tetrodotoxin-sensitive current, recorded under action potential voltage clamp conditions using either canonic- or self-action potentials as command signals.
Under action potential voltage clamp conditions the amplitude of canine and human $I_{Na,late}$ monotonically decreased during the plateau (decrescendo-profile), in contrast to guinea pig, where its amplitude increased during the plateau (crescendo profile).
Facilitation of $I_{Na,late}$ by Anemone toxin II prolonged APD and induced Ca^{2+}-oscillations that led to early and delayed afterdepolarizations and triggered APs; these arrhythmogenic activities were eliminated by buffering intracellular Ca^{2+} with BAPTA.
Conventional voltage clamp experiments revealed that the crescendo $I_{Na,late}$ profile in guinea pig was due to the slower decay of $I_{Na,late}$ in this species. When action potentials were recorded from multicellular ventricular preparations with sharp microelectrode, action potentials were shortened by tetrodotoxin, which effect was the largest in human, while smaller in canine, and the smallest in guinea pig preparations.
It is concluded that important interspecies differences exist in the behavior of $I_{Na,late}$. At present canine myocytes seem to represent the best model of human ventricular cells regarding the properties of $I_{Na,late}$.

W-KB2-5 - DIABETIC REMODELING PROCESSES OF THE INTRAMURAL CORONARY RESISTANCE ARTERIES OF STREPTOZOTOCIN TREATED MICE SHOW CHARACTERISTIC SEGMENTAL SPECIFICITY. POTENTIAL ROLE OF THE CONNECTIVE TISSUE ORGANIZATOR PROTEIN TENASCINC

Nadasy GL[1], Szekeres M[2], Fees A[3], Chang X[1], Hunyady L[1], Szabo LP[4], Podesser BK[4], Kiss A[4].
[1]Physiology Dept, Semmelweis University, Budapest; [2]Dept of Morphology and Physiology, Health Sciences Faculty, Semmelweis University Budapest; [3]Kansas State University, Manhattan, KS, US; [4]Center for Biomedical Research, Medical University of Vienna.

Microvascular damage to the coronary resistance arteries is an important component of diabetic cardiomyopathy. In streptozotocin-treated diabetic mice we studied the alterations in the geometry of the intramural coronary resistance artery network (i) as well as the alterations in the segmental geometry (ii) and wall histology (iii) of arterioles with different positions in the network. Suspecting a role of the connective tissue organizer protein TenascinC (TNC) in diabetic vascular remodeling processes, in addition to wild-type mice investigations were also made on TNC knockout animals (iv). In situ microprepared, pressure-perfused whole network of the left anterior descendent coronary artery was microphotographed, all segments and branchings identified down to 40 µm diameter, inner, outer diameter, wall thickness and position in an orifice-apex Cartesian coordinate system were determined. The whole network was theoretically divided into ring units with 50µm length each (several hundred such units per heart) for statistical analysis. Histological sections were stained for collagen (PicroSirius), elastin (resorcin-fuchsin), smooth muscle actin, collagen type IV, fibronectin and tenascinC (immune-histochemistries) and evaluated colorimetrically. In diabetic networks geometrical aberrations developed in form of trifurcations, and larger branches with broken courses. One typical observation was the thickening of the wall of vessels in the large arteriolar (>220µ) range (hypertrophic segmental remodeling), while arterioles in the range of 100-140 µm had thinned walls (hypotrophic segmental remodeling). In the 100-180 µm range a new population of vessels has arisen also with relative thin walls (vasculogenesis) (All significant with the $\chi 2$ probe.) Diabetic hearts were fibrotic, however, in TNC KO animals, ventricular fibrosis was significantly reduced. Quantitative histological analysis of arterioles in the different diameter range revealed significantly elevated number of cross-sected small arterioles (<75µm, vasculogenesis). Diabetic arterioles (25-125µm) contained more elastin per area cross section, their fibronectin content was reduced in the 75-100 and elevated in the 100-125 µm diameter range. Knocking out the TNC gene reduced arteriolar wall fibronectin, but even these animals retained their abilities to elevate wall fibronectin upon diabetes. Half of the cross section was positive for smooth muscle actin throughout the 25-125 µm range which value was hardly affected by diabetes or knocking out the TNC gene. Our conclusion is that diabetic remodeling affects the different serially connected segments of the intramural coronary resistance artery system producing diverse, but specific geometric and histologic alterations. The TNC gene product seems to have an important role in determining connective tissue organization in coronary resistance arteries both in health and in diabetes. Supported by Hungarian National Grants OTKA TO 32019, OTKA K116954, by the “Aktion Österreich-Ungarn” grants Nos 98öu4, 104öu5; by a grant from the Dean of the Semmelweis University, by the Fonds des Bürgermeisters der Bundeshauptstadt Wien (project-Number: 21001). Alexander Fees was supported by the Fulbright Stipend.

W-K3-1 - INVESTIGATION OF ACUTE RESPIRATORY FAILURE SYNDROME AND ITS RENAL COMPLICATIONS IN A NEW LARGE ANIMAL MODEL

Gajda Ámos[1], Vida Noémi[1], Vigyikán Gyöngyvér[1], Hodoniczki Ádám[1], Varga Zoltán[1], Rutai Attila[1], Móró Vivien[2], Hantos Zoltán[2], Varga Gabriella[1], Érces Dániel[1]
[1]Institute of Surgical Research, University of Szeged [2]Department of Anaesthesiology and Intensive Therapy, Semmelweis University, Budapest

Introduction: In the absence of curative treatment, the cornerstone of medical care for acute respiratory distress syndrome (ARDS) is mechanical ventilation. We aim to develop a complex double-hit large animal model for the standardized investigation of organ failure associated with ARDS, wherein the mechanism of acute kidney injury often seen in ARDS can also be studied. Based on literature data, we hypothesized that the extracellular release of the mitochondrial metabolite succinate facilitates the processes leading to ARDS.

Methods: The experiments were performed on anesthetised, ventilated Vietnamese minipigs. In the first group (n=5), Na-succinate (2.5 mM/kg iv) was administered followed by repeated administration of a 1:1 mixture of oleic acid and 5% glucose (OA) (5x1 ml; iv) 3 h later. The control group (n=5) received solvent treatment (0.9% NaCl; 5% glucose). During the 7 h of continuous observation, haemodynamic measurements and blood gas analysis were performed. Oxygenation index (OI) was determined, hourly urine output was monitored, and renal blood flow was measured using an ultrasound flowmeter. Changes in respiratory mechanics were monitored by low-frequency oscillometry to determine airway resistance (Raw) and tissue elasticity (H). At the end of the experiments, inflammatory mediator (nitrite/nitrate (NO_x); myeloperoxidase (MPO) enzyme activity) levels were measured from lung and kidney tissue samples.

Results: Succinate and OA administration significantly increased heart rate and OI, decreased PaO_2/FiO_2 and renal arterial blood flow. Onset of ARDS was indicated by significantly elevated H values, Raw was moderately elevated. Elevated MPO (lung: 161.1±45.4 vs 87.3±15.6; kidney: 7.27±2.3 vs 3.9±1.1 mU/$mg_{protein}$) and NO_x (lung: 7.1±0.2 vs 6.5±0.2; kidney: 10.7±3.5 vs 2.7±0.6 □M) were measured in tissue samples.

Conclusion: The new model reproduces the clinical picture of ARDS well – administering OA after succinate pretreatment results in ARDS-like airway lesions accompanied by renal complications. The protocol could be suitable for experimental investigation of organ failure associated with ARDS.

Funding: NKFIH-K120232

W-K3-2 - DECYPHERING TISSUE DAMAGE INDUCED PURINERGIC AND CALCIUM SIGNALING PATHWAYS IN ZEBRAFISH

Diána Kaszás[1,2,3], Anna Török[1,3], Benoit Roux[1,3], Fabian Dehne[1,3], László Fazekas[1,2,3], Szimonetta Tamás[1,2,3], Klaudia Vágó-Kiss[1,2,3], Balázs Enyedi[1,2,3]
[1]Department of Physiology, Semmelweis University, Budapest,[2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest,
[3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest

Introduction: Wound detection and early wound healing processes are triggered after tissue injury by a myriad of extracellular regulators and signaling pathways. In our studies, we investigated two important participants of these mechanisms: the release of extracellular ATP (eATP), a previously known DAMP (Damage Associated Molecular Pattern), and intracellular Ca2+ signaling pathways which are rapidly triggered after wounding. Among the many aspects of tissue damage responses which remain unclear, the possible connection between eATP release and Ca2+ signaling pathways is still controversial. It is also not known to what extent they contribute to wound contraction after injury.

Aims: In our study, we attempt to better define the role of extracellular adenosine derivatives in mediating rapid wound healing processes. Therefore, we investigated different P2x and P2y-mediated purinergic signaling pathways. In particular, we studied how different Ca2+ signaling patterns are induced by eATP and eADP with the future goal of delineating which endogenous mediators regulate these Ca2+ signaling pathways during tissue injury.

Methods and results: In our studies, we image genetically encoded fluorescent biosensors to measure real-time eATP and Ca2+ concentration changes in Hek293 cells and zebrafish larvae. To this end, we have created stable Hek293 cell lines and transgenic fish lines expressing the GrabATP and the GCaMP7s reporters for the respective measurements. In order to visualize the immediate responses after tissue injury, we used a pulsed UV-laser during live imaging to ablate cells and wound the tissues.
Our observations suggest that wounding activates multiple pathways simultaneously. During our measurements, three different intracellular Ca2+ signals were detected. First, a long-term high signal next to the wound edge, then a wave spreading deeper into the tissue which was followed by an oscillatory Ca2+ signal in epithelial cells further away from the wound. In connection with these results, we have found that different adenosine derivatives are able to induce different Ca2+ signaling patterns. While exogenous administration of ADP through a prewounded tailfin triggers a Ca2+ wave spreading deep into the tissue, ATP stimulation also recapitulates the high would edge Ca2+ signal. Furthermore, we also found that wound-induced Ca2+ signals are eliminated by blocking purinergic signaling pathways. In addition, we observed that wound contraction is inhibited when purinergic signaling pathways are blocked.

Conclusions and future plans: Our results suggest that the presence of eATP and other adenosine derivatives which activate purinergic signaling pathways are responsible for both the initiation of Ca2+ signaling following wounding and the processes that trigger wound closure. Using inhibitor and CRISPR/Cas9-based knockdown approaches, we are currently investigating which purinergic receptors are responsible for the above phenomena.

W-K3-3 - TRPA1 BUT NOT TRPV1 ION CHANNEL IS EXPRESSED ON THE K7M2 MOUSE OSTEOSARCOMA CELLS AND ITS ACTIVATION REDUCES VIABILITY

Lina Hudhud [1,2], Krisztina Pohoczky [1,2,4], Katalin Rozmer [1,2], Angéla Kecskés [1,2], Éva Szőke [1,2,3], Zsuzsanna Helyes [1,2,3]

[1] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs H-7624 Pécs, Hungary,[2] National Laboratory for Drug Research and Development, H-1117 Budapest, Hungary,[3] Eötvös Lorand Research Network, Chronic Pain Research Group, University of Pécs, H-7624, Pécs, Hungary,[4] Department of Pharmacology, Faculty of Pharmacy, University of Pécs, H-7624 Pécs, Hungary

There is an urgent need to identify novel treatments for high grade, painful bone cancers with poor prognosis, such as osteosarcoma (OS). The key molecular mechanisms are not well understood, therefore data obtained in preclinical models including in vivo experiments to reveal complex interaction in tumor microenvironment are important. Transient receptor potential Ankyrin repeat domain 1 and Vanilloid 1 (TRPA1 and TRPV1) non-selective cation channels have become in the focus of cancer research, but there are few data on OS. In the present study we determined their expressions and function in the highly metastatic mouse K7M2 OS cell line.

Trpa1 and trpv1 mRNA were detected by PCR gel electrophoresis and highly sensitive RNAscope in situ hybridization. Radioactive Ca^{2+} uptake and ATP-based viability were measured to determine the functional expression of TRPA1 and TRPV1 using their agonist allyl-isothiocyanate (AITC) and capsaicin, respectively.

Moderate trpa1 mRNA expression in K7M2 cells was demonstrated by both techniques but trpv1 mRNA showed very low expression with few transcripts detectable in few cells. No co-localization was found between trpa1 and trpv1 mRNA. The TRPA1 agonists AITC (200 µM) induced significant radioactive Ca^{2+} uptake, which was blocked by the antagonist HC-030031 (10 µM). In contrast, capsaicin (100 nM and 1 µM) did not cause Ca^{2+} influx. Viability was significantly and concentration dependently reduced in the presence of 5, 10, 20, 50, 100, and 200 µM AITC, but capsaicin only reduced viability at very high concentrations (20, 50, 100 and 200 µM).

We provide here the first data on functional TRPA1 but not TRPV1 expression on K7M2 mouse OS cells which provide good basis for further in vivo experiments to investigate the role in tumor progression and related pain in a model we have established and characterized.

Funding: Eötvös Loránd Research Network (ELKH); National Research Development and Innovation (NKFI) Fund of Hungary, financed under EGA 16 funding scheme.

W-K3-4 - EFFECTS OF A JANUS KINASE INHIBITOR ON COMORBIDITIES OBSERVED IN A RHEUMATOID ARTHRITIS MODEL OF ZSF1 RATS

Tarjányi Vera[1], Vietrov Tymur[1], Szilágyi Anna[1], Szekeres Réka[1], Takács Barbara[1], Priksz Dániel[1], Trencsényi György[2], Juhász Béla[1], Szilvássy Zoltán[1], Bombicz Mariann[1]
[1]Department of Pharmacology and Pharmacotherapy, University of Debrecen, [2]Medical Imaging Clinic, Nuclear Medicine Department, University of Debrecen

Rheumatoid arthritis (RA) is a non-infectious systemic inflammatory disorder, featured by several comorbidities, such as metabolic syndrome or cardiovascular alterations. According to the literature, these have a pivotal role in the progression, the treatment of rheumatoid arthritis, and in the quality of life also. The objective of this present study was to evaluate the possible positive effects of the Janus kinase Inhibitor tofacitinib (Tofa) on the metabolic syndrome associated with RA on a complete Freund's adjuvant (CFA)- induced rheumatoid arthritis rodent model.

Eight groups were created from 50 ZSF1 rats:
I. Lean (not obese, not treated); II. Lean + CFA (not obese, treated with CFA); III. Lean + CFA + Tofa (not obese, treated with CFA and tofacitinib); IV. Lean + Tofa (not obese, treated only with tofacitinib) Similarly, we created four obese ZSF1 groups (V-VIII).

Digital micrometre was used to evaluate the severity of inflammation by measuring the circumference of the hind paw. The thermo- and mechanonociceptive threshold of the animals was also assessed by hot plate and von Frey methods. To prove the existence of the metabolic syndrome parameters connected to glucose homeostasis, blood pressure, and body weight were estimated as well. To differentiate the metabolic activity of the hearts and tibiotarsal joints of the rat's Fluorodeoxyglucose (FDG)-positron emission tomography (PET) was performed. The study was exterminated on the 28th day of the treatment period.

All of the metabolic syndrome-related parameters were higher in the obese groups than in lean groups including, HgbA1C levels, blood pressure, and body weight of the animals. On the day of the first measurement of the paw volume, significant differences were observable between the CFA-treated and not treated groups. With FDG-PET a greater metabolic activity was observed in the tibiotarsal joint of rheumatoid rats. In consideration of the FDG uptake of the obese animal's heart tissue, we saw lower emission, correlating with the progression of the autoimmune disease and the insulin resistance caused by metabolic syndrome, but an impressive difference was detectable when the animals received tofacitinib treatment. We observed a descending tendency in the thermo- and mechanonociceptive threshold in the ZSF1+Tofa and in the ZSF1+CFA+Tofa groups.

According to our present results, we assume that tofacitinib can recover the insulin resistance caused by metabolic syndrome, or the symptoms of the diabetic foot by lowering the nociceptive threshold without having any effect on the blood pressure. To clarify the underlying mechanisms of our observations and to confirm our results more parameters will be evaluated from blood samples, moreover, molecular and histological examination is under completion.

Project no. TKP2021-EGA-18 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme.

W-K3-5 - THE EFFECTS OF ABT-333 (DASABUVIR) ON THE ELECTROPHYSIOLOGY OF THE CANINE LEFT VENTRICULAR CARDIOMYOCYTES AND EXPRESSED HERG CHANNELS

Zsigmond Kovács[1], Csaba Dienes[1], József Óvári[1], János Magyar[2], Tamás Bányász[1], Péter Pál Nánási[3], Balázs Horváth [1], Ádám Fehér[4], Zoltán Varga[4], Norbert Szentandrássy[5]
[1]department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary
[2] Division of Sport Physiology, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,[3] Department of Dental Physiology and Pharmacology, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary,[4] Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,[5] Department of Basic Medical Sciences, Faculty of Dentistry, University of Debrecen, Debrecen, Hungary

ABT-333 is an anti hepatitis C drug containing a methanesulfonamide group, which can be found in several blockers of the rapid delayed rectifier potassium current (I_{Kr}). Previously we showed concentration dependent lenghtening of action potential (AP) and decreasing maximal velocity of phase 0, 1, and 3 by ABT-333.

Our goal was to investigate these effects of on the level of ion currents and in case with decreased repolarization reserve.

APs and ion currents were recorded using a sharp microelectrode technique and AP voltage clamp (APVC) at 37 °C on canine left ventricular myocytes. In our experiments, before ABT-333 was applied with increasing concentrations (1, 3, 10 and 30 µM, 5-5 min) in a cumulative manner, we used either dofetilide or $BaCl_2$ for 5 minutes. For the APVC measurements we used 10 µM ABT-333. The hERG current was recorded on Kv11.1 channels expressed in HEK293 cells.

Increasing ABT-333 concentrations caused reversible AP prolongation and the irreversible reduction of the maximal velocity of the phase 0 and 1. Effects of ABT-333 were similar when the repolarization reserve was reduced by BaCl2 pretreatment.

With our ion current measurements we found, that the ABT-333 sensitive current contains an early short outward component and an elongated outward component as well. These are probably the results of the ABT-333-induced inhibition of the transient potassium current (I_{to}) and I_{Kr} currents, respectively. ABT-333 inhibited the hERG channels in time and concentration dependent manner, with approximately 3 µM half inhibitory concentration.

In light of our results, it is likely that the effect of ABT-333 on action potential is achieved primarily through the inhibition of potassium currents, mainly I_{to} and I_{Kr}. That is supported by results of our APVC and hERG channels measurements. The effects of ABT-333 were not larger in case of reduced repolarization reserve, so the increased risk of cardiac side effects in patiens with long QT syndrome is unlikely.

THURSDAY
8 JUNE 2023

T-K1 - NOVEL ANTI-INFLAMMATORY DRUGS FOR THE TREATMENT OF RESPIRATORY DISEASES

Page Clive P
Sackler Institute of Pulmonary Pharmacology, Institute of Pharmaceutical Science, King's College London

Asthma and COPD are respiratory diseases with significant unmet needs with current treatments primarily being the use of inhaled bronchodilators (β2 agonists and muscarinic receptor antagonists), along with inhaled glucocorticosteroids as anti-inflammatory drugs. However, there is an urgent need to find novel anti-inflammatory drugs to reduce the need for glucocorticocoids, particularly for the treatment of patients with COPD given recent concerns that the use of this class of drug may lead to an increased pneumonia risk. Whilst there have been a number of novel "biologics" targeting IgE, IL5 or TSLP for the treatment of severe asthma, the recent approval of the orally active PDE 4 inhibitor, roflumilast N-oxide has been the only novel anti-inflammatory drug introduced for several decades for the treatment of COPD. However, this drug has a very narrow therapeutic window which limits its wider use and is only approved for add on therapy for the most severe patients with COPD. Furthermore, it does not cause acute bronchodilation. PDE4 is now recognised as the predominant PDE isoform in most inflammatory cells considered of importance in the pathogenesis of asthma and COPD, but it is PDE3 that is understood to be the predominant PDE found in airway smooth muscle. Inhibition of PDE3 induces airway smooth muscle relaxation and inhibition of PDE4 inhibits the activation of inflammatory cells. This has led to the development of a number of dual acting PDE 3/ 4 inhibitors exemplified by ensifentrine.

Ensifentrine is an inhaled drug exhibiting both PDE3 and PDE4 inhibition in the same molecule that has both bronchodilator and anti-inflammatory activity. This "first in class" "bifunctional" drug has been demonstrated to cause significant bronchodilator activity in patients with asthma or COPD (1). In addition, bronchodilator doses of ensifentrine inhibits LPS-induced recruitment of inflammatory cells into the lungs of healthy volunteers confirming its anti-inflammatory effects (1). Recently ensifentrine has successfully completed a Phase 3 trial in patients with COPD that have shown a very significant reduction in exacerbations (2). Importantly, use of this "bifunctional" drug has not been associated with any significant gastrointestinal or cardiovascular side effects and ensefentrine shows promise as a future treatment strategy for patients with asthma or COPD (1.2).

Another current treatment for the reduction of exacerbations in patients with COPD is the "off label" use of azithromycin (3) for up to 1 year. This treatment approach has been shown to reduce the number of exacerbations, but long term use also induces resistance to the anti-microbial activity of this macrolide which renders the drug less effective when required to treat acute infections normally sensitive to azithromycin. Given that macrolides are now recognized for having other pharmacological effects such as the ability to be anti-inflammatory, immunomodulatory and to have "barrier enhancing" activity in increasing the integrity of epithelial layers (4), another recent approach has been the development of novel azithromycin analogues that lack anti-microbial activity, but retain these other relevant pharmacological properties exemplified by the drug EP395 currently undergoing phase 2 clinical trials (4).

Recent epidemiological studies have suggested that patients exposed to tuberculosis (TB) have a reduced incidence of allergic diseases, including asthma (5). We have identified that a TB derived chaperonin called 60.1 and a peptide isolated from this protein exhibit long

lasting anti-inflammatory and immunomodulatory activity across a range of experimental models (5). Recent pilot data in patients with eosinophilc esophagitis have confirmed that the 60.1 derived peptide is able to reduce the number of intraepithelial eosinophils in the eosphagus (6) suggesting that this drug may have important immune resetting activity in allergic patients and therefore represent a possible new treatment of allergic inflammatory diseases.

1. Franciosi et al. Lancet Respiratory Medicine. 1: 714-727 (2013)
2. www.veronapharma.com (2022).
3. Kricker JA et al. Pharm.Rev. 73: 1404-1433 (2021)
4. Parnham MJ et al. Adv.Pharmacology, Vol 98, 1-28 (2023)
5. Riffo-Vasquez Y et al. Clin.Exp.Allergy 50: 508-419 (2020)
6. www.revolo.com (2023)

T-KA2 - ROLES OF HYDROGEN SULFIDE IN METABOLIC HEALTH AND OBESITY

Andreas Papapetropoulos
Laboratory of Pharmacology, Department of Pharmacy, National and Kapodistrian University of Athens

Hydrogen sulfide (H_2S) has emerged as an important endogenous gasotransmitter, which regulates homeostasis and affects the function of most organs in the body. Endogenously produced hydrogen sulfide is generated through cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), which use cysteine as a substrate, and 3-mercaptosulfurtransferase (3-MST), which utilizes 3-mercaptopyruvate to yield H_2S and polysulfides. H_2S production and/or degradation has been shown to be deregulated under pathophysiological conditions, contributing to the development and progression of disease. Reduced H_2S levels have been documented in several conditions including hypertension, atherosclerosis, preeclampsia, diabetic vascular complications, heart failure and obesity.

The actions of H_2S in cells and tissues have been causatively linked to its ability to trigger posttranslational modifications. H_2S promotes sulfhydration of cysteine residues modifying the function of ion channels, receptors, enzymes and transcription factors. By targeting multiple signaling pathways, H_2S controls fundamental mammalian cellular responses, such as growth, differentiation, migration and death. However, the impact of H_2S on key metabolic pathways have not been systematically investigated. During the presentation, recent and ongoing research from our group regarding the impact of H_2S on cell and whole body metabolism will be discussed. Mechanistic insights for the biological actions of H_2S that support mitochondrial processes will be provided and examples of how changes in cell metabolism translate into alterations of complex biological phenomena will be presented.

T-KB2 - "HIGH SALT DIET AFFECTS THE CEREBRAL VASCULAR REACTIVITY – COMPARATIVE STUDIES IN ANIMALS AND HUMANS"

Ines Drenjančević
Faculty of Medicine Osijek University Josip Juraj Strossmayer Osijek, J. Huttlera 4, 31000 Osijek, CroatiaScientific Center of Excellence for Personalized Health Care University of Osijek, Croatia

A numerous data confirmed that high salt (HS) diet had a deleterious effect on endothelium-dependent vascular reactivity, both in microcirculation and macrocirculation. HS diet physiologically suppresses renin-angiotensin system (RAS). Our results in middle cerebral arteries (MCA) of Sprague-Dawley rats on a HS diet have shown significantly impaired vasodilator responses to acetylcholine (AChID) and flow-induced dilation. The underlying mechanisms were increased vascular and systemic oxidative stress and decreased vascular production of NO, together with altered balance between vasoactive prostaglandins and eicosanoids, such as metabolites of COX1,2 and epoxygenases. This was related to decreased expression of antioxidative defense systems. Scavenging of reactive oxygen species with TEMPOL restored NO production and vascular reactivity. Importantly, chronic low-dose ANG II supplementation in high salt fed rats restored FID of MCAs. ANG II changed the protein/gene expression of COXs, HIF-1α and VEGF and significantly increased GPx4 and EC-SOD antioxidative enzyme expression, decreased systemic oxidative stress, decreased superoxide/ROS levels and increased NO bioavailability in the vascular wall.

Interestingly, similar was observed in our human studies: HS diet led to impaired peripheral microvascular and macrovascular reactivity in response to ACh and post-occlusive hyperemia. Plasma renin activity (PRA) and serum aldosterone level were significantly suppressed after HS diet and markers of oxidative stress were increased. Impaired AChID and increased salt intake, as well as impaired AChID and suppressed RAS were significantly positively correlated. Impaired vasorelaxation and increase in oxidative stress could have been prevented by concomitant intake of antioxidants together with HS diet. Furthermore, in healthy young humans on HS diet, cerebral blood flow in response to orthostatic test was preserved due to altered vascular reactivity of MCA, with increased cerebrovascular resistance and blunted baroreceptor sensitivity and sympathetic activity.

Taken altogether, our results support conclusion that physiological levels of circulating ANG II are crucial to maintain the HIF-1α dependent mechanisms of endothelium-dependent vasodilation and vascular oxidative balance without affecting mean arterial pressure. Suppression of sympathetic activity during HS diet represents a physiological response.

Funding: IP-MEFOS-2023 grant (PI_ Ines Drenjančević)

T-KA3-1 - NIMODIPINE IN SOLUTION OR ASSOCIATED TO pH-SENSITIVE NANOPARTICLES ATTENUATES ISCHEMIC INJURY

Rita Frank[1,2], Orsolya M. Tóth[3], Ákos Menyhárt[1,2], Ferenc Bari[3], Eszter Farkas[1,2]
[1] HCEMM-USZ Group of Cerebral Blood Flow and Metabolism, [2]Department of Cell Biology and Molecular Medicine, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Hungary, [3]Department of Medical Physics and Informatics, Albert Szent-Györgyi Medical School and Faculty of Science and Informatics, University of Szeged, Hungary

Nimodipine, a potent voltage gated Ca^{2+} channel antagonist and cerebral vasodilator was found to be effective against ischemia-coupled vasoconstriction. Nanoparticles yield a novel approach of drug delivery and can be constructed to release drugs in response to specific signals. This affords high drug concentration at the site of ongoing injury with minimized undesirable side effects. We propose that, ischemia induced tissue acidosis can be a relevant signal to initiate nimodipine release from nanoparticles. Also, we hypothesize that nimodipine can exert a direct neuroprotective effect via VGCC antagonism, independent of its vasodilator action.

Anesthetized Sprague-Dawley rats (n=18) were used. After washing a suspension of nanoparticles with or without nimodipine on the exposed brain surface, both common carotid arteries were permanently occluded to create global forebrain ischemia. Spreading depolarizations (SDs) were elicited by 1M KCl to worsen the ischemic insult. Local field potential, cerebral blood flow (CBF) and tissue pH-variations were recorded from the cerebral cortex. Coronal brain slices prepared from C56BL/6 mice (n=16) were perfused with artificial cerebrospinal fluid (aCSF). First, we determined the optimal incubation time of nimodipine (10 µM) treatment by measuring tissue nimodipine saturation with liquid chromatography-tandem mass spectrometry (LC-MS) at increasing incubation times. Low glucose aCSF (5 mM) and transient anoxia (1 min) were applied to elicit SD. Intrinsic optical signal imaging was used to confirm SD occurrence.

Ischemia-induced tissue acidosis initiated nimodipine-release from nanoparticles, which was confirmed by the significant elevation of baseline CBF (29.3 ± 6.9 vs. 47.8 ± 23.7 %; nanoparticle only vs. nimodipine+nanoparticle) Nimodipine significantly shortened the duration of SD (76.2 ± 17.2 vs. 48.1 ± 23.3 s; nanoparticle only vs. nimodipine+nanoparticle), and the associated tissue acidosis (138.3 ± 66.1 vs. 65.5 ± 20.2 s; nanoparticle only vs. nimodipine+nanoparticle), also it enhanced the SD-related hyperemia (2368.0 ± 1324.7 vs. 4604.4 ± 2572.3 %*s; nanoparticle only vs. nimodipine+nanoparticle). In brain slices, 30 min was required for the saturation of the tissue with nimodipine. Nimodipine reduced the focal area of SD (3.38 ± 0.88 vs. 2.37 ± 0.94 %, control vs. nimodipine), decreased the total cortical area affected by SD (39.88 ± 22.42 vs. 17.12 ± 8.63 %, control vs. nimodipine) and curtailed the propagation velocity of SD (1.59 ± 2.29 vs. 0.19 ± 0.79 mm/min, control vs. nimodipine).

Our results show that the delivery of nimodipine targeted to the ischemic nervous tissue with pH-sensitive nanoparticles is a feasible strategy to attenuate secondary brain injury mechanisms such as SD. Moreover, nimodipine exerted direct neuroprotection against the detrimental effect of SD in our *in vitro* ischemia model.

T-KA3-2 - THE SIGNIFICANCE OF PORCINE MODEL PREDICTING HYPERSENSITIVITY REACTIONS TO NANOPARTICLES

László Dézsi[1,2], Gergely Kozma[1,2], Tamás Mészáros[1,2], Tamás Bakos[1], László Rosivall[1,2], Gábor Szénási[1] and János Szebeni[1,2]
[1]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary; [2]SeroScience LTD., Budapest, Hungary

Introduction: Pigs are known to provide a sensitive model for studying complement (C) activation-related pseudoallergy (CARPA), a hypersensitivity reaction (HSR) to various nanomedicines and nanoparticle (NP)-based diagnostics. The major symptoms of CARPA include hemodynamic, cardiopulmonary, blood cell count and skin changes, similar to humans. HSRs limit the clinical use of these agents. Our model can reliably predict HSRs of nanodrugs during development, and can help to establish new therapies to reduce the incidence and severity of HSRs.

Methods: Juvenile domestic pigs sedated with ketamine/xylazine were anesthetized by isoflurane and spontaneously ventilating. Pulmonary arterial pressure (PAP), systemic arterial pressure (SAP), and heart rate (HR) were measured. Hemodynamic parameters and ECG were continuously recorded (PowerLab, ADI). Test substances were administered as an intravenous (i.v.) bolus injection or as an i.v. infusion. Blood samples were collected at pre-determined time points. Samples served for blood cell counting (Abacus, Diatron), and for measuring plasma concentrations of thromboxane (TXB2), complement components (C3a and sC5b-9) and antigens (IgG and IgM).

Results: The effects of single or repeated doses of test substances were studied. Liposomal drugs (Doxil and Ambisome) induced CARPA in all cases at low doses (0.01 to 0.1 mg/kg) and with variable intensity. Main symptoms of CARPA were cardiovascular changes; i.e. transient increase in PAP starting at 1 min post-infusion, rise or decline of SAP, tachy/bradycardia and pathological ECG changes; blood cell changes, i.e. leucopenia or leukocytosis, thrombocytopenia; as well as flash or rash of the skin. Elevated plasma concentrations of TXB2, C, and Ig corresponded with CARPA development. In about 1/10th of the cases, anaphylaxis developed requiring resuscitation. Following repeated administration of Doxil (but not Ambisome) self-induced tolerance (tachyphylaxis) occurred. Slow infusion reduced all CARPA symptoms. SPIONS used as MR diagnostics or iron supplements (Feraheme and Resovist; 0.5 to 5 mg/ml) had similar effects to liposomal drugs. Dextran-coated self-developed SPIONS (SEON Dex, Uniklinik Erlangen) had no effects. Pfizer's LNP-mRNS COVID-19 vaccine (Comirnaty) elicited all CARPA symptoms (at 1 to 5-fold of the human dose).

Conclusion: Our results show that various drugs and chemical entities, which all fall into the size-range of nanoparticles, could induce CARPA in juvenile pigs, which reaction is similar to HSRs in sensitive humans. Revealing the underlying mechanisms of HSRs may enable us to develop new therapies against the adverse events.

Support: EU H-2020 825828 (Expert) and 952520 (Biosafety).

T-KA3-3 - COMPARISON OF LIPOSOMAL DRUG-INDUCED BLOOD PRESSURE CHANGES IN MICE AND RATS

Gábor Szénási[1], Tamás Bakos[1], Erik Őrfi[1], Tamás Mészáros[1,2], László Hricisák[2], László Rosivall[1,2], Péter Hamar[2], Zoltán Benyó[2], János Szebeni[1,2] and László Dézsi[1,2]
[1]Institute of Translational Medicine, Semmelweis University, Budapest, [2]SeroScience Ltd., Budapest, Hungary

Introduction: Liposomal drugs administered intravenously (i.v.) can induce IgE-independent side effects known as infusion reaction, and also termed as complement activation-related pseudoallergy (CARPA).

Aim: We aimed to reveal the basic mechanisms of blood pressure changes after i.v. injection of amphotericin B-containing liposomes (Abelcet, rats: 10 mg/kg; mice: 30 mg/kg).

Methods: Male NMRI mice, and wild type or thromboxane prostanoid receptor deficient (TP KO) mice on C57Bl/6 background, as well as male Wistar rats (anesthetized with pentobarbital) were used (n=6-8/group). Mean arterial blood pressure was continuously monitored. Blood was collected at 0, 1, 3 10 and 30 min in rats, and from separate groups of mice at 3-5 min after treatment. Plasma C3a and thromboxane B2 (TXB2) concentrations were assayed using ELISA. Blood count was obtained using a hematology analyzer (Abacus Vet5).

Results: Abelcet caused transient hypertension in mice (10-15 min) and transient hypotension in rats (20-40 min). Abelcet resulted in leucopenia and thrombocytopenia, and increased plasma complement C3a and TXB2 concentrations in both species. Complement depletion with cobra venom factor (CVF) lengthened the hypertensive effect in mice and abolished the hypotensive effect in rats. Pretreatment with DF2593A (10 mg/kg, i.v.), a C5a receptor (C5aR) antagonist, lengthened the hypertensive effect of Abelcet in mice and elicited a small decrease in the hypotensive effect in rats. Inhibition of C3a receptors with SB290157 (10 mg/kg) attenuated the hypertension in mice and enhanced the hypotension in rats, but both effects were rather small. Macrophage depletion with clodronate liposomes in mice lengthened the hypertensive effect similarly to CVF. Pre-treatment with $GdCl_3$ to inhibit macrophages slightly attenuated the hypotensive effect of Abelcet in rats. Inhibition of mast cell activation by cromolyn or C48/80, decreased the hypotensive response in rats, but induced delayed hypotension in mice, respectively. Inhibition of platelet activation using eptifibatide, a platelet glycoprotein IIb/IIIa (GPIIb/IIIa) receptor inhibitor, lengthened the hypertensive effect in mice, but hardly affected the responses in rats. Abelcet did not change blood pressure in TP KO mice and led to a delayed hypertension after pretreatment with CVF.

Conclusion: Our results suggest that complement activation has a small contribution to liposomal drug-induced hypotension, and both macrophages and mast cells contribute to the release of vasoactive mediators in rats. The early hypertensive effect of TXA2 release in mice was independent from complement activation, and was reversed with some delay mainly by the activation of C5aR. Both macrophages and platelets are substantially implicated in the latter effect.

Support: ÚNKP-19-3-III and OTKA K-101775, K-115623, K-125174, EU H-2020 825828 “Expert” and 952520 (Biosafety).

T-KA3-4 - NOVEL PERSPECTIVES IN THE UTILIZATION OF NANOMEDICINES FOR BRAIN TARGETING VIA INTRANASAL ROUTE

Gábor Katona[1], Ildikó Csóka[1]
[1]Institute of Pharmaceutical Technology and Regulatory Affairs, Szeged

Neuroinflammatory-associated diseases, such as Alzheimer's Disease, Multiple Sclerosis and other autoimmune originated diseases as well as brain tumours (astrocytomas, oligodendrogliomas, and oligo-astrocytomas) belong to the urgent medical needs, and their effective treatment is possessing a severe challenge. Despite the increase in the number of therapeutic agents, the most of these candidates are still insufficient. Antibodies have been shown to be a potent therapeutic tool, however their brain targeting efficacy is limited, particularly because of the defensive function of blood–brain barrier (BBB), which makes brain tissue hard to access by conventional drug-targeting strategies. To overcome these hurdles advanced drug delivery systems are required, moreover alternative delivery routes should be acquired to access the central nervous system (CNS).

Nose-to-brain delivery may represent a non-invasive novel approach that enables the delivery of complex drugs along the trigeminal or olfactory nerves to the CNS, while bypassing the BBB Due to the high surface area and rich vascularization of nasal mucosa, drugs or drug-delivery systems can be easily absorbed from the nasal cavity through the olfactory epithelium, either (i) by axonal transport after internalization into the neurons, (ii) by paracellular transport across the tight junctions between cells and the channels next to the olfactory nerves, or (iii) by transcellular transport across the basal epithelial cells.

The contribution of nanotechnology to this field is crucial since not only it allows the protection of the sensitive therapeutic cargo from enzymatic degradation, but most importantly it improves the uptake by the olfactory mucosa and the access to the CNS, based either on passive or active targeting. As a result, the use of drug delivery nanocarriers has led to enhanced drug concentrations and extended half-life times with the subsequent improved therapeutic effect of the delivered molecules.

Present works aims to represent the novel perspectives in the treatment of neurodegenerative diseases and brain tumour therapy, as well as the most significant results of our research group obtained during the development of intranasally applicable nanocarriers systems. We already designed such smart nanocarrier systems (lipid-, polymer- and protein-based), which successfully improved the nasal absorption and bioavailability of both small molecule and peptide drugs as confirmed by *in vitro* and *in vivo* studies.

Acknowledgement:
This work was supported by Project no. TKP2021-EGA-32 implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

T-KA3-5 - RADIOSENSITIZING EFFECT OF METAL NANOPARTICLES IN COMBINATION WITH HISTONE DEACETYLASE INHIBITORS

Igaz Nóra[1], Szőke Krisztina[1], Bocz Csenge[1], Kovács Dávid[1], Rónavári Andrea[2], Szabó Rita[3], Polanek Róbert[3], Buhala Andrea[4], Vizler Csaba[4], Tiszlavicz László[5], Rázga Zsolt[5], Hideghéty Katalin[3], Kónya Zoltán[2], Kiricsi Mónika[1]
[1]Department of Biochemistry and Molecular Biology, University of Szeged, Közép fasor 52, H-6726 Szeged, Hungary, [2]Department of Applied and Environmental Chemistry, University of Szeged, Rerrich Béla tér 1, H-6720 Szeged, Hungary, [3]ELI-ALPS, ELI-HU Non-Profit Ltd., Szeged, Hungary, [4]Institute of Biochemistry, Biological Research Centre, Szeged, Temesvári krt. 62, H-6726 Szeged, Hungary, [5]Department of Pathology, University of Szeged, Állomás utca 2, H-6720 Szeged, Hungary.

A frequent reason behind the failure of chemo- and radiotherapy is the development of multidrug- or radioresistance. Radiosensitizing agents are utilized to augment the potency of ionizing radiation on cancer cells and attenuate the disadvantageous consequences of irradiation on healthy tissues. Nanoparticles composed of metals with high atomic number are potential radiosensitizers and could be ideal candidates to overcome radioresistance due to the multitude of anti-cancer effects triggered by these agents. Our aim is to reveal the intrinsic radiosensitizing effect of metal nanoparticles in monotreatments and in combination with histone deacetylase (HDAC) inhibitors on radiosensitive and radioresistant tumor cells. Our hypothesis is that HDAC inhibitors induce the formation of a relaxed chromatin structure in cancer cells which makes the DNA more vulnerable to the effect of ionizing radiation. Additionally, irradiation of the intracellular metal nanoparticles triggers the generation and release of reactive electrons from the nanoparticles yielding reactive oxygen species, that significantly enhances the detrimental effects of irradiation.

We have shown that gold nanoparticles (AuNPs) in combination with the HDAC inhibitor suberoylanilide hidroxamic acid (SAHA) synergistically enhance the damage caused by ionizing radiation both in radiosensitive and radioresistant cancer cells in 2D and 3D cell cultures. Combinational treatments of AuNPs and SAHA significantly increased the number of DNA double strand breaks upon irradiation and significantly decreased the colonyforming capabilities of sensitive and radioresistant cancer cells compared to the irradiated control and to the AuNP or SAHA-treated samples. Besides AuNPs, we examine the radiosensitizing performance of platinum (PtNPs) and hafnium oxide nanoparticles (HfO_2NPs) as well on sensitive and radioresistant cancer cells. In contrary to our findings on AuNPs and SAHA treated cancer cells, enhanced radiosensitization by PtNPs or HfO_2NPs and HDAC inhibitors could not be verified. Although the combination of PtNPs and SAHA significantly increased the number of DNA double strand breaks in cancer cells after irradiation compared to the individual treatments and to the control cells, the colonyformation of tumor cells was not affected by this approach. Moreover, HfO_2NP in combination with SAHA did not exhibit augmented anti-tumor features than what was induced by individual HfO_2NP or SAHA treatments.

In summary, metal nanoparticles are promising anti-cancer and radiosensitizing agents and show synergistic interaction with chemotherapeutic agents, however, their effect probably depends on individual nanoparticle as well as cellular properties, on different signalling pathways and cellular events, such as the degree of endoplasmic reticulum stress or senescence, which aspects should be investigated in details prior to the translation of nanoparticle-based radiosensitization approaches in oncotherapy.

The project is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00351/22) and by grants of the New National Excellence Program of the Ministry for Innovation and Technology (ÚNKP-22-5-SZTE-553).

T-KA3-6 - TARGETED DRUG DELIVERY ACROSS THE BLOOD-BRAIN BARRIER BY NANOPARTICLES

Szilvia Veszelka[1], Anikó Szecskó[1], Gergő Porkoláb[1], Emese K. Páli[1], Mária Mészáros[1], Mária A. Deli[1]
[1]Biological Barriers Research Group, Institute of Biophysics, Biological Research Centre, Szeged

The blood-brain barrier (BBB) represents a major obstacle for the pharmaceutical treatment of central nervous system (CNS) disorders, because it restricts the penetration of therapeutics to the brain. Nanoparticles (NP) are in the focus of research efforts to develop successful drug delivery systems for the brain, since there are still no CNS drugs available which are formulated as NPs. The key to efficient brain delivery of NPs is the specific targeting of the BBB, which is unsolved. The number of drug molecules developed for nutrient transporters of the BBB like solute carriers (SLCs) increases, but SLCs as molecular targets are underresearched.

In our previous studies, we revealed the specific expression pattern of SLCs in brain endothelial cells that can potentially be targeted with NPs. We hypothesized that decorating the surface of NPs with dual or triple combinations of different transporter ligands (biotin, glutathione, alanine, glucose, ascorbic acid or leucine) may result in better docking of the particles to the surface of brain endothelial cells, which subsequently triggers a transcytotic process. Using a novel optical tweezer based method developed in our institute, we directly measured the adhesion forces between glutathione targeting ligand and living cells of the BBB. The presence of targeting ligand combinations on NPs increased the uptake of cargo molecules in cultured brain endothelial cells. The cellular uptake was temperature dependent and could be decreased with metabolic and endocytosis inhibitors suggesting an active, energy-dependent process. Making the negative surface charge of brain endothelial cells more positive with a cationic lipid or digesting the glycocalyx with neuraminidase elevated the uptake of the cargo after treatment with targeted nanocarriers. Our new observations indicated that surface charge at the BBB is important in the uptake mechanism of charged NPs. Treatment with NPs increased the plasma membrane fluidity of brain endothelial cells, suggesting the fusion of nanovesicles with cell membranes. Targeting ligands also elevated the permeability of the cargos of NPs across culture models of the BBB and entry into glial cells or brain organoids. It was verified in mice that multiple ligand decoration of NPs resulted in better CNS penetration as compared to single ligand labeling.

Our data indicate that combination of ligands binding different BBB transporters can potentially be exploited for brain targeting of NPs. Our results can lead to the development of new targeted drug delivery systems for further pharmaceutical applications, and to new products and methods to treat CNS diseases.

T-KA3-7 - MULTIPLEXING LIVE-CELL IMAGING AND REAL-TIME IMPEDANCE READOUT WITH RTCA ESIGHT SYSTEM

Agilent Technologies, Inc.
5301 Stevens Creek Blvd. Santa Clara, CA 95051, United States

The xCELLigence RTCA eSight enables comprehensive insight into cell health, cell behavior, cell function and cell biology processes using live, simultaneous, and real time biosensor impedance-based and image-based measurements. The eSight system combines the label-free xCELLigence RTCA technology with live cell imaging in 3 colors (red, green, and blue). This combination allows for informative live cell analysis.

Live cell imaging and real-time biosensor measurements can be performed on the same cell populations, which provides insightful information on cell behavior. The streamlined workflow, high reproducibility, and quantitative kinetics of the eSight system makes it ideal for a wide range of cell-based assays such as proliferation, cytotoxicity, and apoptosis. The powerful RTCA software integrates two data types into one temporal display. Cell analysis can be displayed and exported into diverse formats, which include RTCA images, KT50 (time to reach 50% cytolysis at a given E:T ratio), % Cytolysis dose response, or IC50 dose response curves.

A MILESTONE IN THE BIOMARKERS DETECTION. THE SENSITIVITY OF QUANTERIX Simoa® TECHNOLOGY PROVIDES NEW INSIGHTS INTO THE HEALT-TO-DISEASE CONTINUUM.

Quanterix
900 Middlesex Turnpike, Building 1 Billerica, MA 01821 USA

Quanterix has commercialized platforms that allow researchers to detect protein biomarkers at levels far below the concentrations detected by other types of assays. The sensitivity of Simoa® technology opens new horizons in research to understand and treat diseases in neurology, oncology, immunology, cardiology and infectious diseases. Simoa® has been tested in a wide variety of samples form relatively common samples types including plasma, urine, but also very difficult and challenging matrices like stool and tissue lysates.
The technologies are available in the form of fully automated, high-throughput systems and compact, small footprint platforms for research laboratories.

The Simoa® bead technologies digitize signal detection utilizing magnetic particles and microfluidic detection arrays. The Simoa®HD-X Analyzer™ is the latest model of the fully automated Simoa®bead-based immunoassay platform. The SR-X™ Biomarker Detection System offers researchers access to ultra-sensitive biomarker detection capabilities in a compact and affordable system. The Simoa® planar array technology is proving to be comparable in sensitivity and enables straightforward multiplexing for up to 10 different analytes with minimal sample volume consumption. The SP-X Imaging and Analysis System™ Unleashing the power of next-generation Simoa® Planar array technology for robust multiplex biomarker detection – even at healthy baseline levels.

T-KB3-1 - ANTIDEPRESSIVE EFFECT OF NMDA RECEPTOR ANTAGONISTS VIA THE NORMALIZATION OF THE SLEEP

Attila Tóth
In vivo Electrophysiology Research Group, Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University

Pharmacological effects of ketamine may affect homeostatic sleep regulation via slow wave related mechanisms.
In the present examinations, electrophysiological effects of ketamine applied at anesthetic dose (80 mg/kg) were tested on neocortical electric activity for 24h in freely moving rats. Ketamine effects were compared to changes during control (saline) injections and after 6 h gentle handling sleep deprivation (SD). As circadian factors may mask drug effects, an illumination protocol consisting of short light-dark cycles was applied resulting of the elemination of the circadian sleep rhythm.

Ketamine application induced a short hypnotic stage with characteristic slow cortical rhythm followed by a long-lasting hyperactive waking resulting pharmacological SD. Coherence analysis indicated an increased level of local synchronization in broad local field potential frequency ranges during hyperactive waking but not during natural- or SD-evoked waking. Both slow wave sleep and rapid eye movement sleep were replaced after the termination of the ketamine effect.

Our results show that both ketamine-induced hypnotic state and hyperactive waking can induce homeostatic sleep pressure with comparable intensity as 6 h SD, but ketamine-induced waking was different compared to the SD-evoked one. Both types of waking stages were different compared to spontaneous waking but all three types of wakefulness can engage the homeostatic sleep regulating machinery to generate sleep pressure dissipated by subsequent sleep. Current-source density analysis of the slow waves showed that cortical transmembrane currents were stronger during ketamine-induced hypnotic stage compared to both sleep replacement after SD and ketamine application, but intracortical activation patterns showed only quantitative differences.

These findings may hold some translational value for human medical ketamine applications aiming the treatment of depression-associated sleep problems, which can be alleviated by the homeostatic sleep effect of the drug without the need for an intact circadian regulation.

T-KB3-2 - THE ROLE OF PROLACTIN-RELEASING PEPTIDE (PrRP) IN THE DEVELOPMENT OF DEPRESSIVE-LIKE SYMPTOMS IN RATS

Anita Kovács[1], Evelin Szabó[1], Kristóf László[1], László Lénárd[1], Zsuzsanna Toth[2], Dóra Zelena[1]
[1]Institute of Physiology, University of Pécs, Medical School, Pécs, [2]Laboratory of Neuroendocrinology and In Situ Hybridization, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest

INTRODUCTION: Nowadays, a growing number of nutritional, metabolic and psychological disorders are associated with stress. More and more research results confirm that the RFamide peptides - especially the prolactin- releasing peptide (PrRP) - can play a role in the regulation of stress responses. Based on the close relationship between stress and depression, it can be hypothesized that PrRP also plays a role in depression.

METHODS: Fifteen minutes forced swimming test (FST) was used to induce depression-like symptoms in male Wistar rats. On the next day the animals' behaviour was analysed during a 6-min FST, and based upon the time spent in immobility resilient (low level) and vulnerable (high level) group was formed beside a control, non-FST group. At the end of the experiments frozen brain samples were taken for mRNA measurement by rtPCR. As lipopolysaccharide (LPS) injection, which induces an immune response, also increases the appearance of depression-like symptoms in FST, we examined at the development of PrRP mRNA expression in this paradigm as well. To prove the direct relationship, we studied depression-like symptoms in FST 30 minutes after intracerebroventricular (icv) administration of PrRP and an antagonist. Since our results indicated an increased role of the hypothalamus, we also confirmed these differences on brain samples from human suicides.

RESULTS: In vulnerable animals, PrRP mRNA levels increased in certain brain areas (e.g. A1 region), while resilient animals had levels similar to controls. An increase in inflammatory cytokines (TNFα mRNA) was detected 3 hours after LPS administration, and after 24 hours PrRP mRNA expression also increased in the A1 region, while the hypothalamic expression of its receptors decreased, parallel to the depression-like symptoms appearing in the FST test. In line with this, icv administered PrRP increased immobility (floating) and decreased time spent with active coping strategies (struggling). However, the administration of the non-specific antagonist did not affect the behavior. Human studies have supported a decrease in the level of receptors in the hypothalamic areas.

CONCLUSIONS: Our results support that an increase in the level of PrRP and/or a decrease in its receptor may contribute to the development of depression. At the same time, it is conceivable that PrRP innervation causes opposite effects on different brain areas. Therefore, it seems reasonable to perform further examinations targeting specific regions.

T-KB3-3 - HOW TO PREVENT OBESITY: THE POTENTIAL OF UROCORTIN 2 – BASED ON ANIMAL EXPERIMENTS

Marta Balasko[1], Dora Krisztina Kovacs[1]
[1]Institute for Translational Medicine, Medical School, Unoiversity of Pecs, Hungary

Middle-aged obesity and aging cachexia present healthcare challenges. Central responsiveness to body weight reducing mediators, e.g. leptin change during aging that may promote middle-aged obesity and aging cachexia. Leptin is connected with urocortin2 (Ucn2) an anorexigenic and hypermetabolic member of the corticotropin family.

We aimed to study the role of Ucn2 in middle-aged obesity and aging cachexia.

Food intake, body weight and hypermetabolic responses (oxygen consumption, core temperature) of male Wistar rats (3-, 6-, 12- and 18-month) were tested following intracerebroventricular injections of Ucn2. Following one central injection, Ucn2-induced anorexia lasted for 9 days in the 3-month, 14 days in the 6-month and 2 days in the 18-month group. Middle-aged 12-month rats failed to show anorexia or weight loss.

Weight loss was transient (4 days) in the 3-month, 14 days in the 6-month and slight but long lasting in the 18-month rats. Ucn2-induced hypermetabolism and hyperthermia increased with aging. The age-dependent changes in the mRNA expression of Ucn2 detected by RNAscope in the paraventricular nucleus correlated with the anorexigenic responsiveness.

Our results show that age-dependent changes of Ucn2 may contribute to middle-aged obesity and aging cachexia. Ucn2 shows potential in the prevention of middle-aged obesity.

Funding: ÁOK_KA_2021_37_Dr.Balaskó Márta

T-KB3-4 - SELECTIVE INDUCTION OF KREBS CYCLE ENZYME SUBUNITS IN THE PARAHIPPOCAMPAL CORTEX OF SUICIDE VICTIMS

Fanni Dóra[1,2], Tamara Hajdu[2,3], Éva Renner[1], Krisztina Paál[5], Alán Alpár[1,4], Miklós Palkovits[1], Christos Chinopoulos[5], Árpád Dobolyi[2,3]
[1]Human Brain Tissue Bank, Semmelweis University, Budapest, [2]Laboratory of Neuromorphology, Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, [3]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, [4]Department of Anatomy, Histology and Embryology, Semmelweis University, Budapest, [5]Department of Biochemistry and Molecular Biology, Semmelweis University, Budapest

Abstract
Altered functional connectivity in human brain networks has been reported in mood disorders. A moderating hub between resting state networks (RSNs) and the medial temporal lobe (MTL) is the parahippocampal cortex (PHC), where abnormal activity has been reported in depressed patients and suicide attempters. Alterations in neuronal mitochondrial function may contribute to depression and suicidal behavior, however, little is known about the underlying molecular level changes in relevant structures. Specifically, expressional changes related to suicide have not been reported in the PHC. Here, we compared the protein expression levels of genes encoding tricarboxylic acid (TCA) cycle enzymes in the PHC of suicide victims by reverse phase protein array (RPPA) and mRNA levels by RT-PCR. Postmortem human brain samples were collected from 12 control and 10 suicide individuals. The entorhinal cortex (EC), topographically anterior to the PHC in the parahippocampal gyrus, served as a control. RPPA analysis revealed that the protein levels of DLD, OGDH, SDHB, SUCLA2 and SUCLG2 subunits were significantly elevated in the PHC but not in the EC. Accordingly, the mRNA levels of respective subunits were also increased. The subunits with altered levels participate in enzyme complexes participating in the oxidative decarboxylation branch of glutamine catabolism. Our data hint on a potential role of glutaminolysis in the PHC in the pathophysiology of suicidal behavior.

Keywords:
suicide, parahippocampal cortex, reverse phase protein array, mitochondria, tricarboxylic acid cycle, glutaminolysis

T-KB3-5 - EFFECTS OF ADULT SOCIAL ISOLATION ON THE BEHAVIOR AND BRAIN GENOMICS OF RATS

Vivien Csikós[1], Rashmi Kumari[1], Fanni Dóra[2], Arpád Dobolyi[1]
[1]Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary,
[2]Human Brain Tissue Bank and Microdissection Laboratory, Department of Anatomy, Histology and Embryology, Semmelweis University, 1094 Budapest, Hungary.

Loneliness is a factor that aggravates depression in human. Social isolation also causes negative behavioural changes in other social species. Still, the genomic background of these changes in the brain are unknown. In the present study, the behaviour, gene expression levels and molecular functions in socially isolated and pair-housed rats were analyzed and correlated. One of the brain areas that plays a prominent role in the formation of behaviour is the medial prefrontal cortex, so we investigated the gene expressional changes of this cortical brain area using RNA sequencing methods in male rats kept socially or solitary for 10 days. In addition to RNA analysis, social behaviour was measured in rats using a three-chamber test and a direct social interaction test. The sociability of animals kept in isolation decreased significantly, but in the test measuring social preference and social interaction, they spent significantly more time with their conspecifics than the social group. The anxiety-like behaviour of the animals was assessed using the elevated plus maze and open field tests, which showed that animals kept in isolation were more anxious compared to their heir conspecifics kept in pairs. The depression-like behaviour of the animals was monitored by forced-swim test, which did not show any change.

More than 30 genes differed between groups according to the criteria of $\log2FC > \pm1$ and correlated p-value < 0.05. Based on the KEGG pathway analysis, changing genes play a key role in the development of addiction and in the regulation of behavioural and learning mechanisms. Among these genes, 5 genes were validated by RT-qPCR: Ndst4, Rgs9, HTr2c, Pdyn and Lrrc10b, whose levels decreased by social isolation. Based on the known functions of these genes, HTr2C (5-Hydroxytryptamine Receptor 2C) and Rgs9 (Regulator Of G-Protein Signalling) are of particular interest, as literature suggests that they may play an role in the regulation of social and depression-like behaviours.

Grant support was provided by NAP3 project of the Hungarian Academy of Sciences (NAP2022-I-3/2022), the NKFIH OTKA K134221 research grant, TKP2021-EGA/TKP2021-NVA/TKP2021-NKTA, the ÚNKP-21-3, and the Joseph Cours Scholarship, of Eötvös Loránd University, Budapest, Hungary.

T-KB3-6 - KISSPEPTIN-8 SUPPRESSES LOCOMOTION, INDUCES A TRANSIENT HYPERTHERMIA AND ALTERS GENE EXPRESSION IN THE VENTRAL TEGMENTAL AREA – NUCLEUS ACCUMBENS CIRCUIT IN RATS

Katalin Eszter Ibos[1], Éva Bodnár[1], Szakács Júlia[1], Zsolt Bagosi[1], Zsolt Bozsó[2], Krisztina Csabafi[1]
[1]University of Szeged, Albert Szent-Györgyi Medical School, Department of Pathophysiology, Szeged, [2]University of Szeged, Albert Szent-Györgyi Medical School, Department of Medical Chemisrty, Szeged

The ventral tegmental area (VTA)- nucleus accumbens (NAc) dopaminergic system has been implicated in the regulation of motivation and locomotor activity. The expression of neuropeptide FF receptors (NPFFR1 and NPFFR2) has been detected in both regions, pointing to a possible role of RF-amides in the modulation of the VTA-NAc circuit. We aimed to investigate how an N-terminally truncated RF-amide octapeptide, kisspeptin-8 (Kp-8) affects behavior and body temperature using telemetry and forced swim test (FST), and whether it modulates gene expression in the VTA and NAc in rats.

Male Wistar rats were used for the experiments, following the implantation of an intracerebroventricular (icv) cannula. Before telemetry, an E-mitter was also implanted into the abdominal cavity. The rats were placed on the trays of the telemetry system in their home cages and were injected icv each day at the same time with 0.1, 0.5, 1 or 2 µg of Kp-8 or saline. The FST was performed according to a standard 2-day protocol. The animals were treated icv. with the above-mentioned doses 30 minutes prior to the experiment. For the gene expression studies, 0.1 or 1 µg Kp-8 was injected icv. 2 hours before dissection. The expressions of *Fos*, *DRD1R, DRD2R* (dopamine receptor D1, D2), *GAD65* (glutamic acid decarboxylase 65kD*)*, and *NPFFR1, NPFFR2* genes were determined in the NAc. In the VTA, the expressions of *Fos, DRD1R, DRD2R* and *TH* (tyrosine hydroxylase) genes were analyzed.

In the telemetry, all doses of Kp-8 triggered a transient increase in body temperature lasting for approximately 6 hours after injection. Analysis of the area under the curve revealed a significant decrease in locomotor activity in the animals treated with 1 or 2 µg of Kp-8. The 1 µg dose also induced an increase in immobility in the FST. There was a downregulation of *DRD1R* (1 µg Kp-8)*, DRD2R* (0.1 and 1 µg Kp-8) and *NPFF2R* (0.1 µg Kp-8) in the NAc, but no significant change in gene expression was detected in the VTA.

In our study, Kp-8 caused a downregulation of D1 and D2 receptors in the NAc. Several groups have shown the role of NAc D1 receptors in the regulation of locomotor activity, thus its downregulation might be in the background of hypolocomotion. According to the literature, the depressive-like behavior in FST also correlates with the downregulation of D2 receptors. Based on our gene expression results, it is unlikely that Kp-8 directly influences dopamine release from the VTA. However, it could modulate the activity of the VTA-NAc system by acting on NPFF receptors expressed on GABAergic interneurons in the NAc, suppressing the effect of dopaminergic input from the VTA.

T-KA4 - TRPM3 – FROM THE PERIPHERY TO THE BRAIN

Thomas Voets
Laboratory of Ion Channel Research, VIB-KU Leuven Center for Brain & Disease Research & Dept. of Cellular and Molecular Medicine, KU Leuven, B-3000 Leuven, Belgium

The transient receptor potential (TRP) channel TRPM3 is a calcium-permeable cation channel activated by heat and by the neurosteroid pregnenolone sulfate (PregS). TRPM3 is highly expressed in sensory neurons, where it plays a key role noxious heat sensing. Upregulation of TRPM3 expression and function contributes to hypersensitivity and ongoing pain in a variety of pain models, and TRPM3 antagonists are effective in reducing evoked and ongoing pain in these models. Moreover, *de novo* TRPM3 variants were described in patients with neurodevelopmental disorders characterized by intellectual disability, hypotonia and altered pain sensitivity. Disease associated variants invariably lead to a dominant gain of channel function, by affecting different aspects of TRPM3 gating. These findings indicate an important role for TRPM3 in brain development and function, provide a rationale for the use of TRPM3 antagonists to treat these patients.

T-KB4 - PHYSIOLOGICAL ROLE OF PULMONARY SURFACTANT: MUCH MORE THAN LOW SURFACE TENSION

Calkovska Andrea[1], Hanusrichterova Juliana[2], Kolomaznik Maros[2], Uhrikova Daniela[3]
[1]Department of Physiology and [2]Biomedical Centre Martin, Jessenius Faculty of Medicine, Comenius University, Martin, Slovakia; [3]Faculty of Pharmacy, Comenius University, Bratislava, Slovakia

Pulmonary surfactant is a lipoprotein complex which stabilizes the lung alveoli and small airways and thus reduces the tendency of the lungs to collapse at the end-expiration. The importance of surfactant goes far beyond the alveoli and is not limited to reducing lung surface tension. Newer studies emphasize less known properties of surfactant components related to its anti-inflammatory abilities and the relaxing effect on the airway smooth muscle.

Physiological and pharmacological surfactant research benefits from combination of several models, which provide a relatively comprehensive picture of the studied issue. In series of studies we addressed the effect of bacterial lipopolysaccharide (LPS) on the respiratory system, its interaction with lung surfactant and new treatment options for acute respiratory distress syndrome (ARDS). The most important results include the use of clinically relevant animal models to test and confirm the suitability of exogenous surfactant therapy, verified combinations with other substances (N-acetyl cysteine, NAC; polymyxin B, PxB) and pointed out the risks. Original results were obtained regarding the effect of LPS on cells of the alveolar-capillary membrane. The results support the idea that long-term cultivation of A549 cells could promote a more ATII-like phenotype and thus could be a more suitable model for ATII cells, especially to study surfactant production. The experiments focused on the relaxing effect of surfactant on the airway smooth muscle confirmed the involvement of leukotriene and histamine receptors in the contractile mechanisms of the airways after LPS exposure. The atomic force microscopy of airway smooth muscle cells revealed that the EP4 receptor for the relaxing prostaglandin PGE2 may be involved in the mechanism of airway smooth muscle relaxation by surfactant through the interaction of the surfactant with the airway epithelium.

Physical-chemical studies can expand the possibilities by investigating the mutual relations of exogenous surfactant with other drugs and biologically significant molecules (e.g. N-acetylcystein, NAC; Polymyxin B, PxB; cathelicidin LL-37; SP-B protein). The aim was to clarify the interactions at the molecular level for the potential use of exogenous surfactant as a drug carrier for intratracheal administration. There is possible therapeutic benefit of enriching exogenous surfactant with low PxB content when administered intratracheally in LPS-induced lung damage. NAC does not affect surfactant in an undesirable way, it does not induce significant structural changes. From a physical-chemical point of view, NAC has a stabilizing effect on the structure of surfactant in the fluid state at a low content.

Supported by projects VEGA 1/0223/20, VEGA 1/0097/23 and APVV-17-0250.

T-KA5-1 - A HEAT ACCLIMATION-INDUCED NEURONAL PACEMAKER THAT DRIVES HEAT TOLERANCE

Jan-Erik Siemens
University of Heidelberg, Heidelberg, Germany

Precise body temperature regulation requires the detection of temperature. Current models suggest external/ambient temperatures, detected via cutaneous temperature sensors, to provide “anticipatory” feedforward temperature information that allow thermoregulatory responses to prevent disturbances of thermal homeostasis. These preemptive temperature sensors have been suggested to dominate thermoregulation under normal conditions and several ion channels, in particular TRP ion channels, have been shown to detect ambient temperature changes.

However, internal temperature sensors also exist and a particularly temperature sensitive site is situated deep in the brain, in the hypothalamic preoptic area (POA). It has been a matter of debate to what extent neuronal temperature sensitivity in this deep brain structure is physiological relevant and in laboratory mice, under normal housing conditions, we and others find that experimental POA warming has only a small effect on body temperature regulation.
Intriguingly, we find that long-term exposure to hot ambient temperatures (heat acclimation) induces a dramatic increase in preoptic temperature sensitivity. Heat acclimation is an important adaptive process that allows homeothermic organisms to adapt to long-lasting changes in ambient temperatures. Due to increasing environmental temperatures world-wide, heat acclimation is an especially interesting aspect of such adaptation. CNS changes that occur during heat acclimation are not well understood and their role in driving heat tolerance are unknown.

We identified a discreet population of hypothalamic preoptic area (POA) neurons that fundamentally change their electrophysiological activity over the course of heat acclimation and that not only become intrinsically-driven “pacemaker”-like neurons but that also gain robust warm-sensitivity. We found these adaptive cellular changes to occur over an unusually long time scale, reaching a maximum after mice had been placed for 3-4 weeks at warm temperatures, roughly mirroring the time frame required for the animals to reach a fully heat acclimated state and full heat tolerance.

Indeed, we found the phenotypic shift and the increase in tonic activity to be of vital importance for mice to become heat tolerant.

I will also present electrophysiological experiments that we performed to address the ionic mechanism underlying the intrinsic tonic firing and that is required for the acclimation activated neurons to become warm sensitive.

In conclusion, we identified and characterized a hypothalamic neuron population that plastically alters its electrophysiological properties upon long-lasting heat exposure to promote heat tolerance.

T-KA5-2 - MOLECULAR STRATEGIES FOR SUPERSENSITIVE CENTRAL HEAT DETECTION BY TRPM2 CHANNELS

Bartók Ádám[1], Csanády László[1]
[1]Semmelweis University (Budapest)

TRPM2, a Ca^{2+} permeable non-selective cation channel co-activated by cytosolic Ca^{2+} and ADP ribose (ADPR), was recently identified to play a role in the central regulation of body temperature. The central thermostat must contain a temperature sensor which is able to differentiate temperature fluctuations as small as ±1°C around 37°C. To address whether heat-activation of TRPM2 gating in intact cells is an intrinsic property of the TRPM2 protein, temperature dependence of TRPM2 currents was studied in inside-out patches between 15°C and 40°C, across broad ranges of concentrations of both agonists. For fully liganded TRPM2 pore opening is intrinsically endothermic, the enthalpy of opening is ~180 kJ/mol, due to ~10-fold larger activation enthalpy for opening (~200 kJ/mol) than for closure (~20 kJ/mol). However, the TRPM2 temperature threshold is too high (>40°C) for unliganded, but too low (<15°C) for fully liganded channels. Calculations based on a mechanistic gating model indicate that TRPM2 warmth sensitivity around 37°C is restricted to narrow ranges of agonist concentrations. For ADPR that range (submicromolar-to-micromolar) matches, but for Ca^{2+} (>1 μM) it exceeds, bulk cytosolic values, suggesting that a Ca^{2+} nanodomain drives TRPM2 activation *in vivo*. TRPM2 is Ca^{2+} permeable, and the binding sites for activating Ca^{2+} are near the cytosolic pore entrance. We therefore investigated how the presence of a physiological extracellular [Ca^{2+}] affects temperature dependence of TRPM2 gating between 37°C and 40°C, while bulk cytosolic [Ca^{2+}] was buffered to 100 nM and [ADPR] was set to 2 μM. Under such quasi-physiological conditions P_o was ~0.04 and ~0.34, respectively, at 37°C and 40°C. Because such a large ΔP_o cannot be achieved at any fixed concentration of agonists, these findings demonstrate and quantitate the positive feedback provided by Ca^{2+} influx. The larger P_o at 40°C elevates local [Ca^{2+}] around the activating sites, which in turn further enhances P_o. That positive feedback provides strong amplification to the TRPM2 temperature response (Q_{10}~1000), enabling the TRPM2 protein to autonomously respond to tiny temperature fluctuations around 37°C.

T-KA5-3 - RPS6KA5 (MSK1), IN PRIMARY SENSORY NEURONS, IS AN ESSENTIAL REGULATOR FOR THE DEVELOPMENT AND PERSISTENCE OF HYPERSENSITIVITY TO HEAT IN INFLAMMATION

István Nagy
Imperial College London, University of Decberecen

Tissue injuries are followed by an adaptive response that is characterised by inflammatory reactions and the development of hypersensitivities to mechanical and thermal stimuli. Normally, the tissue injury/inflammation-associated pain ceases when recovery nears completion however, it often persists beyond that. The development and particularly the persistence of hypersensitivities in tissue injury/inflammation depend on transcriptional changes in areas of the nervous system involved in nociceptive processing. However, the regulatory mechanisms, the genes which exhibit altered expression, and the resulting molecular networks are poorly understood at present. I am presenting evidence that the nuclear enzyme, ribosomal protein S6 kinase A5 (also known as mitogen-and stress-activated kinase 1, MSK1), through controlling transcriptional changes in primary sensory neurons, acts as a “master regulator” of transcriptional changes needed for the development of hypersensitivity to heat stimuli. I am also presenting evidence that *Trpv1* that has been shown being pivotal for the development and persistence of inflammatory hypersensitivity to heat stimuli constitutes one of the genes controlled by MSK1. Our findings open novel avenues in mapping, in primary sensory neurons, genes and regulatory mechanisms involved in the development and persistence of pain in tissue injuries.

T-KA5-4 - ANALGESIA VIA LIPID RAFTS - INHIBITION OF ACTIVATION OF TRANSIENT RECEPTOR POTENTIAL ION CHANNELS

Éva Szőke[1], Ádám Horváth[1], Maja Payrits[1], Andrea Nehr-Majoros[1], Gábor Berenkei[1], János Erostyák[2], Zsuzsanna Helyes[1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs; Hungary, [2]Department of Experimental Physics, University of Pécs, Pécs; Hungary

Transient Receptor Potential (TRP) cation channels as the Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) are playing important role in pain sensation, they are thermosensors. The „melastatin" TRP receptor TRPM8 an TRPM3 are also expressed in subgroups of primary sensory neurons. TRP channels are proven to be embedded in tspecific microdomains of the cell membrane, the lipid rafts, that are rich in cholesterol, sphingolipids, and gangliosides. Their integrity can be broken by methyl-β-cyclodextrin (MCD), sphingomyelinase (SMase) and myriocin (Myr) or with our own carboxamido-steroid compound (C1). We previously described that lipid raft disruptors inhibit the activation of TRPV1, TRPA1 *in vitro*, and we also demonstrated analgesic effect *in vivo* via TRPV1/TRPA1. We aimed to test the effect of lipid raft disruptors on the activation of the TRPM8 ion channel *in vitro* and *in vivo*, and their effects on membrane fluidity using fluorescence spectroscopic methods.

For the membrane fluidity studies, native CHO cells were treated with lipid raft disruptors, then they were incubated with 40 µM Laurdan and the decay curves of the Laurdan time-lapse emission spectrum between 410-540 nm were recorded. The microviscosity and the parameters characteristic of Laurdan and its environment were determined, from which we can deduce the membrane fluidity. The role of plasma membrane microdomains of lipid rafts was analysed on isolated trigeminal (TG) neurons by measuring agonists-induced Ca^{2+} transients with ratiometric technique. In *in vivo* experiments, mice were pretreated intraplantarly with MCD, SMase, Myr and C1 before the TRPM8 agonist icilin or the TRPM3 agonist pregnenolon-sulphate (PS)-CIM-0216 combination injection into the hindpaw of animals and we measured the duration of the pain reaction (raising, licking, chewing, shaking). It has been revealed that intracellular Ca^{2+} enhancement evoked by icilin (TRPM8) was inhibited after SMase, MCD, Myr and C1 incubation, but the response to PS (TRPM3) only decreased after C1 treatment. The duration of the icilin-induced acute pain reaction was significantly reduced by SMase, but the other compounds were not effective. The duration of the PS-CIM-0216-induced pain reaction was unaltered. MCD increased, while SMase and Myr decreased the membrane fluidity. We suggest that the hydrophobic interactions between the TRP channel and lipid raft interfaces modulate the opening properties of these channels and therfore, targeting this interaction might be a promising tool for drug developmental puposes.

Support: TKP2021-EGA-16, TKP2021-EGA-13, NKFIH-138936, PTE-ÁOK-KA-2021-09, KTIA_NAP_20017-1.2.1-NKP-2017-00002, ÚNKP-21-3-II New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund,

T-KA5-5 - THE Kv1.3 K^+ CHANNEL IS A THERAPEUTIC TARGET IN THE TREATMENT OF AUTOIMMUNE DISEASES

Gyorgy Panyi[1], Muhammad Umair Naseem[1]
[1]Department of Biophysics and Cell Biology, University of Debrecen

Voltage-gated potassium (K_V) ion channels play a key role in the proper physiological functions of both excitable and non-excitable cells. $K_V1.3$ K^+ channels are expressed in peripheral immune cells and are upregulated in effector memory T (T_{EM}) cells in autoimmune diseases. Antigen-dependent activation of T lymphocytes requires a well-orchestrated sequence of transmembrane signaling events. These include the generation of a Ca^{2+} signal and the maintenance of a negative membrane potential by K^+ channels in order to provide the necessary driving force for sustained Ca^{2+} entry required for proliferation and excessive release of cytokines. Several studies have validated that specific and persistent blockade of $K_V1.3$ suppresses the T_{EM} cell activation and proliferation. This dependence of T_{EM} cells on $K_V1.3$ channels for proliferation brings $K_V1.3$ blockers into the spotlight as a potential therapeutic immunosuppressant to treat a range of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, type 1 diabetes mellitus, psoriasis, and others. Moreover, recent studies have demonstrated that $K_V1.3$ channels are also expressed in microglia, brain-resident macrophages, and are essential for their proliferation.

Several high affinity and specificity Kv1.3 blockers are known; most of them have been engineered based on natural peptides isolated from scorpions and sea anemone. Among these, Vm24 (α-KTx 23.1) is a 36-residue Kv1.3-blocker peptide from the venom of the scorpion *Vaejovis mexicanus smithi*. Vm24 blocks Kv1.3 channel with high affinity (K*d* = 2.9 pM) and displays excellent, over ~1500-fold $K_V1.3$ selectivity against several ion channels. However, at high peptide concentrations (over 10 nM) it also blocks other ion channels including $K_V1.1$, $K_V1.2$ and KCa3.1. Based on the transcriptome analysis of the scorpion venom gland of *V. mexicanus* we have synthetically produced and recently characterized a novel peptide, sVmKTx and found that sVmKTx blocked Kv1.3 channels with high, albeit decreased affinity (Kd = 770 pM) as compared to Vm24. This change in the potency was associated with higher selectivity for Kv1.3 than Vm24 against a large panel of channels. In another recent study, we have characterized a novel peptide, Cm28, that has a unique and unusual primary structure and was shown to be a potent and selective pore blocker of human $K_V1.2$ and $K_V1.3$ channels. In addition, Cm28 did not inhibit a panel of ion channels including K_V, voltage-gated sodium (Na_V), and proton (H_V) channels.

The unique pharmacological properties of these novel peptides may provide novel drug templates for de- signing a highly selective $K_V1.3$ inhibitors that can be targeted to the central nervous system.

T-KB5-1 - COMPUTATIONAL DESIGN OF COVALENT INHIBITORS

György G. Ferenczy[1], Levente Mihalovits[1], György M. Keserű[1]
[1]Research Centre for Natural Sciences,Budapest

Targeted covalent inhibitors (TCIs) have gained increased attention owing to the recognition of distinct therapeutic advantages that include high potency, long residence time and potentially full target occupancy. However, compound reactivity also implies risk factors, including the generation of reactive metabolites, drug-induced toxicity and immunogenicity. Therefore, reactivity and specificity are critical attributes of TCIs and their design can be efficiently supported by computational approaches. Covalent inhibition is a two-step process that includes the formation of the noncovalent complex and the subsequent formation of the covalent bond. Both steps can be characterized by both experimental and computational techniques. Here we present the computational exploration of the binding mechanism of covalent inhibitors to two antibacterial targets, MurA and penicillin binding protein 1b (PBP1b). It was shown that the activation of the catalytic cysteine in MurA is performed by a histidine residue conserved across species. The activation barrier for the reaction between the catalytic cysteine and several fragment-sized covalent compounds was calculated, and it was found that barrier heights tend to separate active from inactive compounds. The catalytic serine of PBP1b is activated by a nearby lysine residue and this allows the binding of the serine to boronic acid inhibitors. Calculation of the activation and the reaction free energies of the reversible covalent inhibition allows the rational design of inhibitors.

T-KB5-2 - NEW SCAFFOLDS FOR TARGETING HIV-1 PROVIRUS REACTIVATION AND SILENCING

Weber Jan
Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Despite the significant success of combination antiretroviral therapy resulting in better clinical outcome and quality of life of HIV-positive patients, the HIV infection persist due to the latent HIV-1 proviruses in many cellular reservoirs. Two main strategies to eliminate or silence these latent HIV-1 reservoirs are extensively explored. First strategy termed “shock and kill” aims to reverse latency and with the help of therapeutic or immune-based approaches clear HIV and achieve complete HIV-1 cure. Second strategy termed “block and lock” aims to achieve a functional HIV-1 cure by inhibiting transcription of HIV-1 proviruses and reaching deep latency state. Here, we present two new compound scaffolds towards both strategies.

The Jurkat cell line J-Lat 10.6, a latency model harboring pseudotyped HIV provirus with GFP under the control of HIV-1 LTR, was used to screen over 5,000 compounds from our in-house library. The subsequent confirmation experiments in Jurkat 2C4 and ACH-2 latency models showed that a oxalamide-based compound was able to reactivate HIV-1 in both models. The initial structure-activity relationships and mechanistic studies indicate that compound reactivates HIV-1 through the activation of the canonical NF-kB pathway via PKC activation. Our effort in opposite strategy lead to identification of helquat-based compounds that inhibit HIV-1 replication at the stage of reverse transcription and provirus expression. Taq polymerase stop and FRET melting assays selected a subset of compounds that demonstrated their ability to stabilize G-quadruplexes in the HIV-1 LTR sequence. In addition, docking and molecular dynamics calculations indicate that the structure of the helquat core greatly affects the binding mode to the individual G-quadruplexes.

Our new compound scaffolds can provide useful tool for determining regulation of HIV-1 transcription, thus improving our understanding of HIV-1 latency and reactivation-

T-KB5-3 - SYNTHESIS OF ANTIBACTERIAL AND ANTIVIRAL GLYCOPEPTIDE ANTIBIOTIC DERIVATIVES

Bereczki Ilona[1,2,3], Lőrincz Eszter Boglárka [1,4], Herczegh Pál [1] and Borbás Anikó [1,2]
[1]Department of Pharmaceutical Chemistry, University of Debrecen, Hungary, ,[2]National Laboratory of Virology, Szentágothai Research Centre, Pécs, Hungary, ,[3]ELKH-DE Pharmamodul Research Team, Debrecen, Hungary, ,[4]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Hungary

There are several dangerous viruses on Earth that can cause pandemics as SARS-CoV-2 do. Unfortunately, there are only a few antiviral drugs available against viral infections and to save patients' lives, hence it is an important task for researchers to find and develop new and more effective antiviral drugs. In addition, antibiotic resistance is a huge healthcare problem, millions of people will die worldwide from infections caused by resistant bacteria in the near future. Teicoplanin is a clinically used glycopeptide antibiotic against resistant Gram positive bacteria and it is emerged as a potential antiviral as well.[1] Based on literature and our experience, its efficacy against bacteria and viruses can be improved with different modifications.[2,3] Lipophilic side chains can increase antiviral activity, therefore we planned to introduce apocarotenoids as nontoxic hydrophobic substances (used as a natural food coloring) as side chains to prepare possible antiviral and nontoxic teicoplanin derivatives. Moreover, we introduced perfluorinated side chains as well, because highly fluorinated alkyl chains are hydrophobic and non-membrane active substances, therefore low cytotoxicity is expected. In this way we prepared several glycopeptide antibiotic derivates with good and excellent anti-SARS-CoV-2 and anti-influenza activity with high therapeutic index. On the other hand, guanidine group(s) can improve antibacterial activity against resistant bacteria[3], therefore guanidine derivatives were also synthesized.

Acknowledgements: This research was funded by the National Research, Development and Innovation Office of Hungary FK 142315 and supported by the ELKH-DE Pharmamodul Research Team.

[1] Zhou, N. et al., Glycopeptide antibiotics potently inhibiti cathepsin L in the late endosome/lysosome and block the entry of Ebola virus, Middle East respiratory syndrome coronavirus (MERS-CoV), and severe acute respiratory syndrome coronavirus (SARS-CoV). *J. Biol. Chem.* 2016, 291, 9218-9232.

[2] Szűcs, Z., et al., Structure-activity relationship studies of lipophilic teicoplanin pseudoaglycon derivatives as new anti-influenza virus agents. *Eur. J. Med. Chem.* 2018, 157, 1017-1030.

[3] Szűcs, Z., et al., *N*-Terminal guanidine derivatives of teicoplanin antibiotics strongly active against glycopeptide resistant Enterococcus faecium. *J Antibiot.* 2020, 73, 603-614.

T-KB5-4 - IN VITRO ANTIVIRAL STUDIES AT THE NATIONAL LABORATORY OF VIROLOGY

Papp Henrietta[1], Kuczmog Anett[2], Kopasz Zoltán László[1], Leiner Krisztina Rebeka[1], Madai Mónika[1], Jakab Ferenc[1,2]
[1]National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Pécs, [2]Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, [3]Doctoral School of Biology and Sportbiology, University of Pécs, Pécs

Antiviral research has gained renewed attention in recent years due to the COVID-19 pandemic. Despite recent advances, there is still a critical need for new antiviral agents to combat emerging and re-emerging viruses. Our laboratory is committed to advancing antiviral research and developing effective treatments against different viral diseases. To assess the in vitro antiviral activity of different compounds, we are using either droplet-digital PCR or a colorimetric assay (MTT) to determine IC50 values. Our laboratory has conducted numerous studies to find potent antivirals against SARS-CoV-2, including drug repositioning screens, validation of in silico studies, and antiviral tests of newly synthesized synthetic, semi-synthetic, and natural compounds suggested by pharmacologists or chemists. In addition to SARS-CoV-2 (B.1.1.5.), our ongoing studies also involve Zika and Chikungunya viruses. Through our diverse antiviral screens, we have identified azelastine hydrochloride and methylene blue as potent antivirals against SARS-CoV-2. Furthermore, fragment hits found by a novel in silico screen blocked the replication of SARS-CoV-2 in vitro. Newly synthesized antibiotic derivatives also showed antiviral activity against both SARS-CoV-2 and the Zika virus (MR766 strain). These findings highlight the potential of these compounds as promising antiviral agents for further development. We believe that cooperation among diverse fields is key to successful drug development campaigns, which is why we welcome collaborations with researchers from different disciplines to help expedite the development of new antiviral agents.

T-KB5-5 - VANCOMYCIN ANTIBIOTICS AND ANTIFUNGAL PROTEINS AS ANTIVIRAL AGENTS ? THE USE OF NMR SPECTROSCOPY AND ARTIFICIAL INTELLIGENCE.

Gyula Batta[1], András Czajlik[1], Gai Jiawei[1], Réka Erdei[1], László Izsépi[1], Pál Herczegh[2] and Ilona Bakai-Bereczki[2]
[1]Institute of Chemistry, UD, Debrecen, [2]Institute of Pharmaceutical Chemistry, UD, Debrecen

The COVID-19 pandemic and SARS-CoV-2 caused more than 5 million victims worldwide from the 2019 outbreak. New influenza viruses also mean global threat, that may cause 1 billion/year infection. Therefore, drug repurposing is an urgent research area. FDA has recently approved new glycopeptides efficient against various microbes. Some of our novel glycopeptide derivatives show promising antiviral and/or antibacterial activities [1,2]. We have recently applied the STD-NMR method proving the binding site of SARS-COV-2 spike protein to anti-cancer drug Rucaparib [3]. We continue intensive NMR and in-silico structure, dynamics, function studies of our antifungal disulfide proteins [4]. Some of them showed anti-corona virus activities [5] and their in-vitro and biological tests are in progress. Now, we started application of AI methods, e.g. AF2 [6] to speed up protein structure determination in line with experimental NMR data.

References:

1. Bereczki, I. et al. Semisynthetic teicoplanin derivatives with dual antimicrobial activity against SARS-CoV-2 and multiresistant bacteria. (2022). Sci Rep 12, 16001 https://doi.org/10.1038/s41598-022-20182-y
2. Izsépi, L. et al. (2021) Bacterial Cell Wall Analogue Peptides Control the Oligomeric States and Activity of the Glycopeptide Antibiotic Eremomycin: Solution NMR and Antimicrobial Studies, Pharmaceuticals (Basel, Switzerland) 14. https://doi.org/10.3390/ph14020083
3. Papp, H. et al. Rucaparib blocks SARS-CoV-2 virus binding to cells and interleukin-6 release in a model of COVID-19, medRxiv, 2022.06. 30.22277079
4. Batta, G. et al. (2009) Functional aspects of the solution structure and dynamics of PAF - a highly-stable antifungal protein from Penicillium chrysogenum, Febs Journal 276, 2875-2890. https://doi.org/10.1111/j.1742-4658.2009.07011.x
5. Huber, A. et al. (2020) Two small, cysteine-rich and cationic antifungal proteins from Penicillium chrysogenum: A comparative study of PAF and PAFB, Biochimica Et Biophysica Acta-Biomembranes 1862. https://doi.org/10.1016/j.bbamem.2020.183246
6. Jumper, J. et al. (2021) Highly accurate protein structure prediction with AlphaFold Nature 596, 7873, 583, https://doi.org/10.1038/s41586-021-03819-2

T-KB5-6 - TOPOGRAPHICAL, MECHANICAL AND MASS CHARACTERIZATION OF BIOLOGIAL SAMPLES WITH AFM

James Berwick
Collaboration between: EPFL, ETHZ, ZHAW, Lund University and Nanosurf AG

One of the tools for characterization of biological samples at the molecular level is the atomic force microscope (AFM). The AFM can work on biological samples in close to physiological conditions, i.e. in buffer or growth medium, at 37°C and in CO_2 and humidity-controlled environment. Due to the fragile nature of the samples, many operations are performed in dynamic mode, in which the cantilever is oscillated and only touches the software at the lower part of the oscillation. With photothermal excitation, the cantilever can be directly excited, preventing the so-called forest of peaks that is obtained by conventional, indirect excitation. Photothermal excitation is compatible with high resolution imaging of double stranded DNA, without being photo-toxic, of which the helical structure is observable.

The mechanism of virus infection is largely unexplored[1]. AFM was used to image Herpes simplex virus type 1 capsids immobilized on isolated and reconstituted cell nuclei. This required a gentle interaction force of the AFM tip with the sample. This has been obtained by oscillating the cantilever under its resonance frequency using a new, modified implementation of photothermal off-resonance tapping (PORT)[2]. Individual capsomeres on the virus capsids can be clearly resolved. The contact phase of the cantilever with the sample contains information on the mechanical properties like tip-sample adhesion or elasticity. To get a more complete idea of the mechanical properties, a frequency sweep of the cantilever can be used to access the viscoelastic properties[3]. The cleanness of the photothermal excitation gives access to a wide frequency range from a few Hertz to tens of kilohertz, a range believed to be relevant at the nanometer and micrometer length scale. First experiments show a difference between protein perturbed and unperturbed HeLa cells adhered to a glass substrate. The same cleanness of the photothermal excitation also provides an unambiguous phase transition over the resonance frequency of the cantilever. The resonance frequency is reduced by an attached cell, thus providing information on the cell mass. This cell mass variation has been used to study the influence of virus infection on cell growth, but also the effect of aquaporin blocking with some tens of milliseconds time resolution[4] or the mass of budding yeast cells[5].

In summary, photothermal excitation is a versatile option for an AFM improving its performance for biological applications and expanding the amount of information that can be obtained on biological systems.

1: Evilevitch, A., & Tsimtsirakis, E. (2022) QRB Discovery 3: e2, 1–8

2: Nievergelt *et al.* (2018) Nature Nanotechnology 13: 696–701

3: Fläschner *et al.* (2021) Nature Communications 12: 2922

4: Martinez *et al.* (2017) Nature 550: 500–505

5: Cuny *et al.* (2022) Nature Communications 13: 3483

T-KA6-1 - CORE CONCEPTS BASED EDUCATION OF PHARMACOLOGY

Pál Riba[1], Zoltán Varga[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

In the past few decades, there have been essential changes in medical education across the European Union. Many universities have moved from horizontal, subject-based training to problem-oriented education. This shift has fundamentally changed the place of traditional subjects in the curricula. In Hungary we continue to teach in a horizontal way, in which pharmacology plays a prominent role. The question arises as to what can ensure that pharmacology retains its relevance in a rapidly changing educational environment. Pharmacological education needs both a research-based consensus on the basic concepts that all graduates should know and understand, and a valid and reliable means of assessing the mastery of these concepts. To achieve these, it is first necessary to clarify which core concepts are essential for medical work and are not taught in other disciplines. Our working group of educational pharmacologists has created a list of concepts, defining core concepts within each concept group. Examples of such concept groups are pharmacodynamics (a set of core concepts describing the effects of a drug on the body), pharmacokinetics (concepts describing the effects of the body on a drug) or types of drug interactions. For each of the basic concepts defined, a short explanatory description has been prepared, and in addition to the more general pharmacological questions, some of these basic concepts will be asked during the exams. These core concepts can both provide a resource to assist in the development of new curricula and the evaluation of existing ones, and facilitate further collaboration within the pharmacology educators to improve teaching material, assessment of knowledge and learning methods.

T-KA6-2 - INTRODUCING FLIPPED CLASSROOM APPROACH IN PHARMACOLOGY FOR MEDICAL STUDENTS – A PILOT FROM SEMMELWEIS UNIVERSITY

Zsófia Onódi[1], Nabil V. Sayour[1], Anikó Görbe[1], Pál Riba[1], Zoltán V. Varga[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Background: Flipped classroom (FC) approach has been shown to improve engagement and student activity. Still, there have been fewer studies reporting on how this approach is received in the undergraduate medical education classroom for pharmacology. We aimed to investigate the effects of FC method on the students' performance as well as teachers' attitude.

Method: One hundred students studying general medicine and 10 teachers participated in this early pilot study as volunteers. Conventional/frontal teaching and FC approach were chosen based on the teachers' preference. Two pharmacology topics were selected for the pilot: positive inotropes and cytotoxic chemotherapeutic agents. Students' performance was estimated by single-choice tests immediately after FC or conventional practice and one week later. Students' and teachers' perceptions were assessed by questionnaires with scales and open-ended questions.

Results: FC approach boosted the active learning and engagement among medical students tendentiously according to the test results (P=0.1 vs conventional; n=38-48). Test results evaluated according to group subclasses showed moderate variability even within conventional and FC models, indicating that other factors than teaching approach can influence significantly the students' performance. Based on the questionnaires, students and teachers had positive perceptions on FC model in general, particularly by facilitating activity, student-teacher and student-student interactions and by boosting self-confidence among students and teachers. However, concerns were raised including time-consuming and variable degree of preparation, as well as lack of time for explaining more complicated topics in details.

Conclusions: FC model can be beneficial in pharmacology course as generally received positive reception and improved engagement. Flipping well-selected topics to more interactive practices could further improve learning and teaching experience in the medical education.

T-KA6-3 - EDUCATIONAL DEVELOPMENT PROGRAMS AT SEMMELWEIS UNIVERSITY PAST-PRESENT-FUTURE

Levente Kiss[1,2]
[1]Department of Physiology, [2]Center of Educational Development, Methodology and Organization

The main objective of the Center of Educational Development, Methodology and Organization – which was established in 2019 - is to improve education through supporting teachers at Semmelweis University. The tasks of the Center include the following:

- organizing trainings for teachers in educational methodology and e-learning
- managing Moodle (e-learning platform) and Semmelweis University Central Authentication (SeKA)
- methodological assistance for departments and clinics
- coordinating and supporting the development of e-learning material
- collecting and publishing the curricula and exam requirements of different subjects.

The lecture aims to summarize the results and take-home messages of the past 4 years, the current developments and the possible future directions.

T-KA6-5 - CHALLENGES IN TEACHING PHYSIOLOGY: PROS AND CONS OF FRONTAL LECTURES AND THE OPPORTUNITIES OF BLENDED LEARNING

Rita Gálosi
Physiology Institute, Medical School of University Pécs, Hungary

The purpose of teaching physiology or any subject is to understand its concepts, to gain knowledge and apply it in a clinical context. We expect the education of professionals who possess a critical mindset and are capable of situation analysis and problem-solving. The training relies heavily on frontal classroom lectures, which is a traditional form of university education. However, the effectiveness of traditional lectures is decreasing within the current educational framework.

In the lecture, as a conversation starter, we will discuss factors significantly affecting the effectiveness of lectures:

1. The question of student motivation: Have statistics shown a change in the proportion of applicants to medical universities compared to the total number of university applicants? How has the position of medical universities changed in the admission ranking?
2. The amount of material to be processed and the cognitive load capacity of the students: Do we need to, and can we, reduce the information load?
3. What depends on the teaching method? Interactive teaching methods, project-based learning, collaborative learning, and personalized learning can be more effective than traditional, frontal teaching methods. International examples from the QS World Ranking top 1-100 medical universities.
4. Processing the material: Is the explicit, structured teaching method or constructive problem-based education more effective?
5. Assessment: To what extent does participation in lectures contribute to exam results? Evaluation of student opinions.

In summary: frontal teaching is currently necessary and fundamental for the structured, guided transfer of knowledge and concepts. We need to identify the factors affecting its effectiveness in order to find its place and form in the current educational environment.

T-KA6-6 - TEACHING PHARMACOLOGY ON INSTAGRAM - TWO YEARS OF EXPERIENCE AT SEMMELWEIS UNIVERSITY

Karádi Dávid Árpád[1], Varga Zoltán[1]
[1]Semmelweis University Department of Pharmacology and Pharmacotherapy, Budapest, Hungary

Recently, in medical education at Semmelweis University, the subject of pharmacology and pharmacotherapy has been split into two separate subjects: "Pharmacology" consisting of two semesters in the theoretical module and "Clinical Pharmacology", a one semester course in the clinical module. Because of digitalisation in recent years, online educational practices and learning methods have emerged which requires a pedagogical shift towards the online space. This process was also accelerated by the COVID-19 pandemic. To reach students with a mainly theoretical background and to facilitate their transition to clinical modules, our institute tries to achieve certain educational goals through social media channels in addition to traditional teaching methods.

Our Instagram® page (@semmelweispharma) was launched in the first semester of the 2021/2022 academic year and content is published almost continuously throughout the teaching periods. The data shown here was generated by the built-in analysis of Instagram and further analysed using GraphPad Prism 9. We have also launched a survey to gain user feedback, information about the personal characteristics of our followers and their online content consumption and learning habits.

Since launch, we have published 117 posts or stories in total with 105 – 2158 accounts reached per content. As of 28 March 2022, we have 1102 followers. Our posts share a short piece of information about pharmacology with our followers or give an insight into the work and thoughts of teachers or researchers associated with the institute. Moreover, with the "story" feature on Instagram, we share short pharmacology quizzes or case studies and related questions and explanations. The latter are made permanently available via the "highlights" feature. Our survey's results showed a generally positive response especially regarding gaining new information that followers have not previously encountered in other settings. Case reports were the most demanded type of content, followed by fun facts and pharmacology quizzes. Respondents cited YouTube, online subscription sites and textbooks as the most useful learning platforms.

Launched in 2021 to complement classroom pharmacology teaching at Semmelweis University, the online pedagogical content built on the Instagram® platform has gained a regular following of around two cohorts and received a significantly positive response in our online survey. Based on the positive test period, we plan to introduce the Instagram page in our English language education too.

T-KB6-1 - REGULATORY ROLES OF RNA BINDING IN HISTONE METHYLTRANSFERASES

Ágnes Tantos
Research Centre for Natural Sciences, Budapest

Histone lysine methyltransferases (HKMTs) perform vital roles in cellular life by controlling gene expression programs through the posttranslational modification of histone tails. Since many of them are intimately involved in the development of different diseases, including several cancers, understanding the molecular mechanisms that control their target recognition and activity is vital for the treatment and prevention of such conditions.

Several HKMTs have been shown to be capable of RNA binding in the absence of a canonical RNA binding domain, indicating that RNA interaction may have a conserved role in the regulation of histone methylation. Nevertheless, the generality and the details of these mechanisms remain largely uncharted indicating the need for directed studies.

Our main focus was on the RNA binding of KMT2D (MLL4) and KMT2F (SETD1A), two members of the KMT2 family that catalyze the methylation of the H3K4 residue.

We identified a broad range of coding and non-coding RNAs associated with both proteins and confirmed their binding through RNA immunoprecipitation and quantitative PCR.

While KMT2F contains a canonical RNA binding domain, KMT2D appears to interact with RNAs through non-canonical RNA recognition elements. In addition to the confirmation of the in cell RNA-binding, we could show *in vitro* RNA interaction for the RNA interacting elements in both proteins.

Analysis of the bound mRNAs revealed that KMT2D and KMT2F interact with a largely non-overlapping set of RNAs within the nucleus, indicating different regulatory roles for the RNA binding in these proteins.

T-KB6-2 - MOLECULAR INVESTIGATION OF CHROMOSOMAL R-LOOPS IN NEURODEGENERATIVE DISEASE

Lóránt Székvölgyi
Lendület Genome Architecture and Recombination Research Group, Faculty of Pharmacy, University of Debrecen

Chromosomal R-loops are formed when a single-stranded DNA (ssDNA) is displaced from its complementary DNA strand by an RNA molecule, resulting in a hybrid structure composed of RNA and DNA. While R-loops are essential for various cellular processes, their dysregulation may contribute to neurodegenerative disorders such as Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD). ALS primarily affects motor neurons leading to muscle weakness and atrophy, while FTD affects the frontal and temporal lobes of the brain, leading to cognitive and behavioral impairment.

To understand the role of R-loop structures in the above pathologies, we used induced pluripotent stem cells isolated from ALS and FTD patients to map the genomic distribution of R-loops. The genome-wide R-loop maps were associated with coding- and non-coding transcriptomes of ALS/FTD samples. Our data to suggest that perturbation of R-loop homeostasis in ALS/FTD leads to misregulation of mRNA and ncRNA transcription, which is likely to contribute to ALS/FTD pathogenesis. These relationships pave the way for the development of R-loop targeting therapeutics to treat neurodegenerative disorders.

T-KB6-3 - COUPLING EPIGENETIC AND CLINICAL DATA OF ADENOCARCINOMA SAMPLES THROUGH A WEB APPLICATION

Müller Dalma[1], Győrffy Balázs[1,2]
[1]Semmelweis University, Department of Bioinformatics, Budapest, Hungary, [2]Research Centre for Natural Sciences Oncology Biomarker Research Group, Budapest, Hungary

Alternations in the methylation pattern during tumorigenesis have been recognized decades ago and the identification of informative methylation patterns continues to be the focus of multiple cancer studies. Given the platform's suitability for screening large cohorts, Illumina Infinium Arrays have been the most popular among methods to identify genom-wide methylation profiles at a CpG-site resolution level. Our goal was to assemble an integrated database containing all available methylation and clinical data for publicly available samples and to analyze methylation changes contributing to altered gene expression in colorectal cancer. In order to enable comfortable examination of the database we also aim to create a web-based interactive platform.

Studies with publicly available raw intensity data files were systematically screened using the GEO Platform browser. After filtering by dataset origin, methylation data from colorectal cancer studies were acquired along with clinical and demographical data. In order to identify differentially methylated regions, gene level analysis was performed. Pearson correlation between methylation and gene expression in colorectal cancer and normal tissues was computed using Illumina HumanMethylation450K and RNA-seq data from the GDC (Genomic Data Commons) database. For web application development we used the shiny R package.

As a result, a database containing 2295 adenocarcinoma, adenoma and normal tissue data was established. When tumor and normal tissues were compared, promoter and first exon regions exhibited the highest fold change. Among the top 20 genes based on AUC value, there were multiple genes previously associated with malignancies such as *POFUT1, DMBT1* and *TDG* in the TSS1500*, PSMD11, LYPD5* and *MIR16* in the TSS200*, CPNE5, SLC9A1* and *WEE1* in the 5'UTR, *NAT8L, MERTK* and *A1BG* in the first exon, *SOD3, C11orf52* and *RAPH1* in the body region and *QPCTL, MEIS2* and *CISH* in the 3'UTR region. Among the genes with differentially methylated regions, the ones with strong negative correlation between methylation and expression ($r < -0.7$) were overrepresented in the case of promoter and first exon regions.

We assembled a sizeable database containing colorectal samples with genome-wide methylation data and established a pipeline for data processing. The presented database and web platform will be a useful starting point for biomarker discovery.

T-KB6-4 - REGULATING THE REGULATORS: MATURATION AND STABILITY OF MICRORNAS

Ábel Fóthi[1], Tamás I. Orbán[1]
[1]Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary

MicroRNAs (miRNAs) are endogenously encoded short (20-24 nt) single stranded RNA molecules which, after being processed from long precursor transcripts, form ribonucleoprotein complexes with Argonaute proteins. These RNA-induced silencing complexes find their messenger RNA (mRNA) targets by sequence complementarity and they either inhibit translation or initiate the decay of the targets. One miRNA may regulate several mRNAs and vice versa, most mRNAs contain several miRNA binding sites, thereby forming a posttranscriptional regulatory network, the complexity of which is comparable to that of transcription factors. miRNAs themselves are also regulated at various levels, and these subtle control mechanisms all contribute to the fine-tuning of gene expression patterns in a particular cell type. Among these processes, recently we were focusing on the transcriptional control of miRNA clusters, as well as on the elegant regulation miRNA stability via the target-mediated miRNA decay (TDMD) mechanism.

When investigating a long miRNA cluster on the human chromosome 19 (C19MC), we could show that tissue-specific promoter activities regulate the expression of the cluster, and there is a gradual decrease in the mature miRNA levels toward the 3' end of the cluster in embryonic stem cells but not in placenta. We could also provide evidence that the local concentration gradient of the Drosha/DGCR8 miRNA processing complex in stem cells is behind this phenomenon. In contrast to that, we could show that in placenta cells, an alternative promoter with a potential "super enhancer" region can efficiently recruit this processing complex, thereby providing a constant distribution and the efficient processing of the precursors throughout the entire miRNA cluster. We are currently investigating whether similar mechanisms could also be involved in the regulation of other, highly expressed tissue specific miRNA clusters.

In addition to the maturation processes, the steady-state levels of miRNAs are also influenced by various posttranscriptional regulatory mechanisms, influencing the stability of these small RNA species. Among them, TDMD was discovered in viruses, where the invasive genetic sequence specifically targets endogenous miRNA species for elimination. We are currently investigating whether SARS-CoV-2 could in fact initiate the decay of cellular miRNAs and contribute to the severe symptoms of the COVID disease. We developed a new algorithm to predict strong TDMD-inducing viral sequences and we are testing the predicted interactions in cell-based assays. If proven to operate, such TDMD-based mechanisms may also provide plausible explanations for the development of the so-called post-covid syndromes. Recent data from our laboratory on this issue will be shown and discussed during the oral presentation of the conference.

miRNA research in our laboratory is supported by the PC-II-12/2022 grant from the Hungarian Academy of Sciences.

T-KB6-5 - EXPLORATION OF THE EFFECT OF HEAT TREATMENT ON EPIGENETIC ALTERATION IN CHICKEN PRIMORDIAL GERM CELLS

Elen, Gócza [1], Nikolett, Tokodyné Szabadi [1]; Roland, Tóth [1]; Maria Teresa Salinas Aponte[1], Bence, Lázár [1,2]; Eszter, Várkonyi [2]; Krisztina, Liptói [2]
[1]Agribiotechnology and Precision Breeding for Food Security National Laboratory, Hungarian University of Agriculture and Life Sciences, Institute of Genetics and Biotechnology, Department of Animal Biotechnology, Gödöllő, Hungary, [2]National Centre for Biodiversity and Gene Conservation, Gödöllő, Hungary

In avian biotechnology, it was a long-awaited goal to establish cell lines originating from the chicken embryo as tools for gene preservation. Meanwhile, primordial germ cells (PGCs) are good in vitro model systems for studying many molecular processes.
Our project aims to investigate RNA and microRNA expression profiles at the molecular level behind the responses to heat stress. We could detect epigenetic consequences of heat stress in PGCs. These changes were passed on to the subsequent generations.

Fertilized eggs from the Transylvanian Naked Neck Chickens were collected from National Centre for Biodiversity and Gene Conservation and then incubated in an incubator at 37.5°C at 60% humidity. These fertilized eggs were collected from hens of three differently treated groups. The control group (C) grew up under normal conditions without heat treatment and stress exposure. The second group (HTHS) was subjected to heat treatment (38.5°C) at the age of 2 days for 12 hours, followed by heat stress (30°C) beginning at the age of 23 weeks, continuing about 12 weeks long. The third group (HS) was heat-stressed (30°C), starting at 23 weeks and continuing at about 12 weeks. The roosters used for mating were from the same groups. We isolated 26 PGC lines from embryos developed in the eggs of control, heat-treated and non-treated hens. We isolated RNA and DNA from the collected PGC lines and performed RNA and WGB sequencing. Expression levels of stem and germ cell-specific genes and heat shock-related factors were compared.

Based on our results, it can be assumed that as the effect of heat treatment and heat stress, epigenetic changes can be detected in the primordial germ cells, which can be passed on to the next generations.

Keywords: heat-treatment, primordial germ cells, epigenetic modification, WGS, miRNA

Acknowledgement: RRF-2.3.1-21-2022-00007; H2020-RUR-2020-1/CSA/101000728 NETPOULSAFE; 2019-2.1.11-TÉT-2019-00036 and ÚNKP-22-3-II.

T-G6-1 - THE ROLE OF THE COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP) IN THE CENTRALISED AUTHORISATION OF MEDICINES

Róbert Pórszász[1,2]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of General Medicine, University of Debrecen, Debrecen, Hungary, [2]National Institute of Pharmacy and Nutrition, Budapest, Hungary

The CHMP is one of the important committee of the European Medicines Agency of the European Union with members and alternates from 27 Member States. The Committee's remit includes proposing to the European Commission the adoption of marketing authorisations for medicinal products for human use. In a centralised procedure, the CHMP is responsible for:

- Carrying out the initial assessment of EU-wide marketing authorisation applications;
- Evaluation of variations or extensions to existing marketing authorisations ('variations');
- Considering the recommendations of the Agency's Pharmacovigilance Risk Assessment Committee (PRAC) on the safety of medicinal products on the market and, where necessary, making recommendations to the European Commission on the variation, suspension or withdrawal of marketing authorisations;
- The CHMP also evaluates nationally authorised medicines that are referred to the EMA to develop a harmonised position in the EU (Referral procedures).

It takes 210 "active" days to process a new medicine application. This active evaluation period is interrupted by at least one "clock stop" during which the applicant prepares and submits a written response to the CHMP's questions. The clock stop occurs after the 120th day, but may also occur after the 180th day, when the CHMP has accepted a list of questions or unresolved issues that the company must answer. The discussion in this regard is included in Agenda Sections 3.2 (List of Outstanding Issues (LoOI) for Day 180) and 3.3 (List of Questions (LoQ) for Day 120).

A bidding list is issued each month for the undertaking of evaluations, which is tendered by Member State authorities in their capacity as Rapporteur or Co-Rapporteur. In many cases, however, multinational teams (MNATs) are formed to evaluate submissions depending on the human resources (assessors) available in each country for the product concerned. The structure of the submissions from the part of pharmaceutical companies is required to follow the format of the electronic Common Technical Document (eCTD), which is checked by EMA's permanent staff during the validation process.

Once the marketing authorisation has been granted, the functions of the (Co-)Rapporteur are not terminated, but remain alive throughout the life cycle of the medicinal product and may be re-assigned for any pharmacovigilance issues (life cycle management).

In this presentation, I will describe the process described above and provide a number of examples of the development and regulatory authorisation of new innovative medicines in the hope of stimulating interest in the work of the assessors among the audience.

T-G6-2 - THE ROLE, TASKS AND ACHIEVEMENTS OF THE HUNGARIAN NATIONAL HEALTH RESEARCH AGENCY IN CLINICAL TRIALS IN HUNGARY

Dr. Judit Tarnai
Managing Director
Hungarian National Health Research Agency

The title of the presentation:

The Hungarian National Health Research Agency (HNHRA) was established by the competent Ministry at the end of 2021.It has a unique position to facilitate excellent cooperation among all actors of the health research sector, such as authorities, health care institutions, universities and pharmaceutical companies.

High-quality health research is increasingly carried out worldwide through expert agencies-like HNHRA -that are in close connection with governments.

HNHRA provides research centers and clinical trial sites with continuous support in their scientific and research activities, and is responsible for supporting the effective cooperation among key figures of this area.

The Hungarian National Health Research Agency shares its research, and pharmaceutical expertise in international cooperation, conveying the knowledge in bilateral and multilateral collaborations.

The presentation explores_the mission, tasks and goals of the HNHRA, which is to support the development of clinical trial infrastructure in Hungary, and to advocate its capabilities to attract a higher number of clinical trials into the country.

T-G6-3 - THE STATUS OF INVESTIGATOR INITIATED TRIALS IN HUNGARY

Kata Mazalin
Novartis Hungária Kft

Clinical research is conducted in Europe mostly under the sponsorship of the pharmaceutical industry (EU average 83 %/all CTs)[1] and with that representing only potential business interests of the affected individual company. This setup does not support the conduction of head-to-head comparison trials from competitor companies, the research of new indications or enhanced use of already generic compounds or underserved diseases like malaria. Further, there is a big regional difference (as usual) between Wester-European countries and our region in both the willingness, opportunities and the number of approved academic trials.

Additionally, the recent change of the centralized European approval procedure does also sets new challenges to the academic community which is especially seen in the pediatric network trials.[2]

Independent academic research provides an unquestionably value for all parties, e.g patients, academic insitutions, pharmaceutical companies and regulatory/governmental bodies and a united approach is needed in Hungary to create a nutruring environment and the necessary expertise.

1 C. Madeira, et al, Investigator-initiated clinical trials conducted by the Portuguese Clinical Research Infrastructure Network (PtCRIN), Contemporary Clinical Trials Communications

2 Fuhrmann et al, First experiences with the implementation of EU Regulation 536/2014 (CTR) from the perspective of non-commercial academic research

T-G6-4 - THE SEMMELWEIS LIPID CENTER FOR HIGH–RISK PATIENTS (SLICK) TRIAL

Alex Ali Sayour[1], Loretta Kiss[1], Zsolt Bagyura[1], Béla Merkely[1]
[1]Heart and Vascular Center, Semmelweis University, Budapest

Atherosclerotic cardiovascular disease (ASCVD) is prevalent in Hungary, yet low density lipoprotein (LDL) cholesterol levels are indaquetly controlled in patients with very high–risk cardiovascular risk.

The Semmelweis Lipid Center for High–RisK Patients (SLICK) is an investigator initiated, randomized, open label, assessor–blind pragmatic trial focusing on complex and timely interventions to reduce LDL cholesterol levels in patients with very high cardiovascular risk.

An overall of 200 patients with very high cardiovascular risk will be enrolled. Patients are eligible if there is evidence of non–obstructive coronary artery disease on coronary computed tomography angiography (CCTA), or had previously suffered a myocardial infarction with residual stenosis on a non–culprit coronary artery, or had previous percutaneous coronary artery intervention (PCI) on at least two coronary arteries. Patients will be randomized in a 3:2 fashion into the lipid center (60%) or the usual care (40%) arms. Patients randomized to the lipid center arm will receive a complex LDL cholesterol–focused intervention at all visits including consultation with dietician (with personalized dietary instructions), psychologist (with personalized psychological intervention), and physician (with tailored state–of–the–art medication schemes aimed at LDL cholesterol lowering). Patients in the usual care arm are referred to their caretaker. All patients will have a baseline carotid and femoral artery ultrasound to assess plaque volume and composition, and laboratory evaluation. During the follow–up, laboratory tests will be performed. At the end of protocol (~21 months), carotid and femoral artery ultrasound scans will be repeated, whereas in the CCTA subgroup, CCTA will also be repeated. The primary outcome is absolute change in LDL cholesterol level from baseline and percentage of patients meeting the LDL cholesterol target in the usual care versus lipid center arm. Exploratory outcomes include plaque volume change.

SLICK will introduce a new layer of patient care at the biggest tertiary cardiovascular center of Hungary to focus on ASCVD risk reduction through a complex LDL cholesterol–lowering team effort. Ultimately, this scalable program aims to tackle soaring cardiovascular death among Hungarian patients.

FRIDAY
9 JUNE 2023

F-KA-1 - "RATIONAL DRUG DESIGN WITH STATE-OF-THE-ART CRYO-EM"

Evgeniya Tsedik
Thermo Fisher Scientific, Eindhoven, the Netherlands

Compared to the age of Earth, the presence of human life on it represents the last 4 seconds of a day. The same probably holds true for rational drug design compared to the immemorial quest of humans for medicines.

Recently, the most spectacular advances in rational drug design have come from the side of single particle cryo electron microscopy (cryo-EM). Cryo-EM images frozen-hydrated samples at cryogenic temperatures. This innovative imaging technology reveals the atomic structures of biomolecules, such as large proteins and dynamic protein complexes, providing another avenue to evaluate protein structures and potential drug targets.

Cryo-EM expands the target space for structure-based drug design. It allows us to support more drug discovery projects that we could imagine previously. The number of near-atomic-resolution structures obtained with it is growing exponentially, historically refractory to crystallography efforts drug targets were obtained. Moreover, cryo-EM has overcome some of its initial limitations: structures have been obtained for proteins with sizes as low as 52 kDa and resolutions as high as 1.2 Å.

F-KB1-1 -SKIN MICROCIRCULATION DURING EXERCISE AND IN THE RECOVERY: AN INSIGHT INTO POTENTIAL MECHANISMS BEHIND

Helena Lenasi
Institute of Physiology, Faculty of Medicine, University of Ljubljana, Ljubljana
e-mail: helena.lenasi.ml@mf.uni-lj.si

Dynamic exercise profoundly impacts skin microcirculation, playing an important role in thermoregulation. Mechanisms inducing alteration in vascular tone are complex; moreover, the time profile as well as the extent of vasodilation significantly differ between glabrous and non-glabrous skin sites, respectively. One of the approaches to study the complex interplay between various mechanisms regulating microcirculation is decomposition of the signals obtained by laser Doppler fluxmetry, the gold standard for skin microcirculation assessment, into basic frequency spectra, each of them corresponding to specific physiological influence: endothelial NO-independent (0.005– 0.0095 Hz), endothelial NO-dependent (0.0095-0.021 Hz), neurogenic (0.021–0.052 Hz), myogenic (0.052–0.15 Hz), respiratory (0.152–0.4 Hz), and cardiac (0.4–2.0 Hz), respectively. Little is known about the regulation of cutaneous vascular response in the early recovery phase to exercise; it has already been demonstrated that skin blood flow undergoes much greater changes in this phase than in the late recovery, suggesting that early recovery likely plays a critical role in re-establishing thermal equilibrium. The talk will present some of the main features of skin microcirculation dynamics in exercise and recovery and potential mechanisms involved with particular emphasis on endothelial component, as evaluated by wavelet analysis. This approach has gained increasing importance as one of the few available to evaluate transient biologic phenomena. The skin microcirculation during exercise and its recovery might be regarded as a suitable model for transient phenomena evaluation.

METABOLIC EXCITABILITY OF ASTROCYTES

Anemari Horvat[1,2], Helena H. Chowdhury[1,2], Marko Kreft[1,2,3], Robert Zorec[1,2], Nina Vardjan[1,2]

[1]Laboratory of Neuroendocrinology-Molecular Cell Physiology, Institute of Pathophysiology, Faculty of Medicine, University of Ljubljana, Slovenia
[2]Laboratory of Cell Engineering, Celica Biomedical, Ljubljana, Slovenia
[3]Department of Biology, Biotechnical Faculty, University of Ljubljana, Slovenia

Email address: nina.vardjan@mf.uni-lj.si

Activation of noradrenergic neurons of the *locus coeruleus* causes global noradrenaline release in the brain, which is associated by activation of brain metabolism. Noradrenaline, when released, preferentially activates astrocytes, homeostatic neuroglial cells in the brain that are rich in adrenergic receptors (ARs). Noradrenergic activation triggers in astrocytes increase in glucose uptake, glycogenolysis, and aerobic glycolysis with production of L-lactate. The latter is released by astrocytes and transported to neurons as a metabolic fuel, which is essential for learning and memory formation. We have shown by using fluorescent sensors for second messengers and metabolites and real-time microscopy that activation of α_1-ARs in astrocytes triggers rapid periodic Ca^{2+} oscillations, whereas activation of β-ARs leads to a tonic, ~10-fold slower increase in cAMP activity without oscillations. Ca^{2+} oscillations were identified as the main trigger for increased aerobic glycolysis and L-lactate production in astrocytes, whereas cAMP played a modulatory role. L-lactate released from glycolytic astrocytes may also act as a signalling molecule. Astrocytes express low levels of the L-lactate sensitive hydroxycarboxylic acid receptor 1 (HCAR1, previously known as GPR81), which is in adipocytes part of an autocrine loop in which G_i protein mediates the reduction of cAMP. However, we have shown that extracellular L-lactate or HCAR1 agonists increase production of intracellular cAMP and L-lactate in astrocytes, both of which are reduced by inhibition of adenylate cyclase. HCAR1 agonists also increased cytosolic cAMP in HCAR1-knock out astrocytes. This suggests that the observed effects are HCAR1-independent and mediated by a novel, as yet unidentified G_s-coupled excitatory L-lactate receptor in astrocytes that enhances aerobic glycolysis and L-lactate production via a positive feedback mechanism (metabolic excitability).

Acknowledgments: This work was supported by the Slovenian Research Agency (P3-0310, J3-2523) and COST Action CA18133 (ERNEST).

F-KA2-1 - SCIENTIFIC, REGULATORY AND ETHICAL CHALLENGES DEVELOPING ADVANCED THERAPY MEDICINAL PRODUCTS (ATMP)

Dr. Sandor Kerpel-Fronius, MD, PhD, DSc, FFPM
Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

The ATMPs represent a large variety of different pharmacologically active agents, gene and somatic cell therapies, tissue engineered products as well as medical device and drug combinations. Due to their wide heterogeneity, their regulatory evaluation is usually done case by case. The demonstration of the feasibility of a new approach frequently requires artificially modified cellular and/or animal models. Many ATMPs are registered by conditional (accelerated) approval and enjoy orphan drug status. The collection of additional clinical data for their final approval needs better-organized cooperation between the pharmaceutical industry and the health-care system. The development of such partnership should help to decrease the very high prices of ATMPs and increase patient access. For the development and clinical application of gene therapies and other targeted therapies many biosamples have to be collected for genomic analysis. The confidential use of the genetic information for avoiding social harm for individuals and/or social groups creates considerable ethical problems which are gradually addressed by various scientific organizations. Finally, it should be emphasized that the clinical application of many ATMPs require the close cooperation of several medically and non-medically qualified scientists which needs the careful harmonization of the differing scientific and ethical concepts of the team members.

F-KA2-2 - REGULATORY CONSIDERATIONS FOR CLINICAL TRIALS OF ADVANCED THERAPY MEDICINAL PRODUCTS

Lengyel Katalin
Országos Gyógyszerészeti és Élelmezés-egészségügyi Intézet

Advanced Therapy Medicinal Products (ATMPs) is used as an umbrella term for gene therapy, somatic-cell therapy and tissue-engineered medicines in the European Union. This innovative class of medicinal products offers groundbreaking opportunities in the treatment of high-burden diseases. The special characteristics of these medicinal products can lead to special problems in their manufacture and clinical development. Taking into account these challenges, the general regulatory framework of medicines was amended by ATMP specific rules. These rules were laid down in the ATMP regulation, and in guidelines. Some of the more important and interesting points of this regulatory framework will be highlighted in the presentation.

F-KA2-3 - CLINICAL APPLICATION OF ADVANCED THERAPEUTIC PREPARATIONS IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Kriván Gergely
Central Hospital of Southern-Pest - National Institute of Hematology and Infectology
Department of Pediatric Hematology and Stem Cell Transplantation

During the last decades hematopoietic stem cell transplantation (HSCT) has become a routinely applied therapeutic approach in the treatment of several malignant and non-malignant hematological disorders, congenital immunodeficiencies and storage diseases, as well as some solid tumors.

HSCT initially was accompanied by significant complications and mortality, however nowadays the treatment of these complications has greatly improved and the mortality rate has decreased.

In addition to many other factors, advanced laboratory methods and cell therapies play a major role in the improvement, and the treatment can be adapted to the special needs of the given patient. In emergency situations or in the absence of an identical donor, with the use of so-called “negative selection” graft selection methods, only those cell are removed from the grafts, that are responsible for vast majority of complications (alpha/beta T and CD19+ B cells). However, cells that support the engraftment and protect the patients from infections after transplant will remain in the graft (eg. gamma/delta T, NK, dendritic cells). Due to the use of this new method, the application of umbilical cord blood stem cells for transplantation has been greatly reduced worldwide.

Viral infections (mainly CMV, EBV, adenovirus) represent significant problems in the severely immunocompromised patients after transplant, especially in children. There are only limited – and highly toxic - antiviral agents available against these infections, so the death rate from viral infections remains very high. Thanks to the virus-specific T cell therapy which can be organized within a few days, these infections have become curable.

Another serious immunological complication of transplantation is the acute graft versus host disease. It is primarily treated with intensive immunosuppression, which increases the risk of infections. The immunomodulatory potential of mesenchymal stem cells (MSC) results in a dramatic improvement in some therapy-resistant cases, so the dose and duration of immunosuppressive drugs can also be reduced.

F-KA2-4 - SVF CELL THERAPY FOR THE TREATMENT OF NON-HEALING LIMB ULCERS

Balázs Bende[1], János Varga[1], Melinda Guba [2], Diána Szűcs [2], Tamás Monostori [2], Lajos Kemény [1,2,3], Zoltán Veréb [1,2]
[1]Department of Dermatology and Allergology, University of Szeged-SZAOK, Szeged,
[2]Regenerative Medicine and Cellular Pharmacology Laboratory, IKIKK, Szeged, [3]HCEMM-Skin Research Group SZTE, HCEMM, University of Szeged, Szeged

INTRODUCTION

Non-healing wounds and ulcers significantly impair the quality of life of patients and pose a challenge in healthcare. When conventional therapies do not yield sufficient results, personalized cell therapy may offer a novel solution. The stromal vascular fraction (SVF), which can be extracted from adipose tissue, can be used in such treatments, as it contains mesenchymal and progenitor cells, white blood cells, vascular pericytes, endothelial cells and vascular smooth muscle cells, which are able to develop into other cells and tissues, promote vascular remodeling and also promote the healing of injuries and wounds. SVF is therefore very beneficial as it contains all the elements needed to regenerate a large, hard-to-heal wound and the proteins produced by the many different cells are beneficial to other cells.

PATIENT MATERIAL AND METHOD

The SVF formulation was prepared by the Cytori Celution system. Cell phenotype was determined by flow cytometer. In vitro mesenchymal stem cell (MSC) cultures were prepared from inoculum taken from the cell therapy preparation. The phenotype and differentiation capacity of the MSC cells were tested in vitro. The gene expression pattern of the SVF fraction was determined by RNASeq and relevant biological pathways were identified by bioinformatic methods (R, KEGG, GOE). We included patients in whom conservative treatment did not lead to wound healing, wound size was between 5 and 100 cm2 and persisted beyond 2 months. In addition to the vital parameters of the patients, we assessed wound size (area) and quality of life before and after treatment for 28 days (5 visits in total).

RESULTS

Five patients (58.8+8 yrs, 3/2 (N/F)) were included in the study. Endothelial and progenitor cells were identified in SVF fractions. The viability of the cells exceeded 85%. After in vitro culture, homogeneous MSC were obtained from the preparation, with MSC specific phenotype (CD90, CD73 and CD105 cell surface markers) and in vitro differentiation capacity in accordance with the standard. The results of RNASeq assays identified several biological pathways important in tissue regeneration. The clinical data showed significant changes in ulcer area size, quality of life and pain during the study period (28 days). In all patients, the target ulcer area reached 50% wound closure 28 days after treatment. Quality of life scores showed a 25% improvement and wound pain scores showed a 50% improvement after cell therapy treatment.

CONCLUSION

Based on the data collected, the cell therapy procedure has no side effects and no safety risk for healing venous leg ulcers. The SVF fraction contains cell populations and factors that promote tissue healing and reduce local inflammation in the wound microenvironment.

F-KA2-5 - CHALLENGES IN BIOPRINTING TISSUES FOR CLINICAL APPLICATION - STAPES

Judit Bóvári-Biri[1,2], Alexandra Steinerbrunnerné-Nagy[1,2], Péter Bakó[3], Márton Kovács[3], Péter Maróti[4], Alexandra Mihócs[5], András Vereczkei[5], Judit E Pongrácz[1]
[1]Institute of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs, Pécs, [2]Szentágothai Research Center, University of Pécs, [3]Department of Otorhinolaryngology, Clinical Centre, University of Pécs, [4]3D Printing and Visualization Center, University of Pécs, Pécs, Hungary, [5]Department of Surgery, University of Pécs, Pécs

Progressive deafness with stapes fixation is caused by Paget's disease and osteogenesis imperfecta, chronic ear infections, and, most frequently, otosclerosis. Otosclerosis is a disease that causes progressive hearing loss due to the fixation of stapes by abnormal bony deposits.

As the stapes is one of the tiny bones in the middle ear, bony deposits lead to impairment of the normal conducting mechanism of the sound conducting system in the middle ear. Otosclerosis occurs in about 10% of the Caucasian population. In about 60% of the affected patients, family members also have otosclerosis, and the disease occurs twice as much in women than in men. Pregnancy has been reported to contribute to the worsening of the symptoms in the affected patients. Otosclerosis can occur in both ears in about 80% of patients, and the most common symptom is a slowly progressive hearing loss. Surgical intervention in the form of partial (stapedotomy) or complete (stapedectomy) removal of the fixed stapes and replacement with a prosthetic device has produced excellent hearing results that remain good for many years and proved to be effective in improving associated tinnitus in the affected patients.

While several materials have been tested to make the implant, bioprinting functional prosthetic stapes has not been applied before. The scanned stapes files were uploaded to the printer, and a PLA (poly-lactic-acid) scaffold was printed and filled with stem cells containing biogel. The setup was incubated in a bioreactor, and bone differentiation markers were tested after the maturation process.

F-KA2-6 - GENERATION OF TRANSPLANTABLE, FUNCTIONAL HUMAN BETA-CELLS FROM IRFP720-EXPRESSING INDUCED PLURIPOTENT STEM CELLS (iPSCs) FOR *IN VIVO* IMAGING

Anita Fehér[1], Andrea Schnúr[1], Suchitra Muenthaisong[1], Tamás Bellák[1,2], Ferhan Ayaydin[3,4], György Várady[5], Kinga Molnár[6], András Dinnyés [1,7,8,9]
[1]BioTalentum Ltd., Gödöllő, [2]Department of Anatomy, Histology and Embryology, Albert Szent-Györgyi Medical School, USZ, [3]Functional Cell Biology and Immunology Advanced Core Facility, HCEMM-USZ, Szeged, [4]Laboratory of Cellular Imaging, Biological Research Centre, ELKH, Szeged, [5]Research Centre for Natural Sciences, Institute of Enzymology, Budapest, [6]Department of Anatomy, Cell and Developmental Biology, Faculty of Science, ELTE, Budapest, [7]Stem Cell Research Group, HCEMM-USZ, Szeged, [8]Department of Cell Biology and Molecular Medicine, USZ, [9]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, MATE, Gödöllő

Stem cell therapy has great potential for replacing beta-cell loss in diabetic patients. However, a key obstacle to cell therapy's success is to preserve viability and function of the engrafted cells. While several strategies have been developed to improve engrafted beta-cell survival, tools to evaluate the efficacy within the body by imaging are limited. Traditional labelling tools, such as GFP-like fluorescent proteins, have limited penetration depths *in vivo* due to tissue scattering and absorption. To circumvent this limitation, a near-infrared fluorescent mutant version of the DrBphP bacteriophytochrome, iRFP720, has been developed for *in vivo* imaging and cell tracking.

Human induced pluripotent stem cells (hiPSCs) using optimized differentiation protocols can be converted into insulin-secreting beta-cells. Here, we present the generation and characterization of iRFP720-expressing human iPSCs, followed by their differentiation into functional beta-cells for *in vivo* imaging in transplantation experiments.

To generate the transgenic iPSCs the CRISPR/Cas9 technology was applied. A puromycin resistance gene was inserted into the *AAVS1* locus, driven by the endogenous *PPP1R12C* promoter, along with the CAG-iRFP720 reporter cassette, which was flanked by additional insulator elements. CAG promoter was used as it enables stable expression of the reporter gene and continuous detection of the transplanted cells, independently of their differentiation status. Proper integration of the transgene into the targeted genomic region was assessed by comprehensive genetic analysis, verifying precise genome editing. We demonstrated that the reporter iPSCs exhibit normal stem cell characteristics, then established an efficient *in vitro* pancreatic differentiation and beta-cell maturation protocol in 3D spheroid culture system. The differentiated cells were characterized in detail and the results confirmed that the mature beta-cell spheroids express cell-type specific marker genes and respond to glucose stimulation with increased insulin secretion. The differentiating cells consistently displayed stable iRFP720 signal which was detected and imaged by near-infrared fluorescence using various *in vitro* and *in vivo* imaging methods.

As the newly developed iRFP720-reporter hiPSCs have retained pluripotency and multilineage differentiation potential, they hold great potential as a cellular model for a variety of biological and pharmacological applications: for real-time imaging and tracking of transplanted cells in preclinical studies to test novel cellular products in regenerative therapies, as well as for disease modelling, drug development and toxicological studies.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 760986 (iNanoBIT) and No. 739593

(HCEMM) and No. TKP2021-EGA-28 from the National Research, Development and Innovation Fund.

F-KB2-1 - POSTTRAUMATIC STRESS DISORDER FROM THE VIEWPOINT OF THE GUT

Dóra Zelena
University of Pécs, Medical School, Institute of Physiology

Posttraumatic stress disorder (PTSD) is a devastating psychiatric condition with increasing prevalence partly due to the COVID pandemic. After a traumatic event only a part of the subjects develops PTSD (vulnerable-V), whereas the resilient (R) ones fully recover. A better understanding of the mechanisms underlying susceptibility and resistance may help in prevention. Due to the recent development on the brain-gut axis we were concentrating on gut-related changes. In a rat model of PTSD using electric footshock first extensive behavioural examination was conducted to identify R-V animals, while controls (C) were not traumatized. Then tissue and fecal samples were collected from the end of the ileum. Based upon haematoxylin-eosin staining the thickness of the intestinal villi was significantly smaller in V compared to R and C animals. On frozen samples rtPCR did not find changes in occludin, a marker of the intestinal wall permeability, however, the C-type regenerating islet derived-3 lectin mRNA, known to defend the mucosa from pathogens, were low both in R and V groups. In contrast, transferrin receptor 1, an important contributor to intestinal homeostasis was increased in R group. There were no changes in the level of inflammatory markers (TNFalpha, IL-1b, IL-10, toll-like-receptor-4). Faeces analysis suggested a shift in microbiota composition in V animals, while R has similar profile as C. Especially the level of Akkermansia muciniphila was increased approximately 10-fold in V group, together with Muc2 mRNA expression increase, which might be a compensation. The small chain fatty acid content of the faeces and blood is known to reflect microbiota activity, but was not altered in our study. Our data clearly confirmed parallel changes in the gut with resiliency and vulnerability to trauma. The causal connection should be further examined by manipulating the microbiome in the gut. As Akkermansia muciniphila was found to have antiobesity and antidiabetic effect, it might be a good candidate to increase resiliency also against trauma.

F-KB2-2 - GUT MICROBIOME COMPOSITION AND GASTROINTESTINAL ACTIVITY IN THE WISKET RAT MODEL OF SCHIZOPHRENIA

Gabriella Kékesi[1], Szonja B. Plesz[1], Leatitia G. Adlan[1], Gyöngyi Horváth[1], Kálmán F. Szűcs[2], Róbert Gáspár[2], Balázs Ligeti[3], Zoltán S. Zádori[4]
[1]Department of Physiology, [2]Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Szeged; [3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest; [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Comorbidities between gastrointestinal (GI) tract diseases and psychiatric disorders, and abnormal stress sensitivity, as an endophenotype of schizophrenia, have been widely reported, with the gut-brain axis hypothesized as a potential biological basis. Thus the imbalances within the gut microbiome may play a key role in the development of schizophrenia, which is barely detected. Rats after repeated ketamine treatment, post-weaning isolation rearing, and also bred selectively ('three-hit' Wisket model) show several schizophrenia-like behavioral phenotypes, like impaired sensory gating, cognitive disabilities, memory impairments, altered social behavior, and decreased pain sensitivity. The present study aimed to compare the taxonomic diversity and the abundance of the gut microbiome in Wisket and control rats; furthermore, to evaluate their basal GI activity and to monitor it during acute stress condition.

Twelve-week-old male and female, control (Wistar) and Wisket rats were investigated. The composition of fecal microbiota was assessed by deep sequencing of bacterial 16S rRNA. Alpha diversities were quantified by using the Shannon index, and principal component analysis was used for visualizing the microbiome composition. The GI activity was recorded in awake rats for three segments (stomach, ileum and cecum) using smooth muscle electromyography. The electrical signals were transformed to spectra by Fast Fourier analysis to reveal the power spectrum density (PsD_{max}) values and to characterize the magnitude of electrical activity in two conditions: two-hours baseline recordings (1) were followed by one-hour immobilization stress (2).

Regarding the alpha diversity, there were no significant differences by group. Microbiome analysis demonstrated significant differences in gut microbial abundance between the Wisket and control groups at the phylum (*Patescibacteria, Campylobacterota*), class (*Campylobacteria, Saccharimonadia*), family (*Helicobacteraceae, Streptococcaceae, Lactobacillaceae, Tannerellaceae*) and genus (*Streptococcus, Limosilactobacillus, Lactobacillus, Helicobacter, Parabacteroides, Prevotellaceae-UCG-003, Lactiplantibacillus*) levels, partially correlated with human and preclinical findings. Wisket animals showed a lower level of GI activity specifically in the stomach and cecum. Stress condition increased the GI activity in both control and Wisket groups that was more pronounced in model animals.

The present study revealed that the Wisket model rats show: complex changes in gut microbiome composition; decreased GI activity; and they are more vulnerable to acute stress. Thus, the Wisket model might be appropriate for investigating the role of the gut-brain axis in the etiology of schizophrenia. The manipulation of gut microbiota with dietary interventions (pre- or probiotic), therefore, may provide a new way to improve health and prevent neuropsychiatric diseases.

F-KB2-3 - THE EFFECT OF CONVENTIONAL NONSTEROIDAL ANTI-INFLAMMATORY DRUGS AND SELECTIVE COX-2 INHIBITORS ON GUT MICROBIOTA COMPOSITION

Arezoo Haghighi[1], András S. Tóth[1], Bernadette Lázár[1], Szilvia B. László[1], Barbara Hutka[1,2], Mahmoud Al-Khrasani[1], Bence Ágg[1], Balázs Ligeti[3], Nóra Makra[4], Eszter Ostorházi[4], Dóra Szabó[4], Klára Gyires[1], Zoltán S. Zádori[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary;[2] Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary (current address), [3]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary; [4]Department of Medical Microbiology, Semmelweis University, Nagyvárad tér 4, 1089, Budapest, Hungary

Background: It has been recognized already in the 60's that nonsteroidal anti-inflammatory drugs (NSAIDs) can cause significant changes in gut microbiota composition. Over the past years, a large amount of data has accumulated on NSAID-induced dysbiosis, in particular due to advances in sequencing technologies. However, several aspects of it remain poorly understood. For example, little is known about the underlying mechanisms or whether bacterial alterations can be avoided by using selective cyclooxygenase-2 (COX-2) inhibitors. Our research aims to provide more insights into NSAID-induced gut dysbiosis.

Methods: Rats were treated with either conventional NSAIDs (indomethacin, ketorolac, naproxen) or selective COX-2 inhibitors (celecoxib, rofecoxib, etoricoxib) for different time periods. We assessed the severity of intestinal damage and inflammation, the expression of some antimicrobial peptides, as well as the changes in luminal pH. The composition of gut microbiota was determined by sequencing bacterial 16S rRNA. The antibacterial properties of NSAIDs were assessed with the broth microdilution method.

Results: Conventional NSAIDs induced intestinal damage and inflammation in a dose-dependent fashion, and increased the expression of tested antimicrobial peptides. The severity of inflammation showed positive correlation with the relative abundance of Gram negative bacteria. However, in some treatment groups dysbiosis occurred without any overt intestinal damage and inflammation. Indomethacin decreased the pH of small intestinal content, but the observed changes in microbiota did not correspond to the reported pH tolerance of bacteria. In addition, indomethacin, in contrast to celecoxib and etoricoxib, had no direct inhibitory effect on the growth of tested bacteria in vitro. Etoricoxib caused mild changes in both the small and large intestinal microbiota of rats, whereas celecoxib and rofecoxib had no effects.

Conclusions: Tissue inflammation is a major factor that contributes to NSAID-induced dysbiosis, but marked bacterial shifts can also occur without significant enteropathy. Changes in pH, or direct antibacterial effects of NSAIDs do not likely have a major role in the development of dysbiosis. Selective COX-2 inhibitors may differ in their effects on gut bacteria.
Grant: NKFI FK 138842.

F-KB2-4 - THE INFLUENCE OF ANTIBIOTICS ON TRANSITORY RESISTOME DURING GUT COLONIZATION WITH MULTIRESISTANT KLEBSIELLA PNEUMONIAE

Dóra Szabó
Institute of Medical Microbiology, Semmelweis University, Budapest, Hungary

Gastrointestinal carriage of multidrug-resistant (MDR) bacteria is one of the main risk factors for developing serious, difficult-to-treat infections. Given that there is currently no all-round solution to eliminate colonization with MDR bacteria, it is particularly important to understand the dynamic process of colonization to aid the development of novel decolonization strategies.

Our aim was to assess the effects of antibiotic administration (ampicillin, ceftazidime, ciprofloxacin) on the establishment and elimination of intestinal colonization with a CTX-M-15 ESBL and OXA-162 carbapenemase producing Klebsiella pneumoniae ST15 (KP5825) in a murine (C57BL/6 male mice) model.

The KP5825 contained four different plasmid replicon types, namely IncFII(K), IncL, IncFIB and ColpVC. IncL (containing the $bla_{OXA-162}$ resistance gene within a Tn1991.2 genetic element) and IncFII(K) (containing the $bla_{CTX-M-15}$ resistance gene) plasmids were successfully conjugated. During ampicillin and ceftazidime treatments, colonization rate of KP5825 increased, while, ciprofloxacin treatments in both concentrations (0.1 g/L and 0.5 g/L) led to significantly decreased colonization rates. The gene copy number $bla_{OXA-162}$ correlated with K. pneumoniae in vivo, while a major elevation was observed in the copy number of $bla_{CTX-M-15}$ from the first day to the fifteenth day in the 0.5 g/L dose ceftazidime treatment group. We have found that the phyla Bacteroidetes and Firmicutes were most dominant in all of the treatment groups; however, Bacteroidetes was more common in the groups treated with antibiotics compared to the control group. Significant differences were observed among the different antibiotic-treated groups in beta but not alpha diversity, implying that the difference is the relative abundance of some bacterial community members. Bacteria from the Lachnospiraceae family (including Agathobacter, Anaerostipes, Lachnoclostridium 11308, Lachnospiraceae UCG-004, Lachnospiraceae NK3A20 group 11318, Lachnospiraceae NK4A136 group 11319, Roseburia, and Tyzzerella) showed an inverse relationship with the carriage rate of the ECKP strain, whereas members of Enterobacteriaceae and the ECKP strain have shown a correlational relationship.

Our results demonstrate that commonly used antibiotics may have diverse impacts on the colonization rates of intestinally-carried CPE, in addition to affecting the gene copy number of their resistance genes, thus facilitating their stable persistance and dissemination. Our results suggest that the composition of the microbial community plays a primary role in the MDR-colonization rate, whereas the antibiotic susceptibility of individual MDR strains affects this process to a lesser extent. Distinct bacterial families have associated into microbial clusters, collecting taxonomically close species to produce survival benefits in the gut. These associations do not develop at random, as they may be attributed to the presence of specific metabolomic networks. A new concept should be introduced in designing future endeavors for MDR decolonization, supplemented by knowledge of the composition of the host bacterial community and the identification of bacterial clusters capable of suppressing or enhancing the invader species.

F-KB2-5 - ANTIBIOTIC-INDUCED MICROBIOTA DEPLETION IN GUINEA PIG INTESTINE REDUCES EXPRESSION OF A_{2B}-ADRENOCEPTORS AND THEIR ACTIVITY IN PERISTALTIC MOTOR INHIBITION

Aitak Farzi[1], Eva Tatzl[1,2], Slave Trajanoski[3], Michael K. Herbert[2], Peter Holzer[1]
[1]Division of Pharmacology, Otto Loewi Research Centre, Medical University of Graz, Graz, Austria, [2]Department of Anaesthesiology and Intensive Care, Medical University of Graz, Graz, Austria, [3]Core Facility Computational Bioscience, Centre for Medical Research, Medical University of Graz, Graz, Austria

Several drugs used in the management of critically ill patients including analgosedative drugs such as α_2-adrenoceptor agonists (e.g., dexmedetomidine, clonidine), impair propulsive GI motility and thereby increase the risk for developing sepsis and multiple organ dysfunction syndrome. While the enteric nervous system is of paramount relevance to the control of GI peristalsis, the intestinal microbiota has also emerged as a factor that can influence GI motility. It has however not yet been analysed in which way antibiotic-induced GI dysbiosis modifies peristaltic motor impairment caused by drugs used in intensive care. Therefore, the current study set out to examine whether the inhibitory effect of α_2-adrenoceptor agonists on peristalsis in the guinea-pig small intestine *in vitro* is altered following depletion of the GI microbiota by enoral antibiotic pre-treatment.

The antibiotics chosen for this purpose were meropenem, vancomycin and neomycin. Antibiotic-induced dysbiosis was confirmed by 16S rDNA sequencing. Evaluation of peristalsis was performed in the isolated guinea-pig small intestine by assessing the peristaltic pressure threshold (PPT), which defines the intraluminal pressure at which a peristaltic wave is triggered. The expression of some factors that may be relevant to the communication between microbiome and the adrenoceptor system were also examined at mRNA (qPCR) or protein level (ELISA).

Antibiotic treatment led to a marked decrease of bacterial load and alpha diversity. Microbial disruption did not significantly affect peristaltic motor activity *per se* but selectively blunted the ability of α_2-adrenoceptor agonists to inhibit peristalsis. Mechanistically, a decrease in small intestinal α_2-adrenoceptors expression could be detected in response to antibiotic treatment, which was associated with a decrease in the immune-related genes Ifn-ɣ and Nos2.

In conclusion, the function of α_2-adrenoceptors is particularly sensitive to antibiotic-induced disturbance of the gut microbiota. Given that inflammatory signals have been reported to affect α_2-adrenoceptors expression, we propose that antibiotic-induced disturbance of the gut microbiota affects intestinal immune signalling leading to downregulation of α_2-adrenoceptors.

F-KB2-6 - A SULFIDE DONOR, DIMETHYL TRISULFIDE, ALLEVIATES EXPERIMENTAL ACUTE PANCREATITIS

Erik Márk Orján[1], Eszter Sára Kormányos[1], Gabriella Mihalekné Fűr[1], Ágnes Dombi[2], Emese Réka Bálint[1], Zsolt Balla[1], Beáta Adél Balog[1], Ágnes Dágó[1], Ahmad Totonji[1], Zoárd István Bátai[2], Eszter Petra Jurányi[3], Ammar Al-Omari[2], Gábor Pozsgai[2], Viktória Kormos[2], Péter Nagy[3], Erika Pintér[2], Zoltán Rakonczay Jr[1], Lóránd Kiss[1]
[1] Department of Pathophysiology, University of Szeged, Szeged, Hungary, [2] Department of Pharmacology and Pharmacotherapy, University of Pécs, Pécs, Hungary, [3] Department of Molecular Immunology and Toxicology and the National Tumor Biology Laboratory, National Institute of Oncology, Budapest, Hungary.

Introduction: Acute pancreatitis (AP) is a potentially life-threatening disease without specific treatment. During AP, endogenous hydrogen sulfide (H2S) production is increased by certain enzymes and is related to AP severity. Interestingly, exogenous slow H2S-releasing organosulfur agents (e.g. diallyl disulfide and GYY4137) exert anti-inflammatory effects. Dimethyl trisulfide (DMTS) also belongs to the organosulfur molecule family; it shows biological activity and decreases carrageenan-induced paw inflammation, but its effect on AP is unknown. Our aims were to investigate the *in vivo* and *in vitro* effects of DMTS in experimental AP.

Methods: AP was induced in FVB/n mice or Wistar rats by intraperitoneal injection(s) of caerulein, ethanol-palmitoleic acid, or L-ornithine-HCl. DMTS treatments were administered subcutaneously simultaneously with AP induction. Disease severity was determined by evaluating pancreatic histological scoring, pancreatic water content and myeloperoxidase activity. Pancreatic heat shock protein 72 (HSP72) expression, sulfide and protein persulfidation were measured. Tetrazolium salt (MTT) and propidium iodide were utilized to assess cellular viability on primary acinar cells. Intracellular concentrations of reactive oxygen species (ROS) and Ca^{2+} were determined by microfluorimetry.

Results: DMTS treatment significantly alleviated the severity of all three AP models. It decreased the pancreatic infiltration of leukocytes and cellular damage. DMTS also reduced the pancreatic myeloperoxidase activity. During AP, DMTS upregulated the HSP72 expression and elevated serum sulfide and protein persulfidation. However, pancreatic sulfide levels were unaltered by AP induction or DMTS treatment. DMTS showed cytoprotection against hydrogen peroxide (H_2O_2) and AP-inducing agents (chenodeoxycholate, L-arginine-HCl) in isolated acini. DMTS reduced ROS levels when acinar cells were treated with H_2O_2 or menadione and modulated physiological, but not pathophysiological Ca^{2+} signalling.

Conclusions: Our results suggest that DMTS is a sulfide donor which has anti-inflammatory and antioxidant effects. The beneficial effects of DMTS in AP could be caused by upregulation of HSP72 expression, by its antioxidant properties, by being a H_2S donor molecule, and/or by reducing leukocyte infiltration. Overall, organosulfur compounds are worth further investigation in this potentially lethal disease.

Acknowledgements: This study was supported by NKFIH, GINOP, MTA, and UNKP.

F-KA3-1 - DEVELOPMENT OF IN VITRO FIBROSIS MODEL FOR THE EVALUATION OF POTENTIAL ANTI-FIBROTIC DRUG CANDIDATES

Csicsely Stefánia[1,2], Jójárt Boldizsár[1,2,3], Szabó Viktória[1,2,3], Maléth József[1,2,3]
[1]Department of Medicine, Szent-Györgyi Albert Medical School, University of Szeged, Szeged, [2]HAS-USz Momentum Epithelial Cell Signaling and Secretion Research Group, Szeged, [3]HCEMM-USz Molecular Gastroenterology Research Group, Szeged

Inflammatory Bowel Disease (IBD) is a chronic immune-mediated disease which caused by the pathological immunological activation of the intestinal mucosa. Fibrosis is evolved through pro-fibrotic signals and is a common secondary complication of IBD. As result of the fibrotic process the affected segment of the intestine is narrowed, and the motility is impaired. Currently there are no treatment for the prevention of fibrosis, and the affected segment of the colon must be removed by surgery. Our previous research showed that expression of Plasminogen activator inhibitor-1 (PAI-1) is increased in mucosa of active IBD patients, which may contribute to the development of fibrosis. Our hypothesis is that inhibition of PAI-1 could be a potential resolution to decrease fibrotic progression. The **aim** of our research is to develop an *in vitro* fibrosis model for identification of potential drug target.

HeLa cell culture was treated up to 48 hours with different concentrations (0,1; 1; 5; 10 ng/ml) of Transforming growth factor β (TGF-β) to induced fibrotic signalisation. Fibrotic phenotypes were characterised by immunofluorescent staining and qRT-PCR. Afterwards we applied TM5275 PAI-1 inhibitor in different concentrations (0,1; 1; 10 and 100 µM) for also 48 hours.

The fluorescent intensity of Vimentin (a fibrosis marker) and PAI-1 are increased after the TGF-β treatment. In addition, the gene expression of other fibrotic marker (α-Smooth Muscle Actin and Fibronectin 1), TGF-β and PAI-1 were also upregulated. If we treated the cells simultaneously with the TM5275 inhibitor the gene expression of PAI-1 and fibrotic markers are decreased at the dose of 10 and 100 µM.

Our results suggest that we successfully developed a TGF-β induced *in vitro* fibrosis model. Furthermore, the PAI-1 inhibitor reduced the expression of the fibrosis markers. With the help of this model, we can assess potential antifibrotic drug candidates with improved throughput that accelerates the selection of potential drugs.

F-KA3-2 - INVESTIGATION OF THE PROTECTIVE EFFECT OF HUMAN MILK OLIGOSACCHARIDES (HMO) IN BACTERIAL ENDOTOXIN-INDUCED FEVER

Bíbor Gárdos[1], András Garami[1], Eszter Pákai[1]
[1]Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pécs, Hungary

Introduction: Sepsis - often caused by Gram-negative pathogens - is one of the most common causes of neonatal mortality. Oligosaccharides in breast milk (human milk oligosaccharides: HMO) are biologically active substances that may play a protective role in neonates. The aim of our study was to investigate the potential antipyretic effect of different HMOs in bacterial lipopolysaccharide (LPS)-induced fever in mice.

Methods: Mice were implanted with intraperitoneal cannula after two weeks of preconditioning. Prior to induction of fever, different HMOs dissolved in physiological saline were administered intraperitoneally at equimolar doses. Two hours later fever was induced by intraperitoneal administration of low-dose (120 µg/kg) LPS. Control groups received physiological saline. The core temperature of the animals was detected by thermocouples at a thermoneutral ambient temperature.

Results: As expected, animals pretreated with physiological saline showed a large temperature rise after LPS administration, peaking at 38.7±0.6°C 120 min after administration ($p<0.05$). However, animals pretreated with two types of HMOs showed a significant ($p<0.05$) decrease in fever compared to control pretreatment, with maximum values of 37.5±0.4 and 37.6±0.3°C, respectively. No reduction in fever response was detected for the other HMOs tested ($p>0.05$).

Conclusions: Our results showed that the HMOs tested did not affect body temperature per se, but two of them significantly reduced the LPS-induced fever response, whereas no antipyretic effect was observed for the others.

Our results confirm that breastfeeding is of paramount importance against bacterial infections, in which HMOs play a crucial role.

F-KA3-3 - THE METABOLIC EFFECTS OF THE BMP-INDUCER TILORONE IN *IN VITRO* AND *IN VIVO* MODELS

Balázs Szenci-Kaszás[1], Norman Noel Tanner[1], Zoltán Márton Köhler[1], Kitti Szabó[1], György Trencsényi[2], Anikó Keller-Pintér[1]

[1] Department of Biochemistry, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary, [2] Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

The reduced glucose uptake of skeletal muscle plays an important role in the pathogenesis of type 2 diabetes mellitus. Tilorone is an antiviral agent that has been used for the treatment and prophylaxis of certain viral infections since the 1970s. Additionally, it induces the transcription of bone morphogenic proteins (BMPs) whose role in the regulation of ontogenesis has been known for a long time. However, based on recent scientific data, BMPs might regulate metabolism as well. BMPs can enhance the expression of glucose transporter type 4 (GLUT4) via the SMAD1/5/8-SMAD4-PPARγ signalling pathway. Moreover, they can also promote the translocation of the transporters through the activation of the key molecules of insulin signalling by PI3K–Akt2–AS160. The main aim of our study was to examine the metabolic effects of tilorone on differentiated myotubes *in vitro* and in an animal model *in vivo*.

For *in vitro* experiments we used differentiated C2C12 myotubes, which were treated with tilorone (20 nM) for 2 or 5 hours and with insulin (100 nM) for 10 minutes. The glucose uptake of myotubes was measured with 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}FDG). For *in vivo* experiments we used C57BL/6 mice, which were randomly divided into a control, a high-fat diet (HFD) and an HFD+tilorone (25 mg/kg tilorone, *ip.*, every third day) group. We measured the body weight and the fasting blood glucose weekly of each animal. On the last week of the experiment, we performed intraperitoneal glucose tolerance test and determined the distribution of tissue glucose uptake with ^{18}FDG-PET/MRI. Upon the termination of the experiment, we measured the abdominal fat. We used immunoblot to investigate changes in protein expression and activation.

The phosphorylation of Akt2 and the glucose uptake of myotubes was increased by insulin which was further augmented by tilorone. Respectively, the phosphorylation of AS160 was increased in treated myotubes. *In vivo* HFD resulted in increased body weight, abdominal fat mass, fasting blood glucose and impaired glucose tolerance, which were mitigated by tilorone treatment. We observed increased Akt2 and AS160 activity and GLUT4 expression in quadriceps femoris muscle samples of the treated group. Treatment enhanced the ^{18}FDG uptake of skeletal muscle, adipose tissue, liver and myocardium.

Based on our results, tilorone increases the activity of the signalling pathway involved in the regulation of GLUT4 translocation, thus it has an *in vitro* insulin sensitizer effect. Furthermore, tilorone moderates the unfavourable changes in body mass, abdominal fat mass and glucose homeostasis caused by HFD. We hope that exploiting BMP signalling will open new horizons in the development of novel antidiabetic drugs.

Funding: NKFI FK 134684, TKP2021-EGA-28, National Academy of Scientist Education (FEIF/646-4/2021-ITM_SZERZ) and ÚNKP-22-2-SZTE-229 New National Excellence Program.

F-KA3-4 - THE ROLE OF NEUROMODULATORY SYSTEMS IN IMPLICIT LEARNING

Franciska Benyó[1], Bálint Király[1], Vivien Pillár[1], Anna Velencei[1] & Balázs Hangya[1]
[1]Institute of experimental medicine, Budapest

Neuromodulators are central to normal cognitive functions. Lesions to the cholinergic, dopaminergic and serotonergic systems all impair memory and learning processes. Additionally, disorders of these systems underlie certain degenerative neurological conditions like Alzheimer's and Parkinson's diseases. While neuromodulators are crucial for explicit learning, their participation in implicit learning is poorly understood. To address this issue, we have developed a mouse model of implicit learning and examined the release of neuromodulators in brain areas heavily involved in learning.

We trained mice (n=8) in a custom-built automated training system on a sequential learning task with blocks of trials in which the stimuli followed each other in random order. Meanwhile, we used fiber photometry to measure acetylcholine, dopamine and serotonin release in the basolateral amygdala, the ventral striatum and the medial prefrontal cortex.
Animals performed the task faster and with better accuracy in the sequential blocks. We observed robust and strongly correlated cholinergic and dopaminergic activation during learning and the execution of the task. However, while cholinergic activation was more pronounced during task execution, the dopaminergic response had a more robust reinforcement-evoked component. In contrast to the positive cholinergic-dopaminergic correlation, serotonin levels were negatively correlated with both other neuromodulators. Furthermore, we found that neuromodulatory levels precisely represented the current stage of the animal in the sequence. Surprisingly, we found characteristic differences between cortical and subcortical neuromodulatory signals.

Our results indicate that implicit learning relies on a precisely coordinated release of various neuromodulators, which act on a heterogeneous manner across different brain areas.

Financial resources: Új Nemzeti Kíválóság Program, Lendület Program
Ethical licence: PE/EA/1212-5/2017

F-KA3-5 - INVESTIGATING THE PRODUCTION AND ROLE OF LEUKOTRIENE B_4 IN NEUTROPHILS

Boldizsár Vámosi[1], Szimonetta Tamás[1,2,3], Benoit Roux[1,3], Balázs Enyedi[1,2,3]
[1]Department of Physiology, Semmelweis University, Budapest, [2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, [3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest

Introduction: As a chemoattractant, leukotriene B_4 (LTB_4) plays an important role in the initial steps of inflammation and wound healing. The intracellular signaling pathways activated by this mediator are well established, but little is known about its endogenous release pattern and its role in neutrophil swarming.

Aims: Our aim is to better understand the endogenous release pattern of LTB_4 and to decipher how it mediates neutrophil migration and swarming.

Methods: We inserted a circularly permuted green fluorescent protein into the third intracellular loop of the endogenous receptor of LTB_4 (BLT-1/block lipid transport-1) to generate a fluorescent biosensor (GEM-LTB_4) in which the presence of LTB_4 induces a fluorescence intensity change. Our sensor was optimized and characterized using a GEM-LTB_4-expressing stable HEK293A cell line. Neutrophil granulocytes isolated from mouse bone marrow were stimulated with fMLP and the secretion of LTB_4 was assessed using our sensor. As a control, the production of LTB_4 by neutrophils was verified by ELISA. We also generated a transgenic zebrafish line expressing GEM-LTB_4 and assayed the production of LTB_4 by neutrophil granulocytes in response to sterile wounding. Furthermore, we investigated the LTB_4 production and intracellular Ca^{2+} levels in neutrophils in response to the calcium ionophore Calcimycin.

Results: Neutrophil granulocytes isolated from mouse bone marrow produced LTB_4 in response to fMLP. Using our sensor, we detected LTB_4 waves produced by individual neutrophils. In parallel, we detected pseudopodia formation towards the source of LTB_4 production in the surrounding neutrophils. Following sterile tail fin wounding in transgenic zebrafish expressing GEM-LTB_4, we measured variable amounts of endogenous LTB_4 release. LTB_4 production was enhanced by the calcium ionophore Calcimycin. A similar response was not observed in larvae pretreated with zileuton, an inhibitor of LTB_4 production.

Conclusion: We have created a novel fluorescent biosensor which allows us to investigate the spatiotemporal pattern of endogenous LTB_4 release and its effect on neutrophil granulocytes both in vitro and in vivo. Using this tool we also assessed the effect of intracellular Ca^{2+} level changes on neutrophil-derived LTB_4 production. In future experiments we seek to understand the reasons of variable LTB_4 production that we observed during sterile wounding.

F-KA3-6 - EFFECTS OF SUMATRIPTAN ON P2X7 PURINERGIC RECEPTOR-MEDIATED SIGNALING IN AN AMPHETAMINE-INDUCED ACUTE MANIA MOUSE MODEL

Fruzsina Maácz[1,2], Flóra Gölöncsér[1], Beáta Sperlágh[1]
[1]Institute of Experimental Medicine, Budapest, Hungary, [2]Eötvös Lóránd University, Budapest, Hungary

Acute mania is a phase of bipolar disorder. Symptoms include, among others, elevated mood, fast talking, engaging in risky behaviours, overconfidence in abilities and intense energy. Dysfunction of purinergic signalling plays a role in the pathophysiology of acute mania. The P2X7 receptor (P2X7R) affects neurotransmitter release and mania-like behaviour in mouse models.

Our research investigated amphetamine-induced hyperactivity in wild-type (WT) and P2X7R gene knockout (P2X7KO) mice using open-field (OF) and elevated plus maze (EPM) assays, changes in c-fos expression in striatum using immunohistochemistry. We also investigated dopamine release released from the striatum.

In behavioural assays, the serotonin 5-$HT_{1A/1B/1D}$ receptor agonist sumatriptan and the P2X7R antagonist JNJ47965567 reduced amphetamine-induced hyperlocomotion in WT mice, whereas sumatriptan had no effect in P2X7KO mice. However, co-administration of sumatriptan and JNJ47965567 did not affect hyperactivity in WT mice. C-fos expression was increased by amphetamine in both WT and P2X7KO mice. The expression level was reduced by adding sumatriptan in WT mice, whereas sumatriptan had no effect in P2X7KO mice. Dopamine release was increased by amphetamine in both WT and P2X7KO mice. The effect of sumatriptan was reduced in WT mice, whereas it had no effect in P2X7KO mice.
Our results suggest that sumatriptan inhibits mania-like behavior in mice and that P2X7R plays a role in mediating its modulatory effect, thus sumatriptan may be effective not only in the treatment of migraine but also in the treatment of mania.

F-KB3-1 - THE EPIGENETIC BASES OF UNIQUE INFLAMMATORY RESPONSIVENESS IN THE ALTERNATIVELY POLARIZED MACROPHAGES

Zsolt Czimmerer[1,2,12,*], Laszlo Halasz[3,12], Bence Daniel[3,11,12], Zsofia Varga[2], Krisztian Bene[1], Apolka Domokos[1,4], Marten Hoeksema[5], Zeyang Shen[5,6], Wilhelm K. Berger[3], Timea Cseh[1], Karoly Jambrovics[1], Zsuzsanna Kolostyak[1,4], Ferenc Fenyvesi[7], Judit Varadi[7], Szilard Poliska[1], Gyorgy Hajas[8,9], Istvan Szatmari[1], Christopher K. Glass[5,10], Attila Bacsi[8,9] and Laszlo Nagy[1,3,*]
[1] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2] Institute of Genetics, Biological Research Centre, Eötvös Loránd Research Network, Szeged, Hungary, [3] Departments of Medicine and Biological Chemistry, Johns Hopkins University School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA, [4] Molecular Cell and Immunobiology Doctoral School, Faculty of Medicine, University of Debrecen, H-4032, Debrecen, Hungary, [5] Department of Cellular and Molecular Medicine, School of Medicine, University of California, San Diego, La Jolla, CA, USA [6] Department of Bioengineering, Jacobs School of Engineering, University of California, San Diego, La Jolla, CA, USA, [7] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [8] Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [9] ELKH-DE Allergology Research Group, Debrecen, Hungary, [10] Department of Medicine, School of Medicine, University of California, San Diego, La Jolla, CA, USA, [11] Present address: Stanford University School of Medicine, Department of Pathology, Stanford, CA, USA, [12] These authors contributed equally to this work.,
*Correspondence: lnagy@jhmi.edu, czimmerer.zsolt@brc.hu

Prior exposure to signals can fundamentally change the response of innate immune cells to subsequent stimuli underlying phenomena such as cellular memory, tissue type-specific reactions, cytokine storm, or anergy/tolerance. Our understanding of the molecular nature of such interactions, especially at the epigenomic and gene expression level, is fragmented. It is believed that alternative macrophage polarization and inflammatory signals-activated transcriptional programs largely antagonize each other, and no significant convergence has been identified between them.

In contrast, here we show that IL-4 polarized macrophages, irrespective of their origin, establish a unique inflammatory gene expression program upon lipopolysaccharide exposure. This interaction, we termed extended synergy, depends on IL-4-induced STAT6-directed epigenomic remodeling, the vast expansion of the LPS-activated NFκB-p65 cistrome, increased chromatin accessibility, and enhancer activity. The EGR2 transcription factor contributes to regulating the LPS-induced de novo and enhanced NFκB-p65 binding and synergistic gene activation in a macrophage subtype-specific manner. Consequently, the previously alternatively polarized macrophages produce extreme levels of immune-modulatory factors, including CCL17, CCL22, CCL2, and EDN1, both *in vitro* and *in vivo* in a murine Th2-type airway inflammation model upon LPS exposure.

Thus, our findings establish that the IL-4-induced epigenetic reprogramming is responsible for developing robust inflammatory hyperresponsiveness to TLR activation and likely contributes to lung pathologies, such as exacerbation of Th2 inflammatory disease or pneumonia.

F-KB3-2 - THE TRANSCRIPTION FACTOR BACH1 REGULATES MACROPHAGE DEVELOPMENT AND INFLAMMATORY FUNCTION

Krisztián Bene[1], Petros Tzerpos[1], Dóra Bojcsuk[1], Noemi Caballero Sanchez[1,2], Mohamed Osama[1], László Halász[3], Andreas Patsalos[3], László Nagy[1,3]
[1]Nuclear Receptor Research Laboratory, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen. Hungary, [2]Doctoral School of Molecular Biology and Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [3]Departments of Medicine and Biological Chemistry, Johns Hopkins University School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, Florida, USA

Introduction: The development and the inflammatory response of macrophages (MFs) are controlled at transcriptional level to provide the appropriate adaptation to tissue microenvironment and to the response to various inflammatory stimuli, respectively. MF continuously monitor their local microenvironment and integrate extra-and intracellular signals to regulate their transcriptional landscape. The transcriptional activation has well-established roles in shaping MF gene expression, but the roles of transcriptional repressors such as the nuclear heme-sensor BACH1 protein is less known in a complex and changing microenvironment.

Results: We show that the nuclear BACH1 protein is part of the core transcriptional program of MF shaping the chromatin accessibility of thousands of distal regulatory elements. Furthermore, BACH1 acts as an extensive, dynamic, and contextual regulator of both inflammatory and anti-inflammatory gene expression programs in bone-marrow-derived MF with different polarization states. Our data also show that BACH1 is required for competitive fitness and tissue adaptation of tissue-resident MF populations, which supports the roles of BACH1 in acquiring and maintaining the functional specificity and diversity of tissue macrophages. Furthermore, we demonstrate that myeloid BACH1 is indispensable in different inflammatory settings including the sterile inflammation of the muscle induced by cardiotoxin, and the type 2 airway inflammation induced by ragweed pollen-extract.

Conclusion: We provide evidence that BACH1, a heme-sensitive transcriptional repressor acts as a pervasive epigenomic nuclear protein to shape the identity and function of MF on a context dependent manner.

F-KB3-3 - SINGLE-CELL RNA PROFILING OF IMMUNE CELLS UNCOVERS THE CRITICAL ROLE OF BACH1 DURING MACROPHAGE POLARIZATION IN MUSCLE REPAIR.

Noemí Caballero-Sánchez[1,2], Petros Tzerpos[1], Krisztian Bene[1], Laszlo Halasz[3], Andreas Patsalos[3], Darby Oleksak[3], Dóra Bojcsuk[1], Gergely Nagy[1], Laszló Nagy[1,3]
[1]Department of Biochemistry and Molecular Biology, Nuclear Receptor Research Laboratory, Faculty of Medicine, University of Debrecen, Hungary, [2]Doctoral School of Molecular Cell and Immunobiology, Faculty of Medicine, University of Debrecen, Hungary, [3]Institute For Fundamental Biomedical Research, John Hopkins All Children's Hospital, St Petersburg, Fl, United States

The immune system plays a central not only in fighting infections but also in maintaining homeostasis and tissue repair. Specifically, upon injury, inflammation is driven by an initial pro-inflammatory response that timely converts into anti-inflammatory proregenerative one through a coordinated infiltration of diverse cell types and the secretion of growth factors, cytokines, and lipids mediators. In order to study this dynamic process, we have utilized skeletal muscle as a model due to its remarkable regenerative capacity. Our previous research, as well as others, have discovered different metabolic and transcriptional switches within macrophages that enable them to adjust to alterations of the microenvironment. Therefore, macrophages modulate their gene expression, function and cellular interactions, which driving the regenerative process and promoting tissue repair. To delineate the transcriptional changes, we used the cardiotoxin (CTX) acute muscle injury model and profiled the chromatin accessibility (ATAC-seq) and gene expression (RNA-seq) in sorted macrophage populations at different time-points during muscle regeneration. We identified BACH1 a heme-responsive transcriptional repressor, as a novel regulator of this process. Histological analysis reveals that Bach1 myeloid-specific knockout mice present a persistent necrotic fiber as well as delayed tissue repair while FACS and bulk RNA-seq show an abnormal macrophage phenotype switch and deregulation of critical genes involved in regenerative inflammation. To study how BACH1 affects the macrophage subtype specification and cellular composition during muscle repair, we have performed single-cell RNA-seq (scRNA-seq) experiments in control and myeloid specific Bach1 knockout models at different time points post CTX injury in mice. Our results indicate significant differences in the macrophage subsets composition and gene expression profiles demonstrated by the magnitude of expression of critical inflammatory and repair-related genes. In conclusion, by combining single-cell transcriptomics and macrophage-specific knockouts, we revealed a crucial role for BACH1 in regulating macrophage subtype specification and subsequently skeletal muscle regeneration

F-KB3-4 - THE EFFECT OF TRANSFECTED SMALL MEMBRANE PEPTIDES IN MUSCLE REGENERATION

Lulu Alsheikh Hussein[1], Ernő Zádor[1]
[1]Institute of Biochemistry, Albert Szent-Györgyi Faculty of Medicine, University of Szeged

The sarcoplasmic reticulum Ca^{2+}ATPase (SERCA) is a membrane protein pump that moves Ca^{2+} from the cytosol into the lumen of the sarco/endoplasmic reticulum. utilizing ATP energy. The neonatal isoform, SERCA1b is expressed in myotubes and developing fibers and it's in vivo and in vitro suppression reduces fiber growth by affecting Ca^{2+} dependent signal pathways and gene expressions essential for muscle differentiation.

For many years, solely two membrane peptides—phospholamban (PLB) and sarcolipin (SLN)—were thought to control SERCA pumps. Recently, transcripts formerly classed as lncRNAs have been shown to encode the micropeptides myoregulin (MLN), endoregulin (ELN), dwarf open reading frame (DWORF), and another-regulin (ALN). These findings also suggest that MLN may influence fiber size growth and muscle regeneration by interfering with numerous signal pathways based on the above-described SERCA1b functions. Our laboratory has developed a muscle regeneration model in which silencing SERCA1b in a few fibers had a wilder effect as it increased growth in the whole regenerating rat soleus.

Our aim is to investigate the effect of myoregulin and the other micropeptides on SERCA1b and muscle regeneration when transfected by intramuscular injection into regenerating rat soleus. Based on the results of fiber size measurement, the regenerating soleus muscle transfection with YFP-SLN caused a decrease in fiber size compared to control regenerating muscle transfected with EGFP expressing vector. Furthermore, there was no significant difference between the muscle transfected with YFP- MLN or the control.

F-KB3-5 - REGULINS, CALCIUM METABOLISM AND MUSCLE REGENERATION

Ernő Zádor
Institute of Biochemistry, Albert Szent-Györgyi Faculty of Medicine, University of Szeged

Skeletal muscle regeneration involves the remodelling of compartmentalization and the regulation of calcium mediated processes. A number of players contribute to intracellular handling of calcium, among them are the sarco/endoplasmic reticulum calcium ATP-ases (SERCAs). These molecules function in the membrane of ER/SR pumping Ca^{2+} from cytoplasm into the lumen of the internal store. Removal of calcium from the cytoplasm is essential for signalling and for relaxation of skeletal muscle and heart. There are three genes and over a dozen isoforms of SERCA in mammals. These can be potentially influenced by small membrane peptides, also called regulins. The discovery of micropeptides has increased in recent years, mostly because of the small ORFs found in lncRNAs. Several excellent works have analysed the mechanism of interaction of micropeptides with each other and also with the best known SERCA1a (fast muscle) and SERCA2a (heart, slow muscle) isoforms. However, the array of tissue and developmental expressions of these potential regulators raises the question of interaction with other SERCAs. For example, the most abundant calcium pump in neonatal and regenerating skeletal muscle, SERCA1b has never been looked at with scrutiny whether influenced by micropeptides. The possibilities of such interactions will be reviewed in this talk.

F-KA4-1 - OPTOGIN (OPTICALLY GUIDED INDUCED NEURON): A NEW OPTOGENETIC APPROACH TO THE GUIDED DEVELOPMENT OF HUMAN NEURONS, IN VITRO

Krisztina Bauer[1], Ben Mahmoud Maissa[1], Anikó Rátkai[1], Attila Szücs[1], Katalin Schlett[1], Krisztián Tárnok[1]
[1]Department of Physiology and Neurobiology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

The application of neuronal cultures derived from neural stem cells is a useful approach for a number of pharmacological studies, especially in neurodevelopmental disease paradigms. However, ensuring a sufficiently mature phenotype of neural cells presents a number of challenges. The phenotype of a terminally differentiated neuron is determined partly by its own genetic history and partly by the environmental influences. Recent investigations have convincingly demonstrated that the intrinsic biophysical and physiological properties of neurons are strongly influenced by the tonic or synaptic inputs that they receive. This suggests that depolarization “training” by optogenetics using similar environmental stimulation patterns that occur in the developing nervous system may be useful to direct and/or activate cell differentiation and maturation.

In this work, we have systematically analyzed the progress of neuronal development of immature neurons differentiated from the NE-4C mouse neuroectodermal stem cell line and how it is influenced by prolonged (48 h) application of different depolarization patterns. To achieve this, all-trans retinoic acid-induced NE-4C cells were transfected with channel rhodopsin 2 (ChR2-H134R-YFP) plasmid construct on the 6th day of induction, and 24 h later cultures were illuminated for 48 h in the tissue culture incubator with different illumination patterns. As controls, parallel cultures were kept in the dark for 48 hours. For training, we used both an oscillatory (which mimics the patterns that neurons are receiving in the embryonic brain) and a random-distributed light flash sequence (as illumination control).

Depolarization training with oscillatory pattern increased the percentage of neurons exhibiting strong action potential output and altered the cells’ intrinsic membrane properties. Action potential amplitudes increased, and the active membrane properties of the cells proved a more mature neuronal phenotype compared to cells kept in dark. The presence of the inward rectifying K-current (KIR) also increased in response to training. When random distribution pattern was used, values were similar to the dark control. Analyses of KIR2.x channel-subtypes expression by qRT-PCR also showed increased KIR channel expression but only following oscillatory training and not random-illumination.

In conclusion, the applied oscillatory pattern proved to be effective in controlling the differentiation of neurons derived from mouse neural stem cells, promoting the formation of cells with more mature electrophysiological properties and selectively increasing the occurrence of a specific voltage-activated K-current.

The present work is funded by Gedeon-Richter Plc. grant RG-IPI-2020-TP14/025 to K.T., by the VEKOP-2.3.3-15-2016-00007 grant supported by the National Research, Development and Innovation Office, and by the ELTE DT2.0 Cognitive and Molecular Neuroscience Program to K.S.

F-KA4-2 - UNMET TREATMENT NEEDS IN SCHIZOPHRENIA AND NEW DRUG DEVELOPMENT

Thomas Brevig, MD, PhD, HD
Gedeon Richter Plc

Schizophrenia is a chronic mental disorder characterized by early-in-life onset of severe disturbances in thought, perception and behaviour. Symptoms experienced by patients, and their response to treatment, varies greatly between patients and over time in the individual. Despite this heterogeneity, medicines approved for the treatment of schizophrenia are relatively similar. They all reduce dopamine D2 receptor neurotransmission, acting as an antagonist or a partial agonist on this target. Additional receptor actions of these drugs (antipsychotics) differentiate them further at the pharmacological level, but only limited differences in clinical utility have been demonstrated. Perhaps the best example of clinical differentiation is with clozapine, which is approved for the treatment of schizophrenia not responding to other antipsychotics (treatment-resistant schizophrenia). However, side effects are common with clozapine and range from the benign to the potentially lethal (less than 1% experience agranulocytosis, which makes patients susceptible to infections). Because of clozapine's differentiated clinical profile, but limited and typically late use, there has been great interest in its non-D2 receptor actions. The presentation will review key developments to generate new treatment options (monotherapies or adjunctive treatments to be used together with an antipsychotic) based on the pharmacological profile of clozapine as well as new potential mechanisms. It will also outline the unmet needs that these new treatments should address, including partial antipsychotic response with broad or specific persistent symptoms and treatment resistance to the currently available antipsychotics.

F-KA4-3 - FROM PERINATAL INFECTIONS TO SYNAPTIC DYSFUNCTION: NOVEL THERAPEUTIC TARGETS FOR THE TREATMENT OF AUTISM SPECTRUM DISORDER

Beáta Sperlágh[1,2] Dorottya Szabó[1,2], Paula Mut Arbona[1,2], Pál Tod[1], Flóra Gölöncsér[1]
[1]Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Budapest, Hungary, [2]János Szentágothai Doctoral School, Semmelweis University, Budapest, Hungary

Autism spectrum disorder (ASD) is a complex neurodevelopmental condition caused by interactions of environmental and genetic factors. Recently we showed that activation of the purinergic P2X7 receptors is necessary and sufficient to convert maternal immune activation (MIA) to ASD-like features in male offspring mice. Our aim was to further substantiate these findings and identify downstream signaling pathways coupled to P2X7 upon MIA. Maternal treatment with the NLRP3 antagonist MCC950 and a neutralising IL-1β antibody during pregnancy counteracted the development of autistic characteristics in offspring mice. We also explored time-dependent changes of a widespread cytokine and chemokine profile in maternal blood and fetal brain samples of poly(I:C)/saline-treated dams. MIA-induced increases in plasma IL-1β, RANTES, MCP-1, and fetal brain IL-1β, IL-2, IL-6, MCP-1 concentrations are regulated by the P2X7/NLRP3 pathway. Offspring treatment with the selective P2X7 receptor antagonist JNJ47965567 was effective in the prevention of autism-like behavior in mice using a repeated dosing protocol. Moreover, using a modified protocol of MIA offspring schizophrenia-like behavior alterations could also be alleviated by P2X7 receptor inhibition. Our results highlight that in addition to P2X7, NLRP3, as well as inflammatory cytokines, may also be potential biomarkers and therapeutic targets of phenotype elements observed in autism spectrum disorder and other neurodevelopmental psychiatric disorders.

Acknowledegement. This work was supported by the Hungarian Research and Development Fund [grant number 131629], Hungarian Brain Research Program [grant number NAP2022-I-1/2022 to B.S.], the Recovery and Resilience Facility of the European Union within the framework of Programme Széchenyi Plan Plus [grant number RRF-2.3.1-21-2022-00011, National Laboratory of Translational Neuroscience and grant number RRF-2.3.1-21-2022-00015, National Laboratory of Drug Research and Development to B.S.], and the Gedeon Richter plc.

F-KA4-4 - MICROGLIAL DYSFUNCTION AS A POSSIBLE DRIVER OF COVID-19-RELATED NEUROPATHOLOGIES

Ádám Dénes
Institute of Experimental Medicine

COVID-19 is associated with numerous neurological symptoms, including loss of smell and taste, headache, dizziness, nausea, seizures, respiratory distress and autonomic nervous system dysfunction among others. While CNS pathologies are likely to be important contributors to acute illness caused by SARS-CoV-2, complex neurological abnormalities in long-COVID may also persist for several months, with severe impact on the quality of life and overall outcome. At present, the mechanisms underlying COVID-19-related neuropathologies are largely unclear. While the involvement of central and systemic inflammation have been proposed, it is not known how microglia, the main resident immune cells of the CNS parenchyma may contribute to acute- and long-lasting neurological symptoms. To this end, we established a unique autopsy platform allowing the integration of molecular anatomy-, protein- and mRNA data sets in parallel fixed and unfixed post-mortem brain and peripheral organ samples. Nanoscale microscopy revealed heterogenous microglial pathologies at key neuronal- and vascular contact sites in the severely affected medullary autonomic nuclei, cortical and subcortical sites proportionally with viral load, virus-containing intravascular immune cells and vascular inflammation. Microglial dysfunction is linked with diverse neuropathologies. Development of a generalized, but regionally heterogenous proinflammatory response across the brain parallels changes in microglial states. Moreover, central inflammation and microglial states strongly associate with multiorgan virus load-related systemic inflammation via virus-sensing pattern recognition receptors and inflammasomes. This data suggest a considerable role for infection-induced central and systemic inflammation in promoting microglial dysfunction and related neuropathologies via distinct mechanisms.

F-KA4-5 - ASTROCYTE- AND NMDA RECEPTOR-DEPENDENT SLOW INWARD CURRENTS DIFFERENTLY CONTRIBUTE TO SYNAPTIC PLASTICITY IN AN AGE DEPENDENT MANNER IN MOUSE AND HUMAN NEOCORTEX

Andrea Csemer[1], Adrienn Kovács[1], Baneen Maamrah[1], Krisztina Pocsai[1], Kristóf Korpás[1], Álmos Klekner[1], Péter Szücs[1], Péter P. Nánási[1], Balázs Pál[1]
[1]University of Debrecen

Slow inward currents (SICs) are neuronal excitatory events elicited by astrocytic glutamate *via* activation of extrasynaptic NMDA receptors.
By using slice electrophysiology, we showed that SICs can elicit synaptic plasticity. Age-dependence of SICs and their impact on synaptic plasticity was also investigated in both on murine and human cortical slices.

It was demonstrated that SICs elicit a moderate synaptic plasticity with features similar to spike timing dependent plasticity. Furthermore, SIC activity clearly declined with aging in humans and completely disappeared above 70 years of age. While SICs contribute to a form of astrocyte-dependent synaptic plasticity both in mice and humans, this plasticity is differentially affected by aging. In humans, the SIC activity itself is affected, whereas the chance of SICs to elicit synaptic plasticity is decreased in mice.

In conclusion, SICs are likely to contribute to age-dependent physiological and pathological alterations of synaptic plasticity.

F-KB4-1 - CRH AS A REGULATOR OF GASTRIC MUCOSAL SENSITIVITY TO ULCEROGENIC STIMULI

Ludmila Filaretova
Pavlov Institute of Physiology of Russian Academy of Sciences, nab. Makarova, 6; St.Petersburg 199034; Russian Federation. e-mail: filaretovalp@infran.ru

Corticotropin-releasing hormone (CRH) plays a key role in regulating the stress response by stimulating the hypothalamic-pituitary-adrenocortical (HPA) axis. The primary receptors that mediate CRH-induced increase in ACTH levels in stress belong to type 1 CRH receptors on pituitary corticotrophs. ACTH stimulates the adrenal gland to release glucocorticoids that in turn provide life-saving processes of the body. It was also established that CRH exerts a number of biological actions independently of the HPA activation. Gastric ulcer disease remains a serious problem in clinic and stressful lifestyle and non-steroidal anti-inflammatory drugs (NSAIDs) make significant contributions to this disease. Therefore, further clarification of gastroprotective mechanisms is required. We found previously that stress-produced glucocorticoids play a critical role in maintaining the integrity of the gastric mucosa providing gastroprotective effects in several experimental models of gastric injury. Exogenous CRH induces an increase in glucocorticoid production and also may protect the gastric mucosa against stress-induced injury. However, it remained unknown whether glucocorticoids released in response to CRH contribute to the gastroprotective effect of CRH. Our findings on gastroprotective role of glucocorticoids produced during stress allowed us to hypothesize that glucocorticoids may contribute to gastroprotective effect of CRH. To test the participation of corticosterone in the gastroprotective effect of exogenous CRH (1.25 g/kg, i.p., before 3 h cold-restraint stress in rats) two approaches were used: pretreatment by the inhibitor of glucocorticoid synthesis, metyrapone (30 mg/kg, i.p.), and the antagonist of glucocorticoid receptors RU-38486 (20 mg/kg, i.p.). Metyrapone injected shortly before CRF administration caused a fast inhibition of CRF-induced corticosterone response and reversed the protective effect of CRH on the gastric mucosa against the stress-induced erosion. The gastroprotective effect of CRH was also attenuated by the pretreatment rats with glucocorticoid receptor antagonist RU-38486. The results obtained suggest that exogenous CRF may protect the gastric mucosa against stress-induced gastric injury through involvement of glucocorticoids. To extend this idea to indomethacin-induced gastric injury we also studied whether CRH may protect the gastric mucosa against ulcerogenic action of indomethacin (IM) through involvement of glucocorticoids. CRH administration (1.25 µg/kg and 2.5 µg/kg, i.p.) markedly, dose-dependently, increased plasma corticosterone level and significantly, dose-dependently, suppressed the occurrence of gastric erosion induced by IM (35 mg/kg, s.c.) in rats. To estimate the role of glucocorticoids in CRH-induced gastroprotection against IM-induced injury we also used metyrapone and RU-38486 and additionally CRH receptor type 1 antagonist NBI 27914 (10 mg/kg, i.p.). Both metyrapone and NBI 27914 injected shortly before CRH administration caused an inhibition of CRH-induced corticosterone response and prevented protective effect of CRH on the gastric mucosa against the IM-induced erosion. The gastroprotective effect of CRH was also eliminated by the pretreatment with RU-38486. The results obtained suggest that CRH may protect the gastric mucosa against IM-induced gastric injury through involvement of glucocorticoids. In summary, CRH administration may protect the gastric mucosa against stress- and IM-caused injury and CRH-induced glucocorticoids are involved in these

gastroprotective effects of CRH. These data extend our previous understanding of the role played by CRH and glucocorticoids in the maintenance of gastric mucosal integrity. The findings provide further support an idea that activation of the HPA axis plays gastroprotective role.

This study was supported by the State Program 47 GP "Scientific and Technological Development of the Russian Federation "(2019-2030), theme 0134-2019-0001

Symposium
“Renaissance of the corticotropic secretion-stimulating hormone family”
Chair: Zelena Dora
June 9, 2023 (14:40 – 16:10)

F-KB4-2 - THE ROLE OF HYPOTHALAMIC CRH NEURONS IN DIFFERENT STRESS MECHANISMS

Alpár Alán
Department of Anatomy, Histology and Embryology, Semmelweis University, Faculty of Medicine

The hypothalamic-pituitary-adrenal axis is the cascade of the classic stress mechanism. The starting elements of this are corticotrophin-releasing hormone (CRH)-containing neurons located in the paraventricular nucleus, whose hormones reach the anterior lobe of the pituitary gland via the vascular route, the portal circulation. In our work, we have shown that the secretion of CRH is regulated by the calcium sensor protein secretagogin, the disruption of which causes a failure of the stress reaction. At the same time, the CRH neurons of the paraventricular nucleus can also stimulate the ependymal cells of the third cerebral ventricle through a glutamatergic neurotransmission, which secrete ciliary neurotrophic factor (CNTF) into the cerebrospinal fluid. In the fourth cerebral ventricle CNTF reach the extensions of the noradrenergic cells of the locus coeruleus that reach the ventricular wall, and bind to their receptors. They trigger a prolonged stress response that occurs hours later through the activation of the pyramidal cells of the prefrontal cortex. In addition to the stress reaction that is activated within seconds, we were able to demonstrate the neuroanatomical basis of a prolonged stress reaction.

F-KB4-3 - UROCORTINERGIC NEURONS OF THE EDINGER-WESTPHAL NUCLEUS IN ACUTE ALCOHOL EXPOSURE

Al-Omari Ammar[1], Kecskés Miklós[2], Gaszner Balázs[3], Pintér Erika[1], **Kormos Viktória**[1]
[1]University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, [2]University of Pécs, Medical School, Department of Physiology, [3]University of Pécs, Medical School, Department of Anatomy

Introduction: The centrally projecting Edinger-Westphal nucleus (EWcp) expresses the neuropeptide urocortin 1 (UCN1), which belongs to the corticotropin-releasing hormone family. EWcp contributes to the control of alcohol consumption by its UCN1 and cocaine- and amphetamine-regulated transcript (CART) co-expressing peptidergic neurons. We recently showed that the urocortinergic EWcp is the primary seat of central transient receptor potential ankyrin 1 (TRPA1) cation channel mRNA expression. Here we aimed to examine the functional activity of TRPA1 in the EWcp and its possible role in a mouse model of acute alcohol exposure. We hypothesized that alcohol influences the UCN1/EWcp via TRPA1.

Methods: Acute EWcp slices of C57BL/6J male mice were subjected to electrophysiology to prove the functional activity of TRPA1 using a selective and potent agonist, JT010. Male *Trpa1* knockout (KO) and wild-type (WT) mice were compared upon acute alcohol treatment. In both genotypes, half of animals was treated intraperitoneally with 1g/kg 6% ethanol vs. physiological saline controls. Transcardial perfusion was performed 2 hours after the treatment. EWcp neuronal activity was assessed by FOS immunohistochemistry. *Trpa1, Cart* and *Ucn1* mRNA expression as well as UCN1 and CART peptide content was semi-quantified by RNAscope *in situ* hybridization combined with immunofluorescence.

Results: JT010 activated TRPA1 channels of urocortinergic cells in acute slices. Alcohol treatment significantly activated FOS in both genotypes and decreased the *Trpa1* mRNA expression in WTs. Lower UCN1 peptide immunoreactivity was observed in saline-injected KO mice compared to WTs. Alcohol affected the UCN1 peptide content genotype-dependently with decrease in WTs and increase in KO mice. Alcohol exposure influenced neither *Cart* and *Ucn1* mRNA expression nor the EWcp/CART peptide content.

Conclusion: We proved the presence of functional TRPA1 receptors in EWcp/UCN1 neurons. Reduced *Trpa1* mRNA expression and UCN1 peptide content suggest the regulatory role of TRPA1 in UCN1 release upon acute alcohol treatment. The role of EWcp/TRPA1/UCN1 in chronic alcohol consumption and addiction models is under investigation.

Funding
V.K. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00750/22/5), by the New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund (ÚNKP-22-5-PTE-1740) and the Research grant of Medical School, University of Pécs (KA-2022-29).

F-KB4-4 - ROLE OF BRAINSTEM CRH IN STRESS REGULATION

Dóra Zelena
University of Pécs

Adaptation to stress is extremely important for maintaining homeostasis and thus life itself. The prevalence of stress-related disorders is increasing, emphasizing the importance of research exploring stress adaptation. Corticotropin-stimulating hormone (CRH) produced in the hypothalamus plays a fundamental role in the regulation of the stress axis, but CRH is also found in many other brain areas. Although CRH-positive cells are also present in some parts of the brainstem, sometimes even in similar quantities as in the hypothalamus, not much is known about their contribution to stress adaptation. Two nuclei contain a particularly large number of CRH-producing neurons. Barrington's nucleus, which regulates urination, may play a role in stress incontinence, while the inferior olivary complex, which is important in fine motor control, may play a role in stress-related motor problems. The raphe nuclei are primarily known for their serotonin content, but we were able to demonstrate the presence of CRH in the median raphe region (MRR) not only in mice, but also in human samples. Stereological studies using CRH-Cre and GAT-flp mouse strains and appropriate viral vectors confirmed that about 70% of MRR-CRH cells were GABAergic, while another, more ventral population was glutamatergic. We characterized these CRH-containing cells at the mRNA level using RNAscope technique, and determined the inputs and outputs of MRR-CRH cells by combining virus vectors and immunohistochemistry. Chemogenetic stimulation of cells acutely increased the level of stress hormones in the blood. Thus, it is not surprising that in some behavioral tests a more anxious phenotype was also detected in these animals. However, we failed to prove the development of a depression-like behavior pattern after repeated MRR-CRH stimulation in parallel with the fact that not the level of CRH, but its receptor mRNA increased in human suicides. All in all, we managed to identify a new group of brainstem nuclei important in the regulation of acute stress, which can complement the function of the hypothalamus.

F-KB4-5 - MODERATE CHRONIC KIDNEY DISEASE INDUCES ANXIETY IN RATS

Éva Bodnár[1], Katalin Eszter Ibos[1], Hoa Dinh Thi Thanh[2], Zsolt Galla[3], Péter Monostori[3], Júlia Szakács[1], Márta Sárközy[2#], Krisztina Csabafi[1#]
[1]University of Szeged, Albert Szent-Györgyi Medical School, Department of Pathophysiology, Szeged, [2]University of Szeged, Albert Szent-Györgyi Medical School, Department of Biochemisrty, Szeged, [3]University of Szeged, Albert Szent-Györgyi Health Centre, Department of Pediatrics and Pedriatic Health Center, Szeged
[#]Contributing equally

Aims: Chronic kidney disease (CKD) is associated with anxiety, however the exact mechanism is not well understood. Therefore the aim of the present study was to assess the effect of moderate CKD on anxiety in rats.

Methods: CKD was induced by two-phased 5/6 nephrectomy in male Wistar rats, 7 weeks after of which behavioral tests (elevated plus maze (EPM), computerized open field (OF) and marble burying (MB) tests) were performed to assess anxiety-like behavior. At week 9 animals were sacrificed and samples were collected for further analysis. Serum creatinine and carbamide, serum electrolyte concentrations and urinalysis were measured to confirm the presence and severity of CKD. Since amygdala has a major role in the regulation of anxiety, amygdala samples were obtained, in which we measured the gene expression and the CRH system (CRH, CRH receptors 1 and 2). Furthermore, plasma metabolites were measured: uremic toxins (p-cresyl-sulfate, indoxyl-sulfate), compounds of tryptophan (kynurenine, kynurenic acid, 3-hydroxykynurenine, anthranilic acid, picolinic acid, quinolinic acid, and xanthurenic acid) and tyrosine (tryptophan, tryptamine, 5-hydroxytryptophan, serotonin and tyrosine) metabolism.

Results: Laboratory tests showed that the serum creatinine and carbamide levels significantly increased in the 5/6 nephrectomized rats. Serum calcium, magnesium and cholesterol concentrations were elevated, whereas urine creatinine level decreased in the CKD group. In the EPM test, the total number of entries and central time of CKD animals decreased compared to control, whereas the closed arm time increased. In the OF test rearing activity and central ambulation distance and time decreased in the CKD group. Furthermore, in the MB test, CKD animals showed less interaction with the marbles. In the amygdala CKD evoked an increase in CRH, CRH receptors 1 and 2 gene expression. Finally, compared to the control group, in the CKD group a significant increase was found in the concentrations of p-cresyl-sulfate, indoxyl-sulfate, kynurenine, kynurenic acid, 3-hydroxykynurenine, anthranilic acid, xanthurenic acid, 5-hydroxyindoleacetic acid, picolinic acid and quinolinic acid. There was a significant decrease, however, in the levels of tryptophan, tryptamine, 5-hydroxytryptophan, serotonin and tyrosine.

Conclusion: Moderate CKD evoked anxiety-like behavior that might be mediated by the shift of the metabolism of tryptophane from the indole and serotonin pathways to the kynurenine pathway as well as the increased expression of the CRH system in the amygdala evoked by CKD.

F-KA5-1 - THE ROLE OF PATTERN RECOGNITION RECEPTORS IN PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL PROCESSES

Szilvia Benkő
Inflammationphysiology Research Group, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Nod-like receptors (NLRs) are cytosolic pattern recognition receptors that sense pathogen- or danger-associated molecular patterns (PAMPs, DAMPs). They may form protein complexes (inflammasomes) that drive IL-1b pro-inflammatory cytokine and pyroptosis; or regulate signal transduction pathway leading to the activation of gene expression, cell division, autophagy/mitophagy or cytokine production during inflammation. Many of the NLRs are expressed mainly in the cells of the innate immune system (like monocytes, macrophages), however some of the NLRs also present in other immune competent cells (like lymphocytes) or non-immune cells (like epithelial or muscle). A brief overview of the field, and some of our recent findings related to macrophages and skeletal muscle will be shown in the presentation.

Financial support: NKFIH-OTKA K131844

F-KA5-2 - TYROSINE KINASE PATHWAYS IN MONOSODIUM URATE CRYSTAL-INDUCED INFLAMMATORY PROCESSES

Krisztina Futosi[1,2], Tamás Németh[1,3,4,5], Ádám Horváth[6,7], Zsuzsanna Helyes[6,7,8] and Attila Mócsai[1,2]
[1]Department of Physiology, School of Medicine, Semmelweis University, Budapest, Hungary; [2]ELKH-SE Inflammation Physiology Research Group, Eötvös Loránd Research Network and Semmelweis University, Budapest, Hungary; [3]MTA-SE "Lendület" Translational Rheumatology Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, Hungary; [4]Department of Rheumatology and Clinical Immunology, Semmelweis University, Budapest, Hungary; [5] Department of Internal Medicine and Oncology, Semmelweis University, Budapest, Hungary; [6]Department of Pharmacology and Pharmacotherapy, Medical School, [7]János Szentágothai Research Centre, Centre for Neuroscience, University of Pécs, Pécs, Hungary and [8]PharmInVivo Ltd., Pecs, Hungary.

Gout is the most prevalent form of inflammatory arthritis, characterized by monosodium urate (MSU) crystal-induced activation of innate immune cells. Despite its high prevalence, the molecular mechanisms of gouty arthritis is poorly understood. Here we show that the myeloid Src-family kinases Hck, Fgr and Lyn play a critical role in MSU crystal-induced neutrophil and macrophage activation and the in vivo development of MSU crystal-induced arthritis.

The *Hck*$^{-/-}$*Fgr*$^{-/-}$*Lyn*$^{-/-}$ triple knockout mutation abrogated MSU crystal-induced ROS production of neutrophils and macrophages and diminished neutrophil chemokine, cytokine and LTB_4 release. *Hck*$^{-/-}$*Fgr*$^{-/-}$*Lyn*$^{-/-}$ mice were strongly protected from various signs of MSU crystal-induced in vivo arthritis including edema formation, leukocyte infiltration and local accumulation of ROS, chemokines and cytokines. Analysis of single and double knockout mice indicated substantial functional overlap between Hck, Fgr and Lyn both in vitro and in vivo. Mechanistically, the *Hck*$^{-/-}$*Fgr*$^{-/-}$*Lyn*$^{-/-}$ mutation inhibited phagocytosis of MSU crystals and ERK/p38 MAP kinase activation but did not affect MSU crystal-induced NETosis, a putative limiting component of gouty arthritis. Dasatinib, a clinically used Src-family kinase inhibitor, abrogated MSU crystal-induced functional responses of human neutrophils and inhibited experimental gouty arthritis in both prophylactic and therapeutic settings.

Taken together, our results identify the myeloid Src-family kinases Hck, Fgr and Lyn as critical components of MSU crystal-induced in vitro and in vivo inflammatory responses and suggest myeloid Src-family kinases as potential therapeutic targets in acute gout flares.

F-KA5-3 - THE SOLUBLE PATTERN RECOGNITION MOLECULES PENTRAXINS, COMPLEMENT AND THEIR COLLABORATION IN HEALTH AND DISEASE

Mihály Józsi[1,2]
[1]Department of Immunology, ELTE Eötvös Loránd University, Budapest, [2]MTA-ELTE Complement Research Group, Eötvös Loránd Research Network (ELKH), at the Department of Immunology, ELTE Eötvös Loránd University, Budapest

Soluble pattern recognition molecules, to which the family of pentraxins and proteins of the complement system also belong, are ancient components of the innate immune system. Pentraxins can be considered innate immune analogs of antibodies, because they recognize selected pathogens and host structures, can bind to Fc-receptors and activate complement. Some pentraxins are upregulated systemically upon infection and inflammation, while others are expressed in a more cell- and tissue-specific manner. Petraxins can bind the initiator or recognition molecules of the various complement pathways, but also interact with soluble complement regulators, allowing for a fine-tuned balance of complement activation and inhibition, which results in an optimal extent of opsonization of target surfaces. The presentation will provide a brief overview of the relations of pentraxins and the complement system, exemplified by the interactions between various pentraxins and members of the human complement factor H protein family, and how these influence complement activation and opsonization under normal and disease-related conditions.

F-KA5-4 - THE EPIGENETIC BASES OF DISTINCT IMMUNOMODULATORY FACTORS AND PATHOGEN-DERIVED MOLECULES-REGULATED PD-L1 AND PD-L2 EXPRESSION IN MURINE AND HUMAN MACROPHAGES

Zsofia Varga[1], Eszter Varadi[1,2], Marta Toth[3], Anett Mazlo[3], Attila Bacsi[3,4], Gergely Nagy[5], Laszlo Nagy[5,6], Gabor Szebeni[7], Balint Csorgo[8], Bence Daniel[9], Zsolt Czimmerer[1,10]
[1] Institute of Genetics, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [2] Doctoral School in Biology, University of Szeged, Szeged, Hungary, [3] Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen,Hungary, [4] ELKH-DE Allergology Research Group, Debrecen, Hungary, [5] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [6] Departments of Medicine and Biological Chemistry, Johns Hopkins University, School of Medicine, Institute for Fundamental Biomedical Research, Johns Hopkins All Children's Hospital, St. Petersburg, FL, USA, [7] Laboratory of Functional Genomics, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [8] Institute of Biochemistry, Biological Research Centre, Eotvos Lorand Research Network, Szeged, Hungary, [9] Department of Pathology, Stanford University, Stanford, CA 94305, USA, [10] Corresponding author: czimmerer.zsolt@brc.hu

Macrophages are phenotypically and functionally diverse elements of the innate immune system driven by microenvironment-induced complex polarization programs. Two endpoints of polarization are interleukin-4 (IL-4)- induced alternatively and interferon (IFN)γ or lipopolysaccharide (LPS)-activated classically activated macrophages.

Programmed death ligand 1 (PD-L1) and PD-L2 interact with the PD-1 receptor and operate as negative costimulatory proteins involved in the functional activity of T cells. Macrophages also express these PD-1 receptor ligands, ensuring their effect in the control of T cell tolerance. However, the regulating role of macrophage polarization signals on the expression of PD-L1 and PD-L2 is poorly understood.

We investigated the regulatory role of macrophage polarization signals on the expression of PD-L1 and PD-L2 genes at the transcriptional and epigenetic levels in bone marrow-derived murine and monocyte-derived human macrophages. According to RT-qPCR and flow cytometry results, macrophage polarization signals triggered the induction of PD-L1 at the gene and protein levels, which is significantly higher in LPS- or IFNγ-treated cells. PD-L2 shows increased expression in IL-4-polarized murine macrophages, but in human macrophages IFNγ is also involved in its regulation.

Based on Hi-C and ChIP-sequencing data, we identified the genomic regions responsible for regulating *Cd274/Pdl1* and *Pdcd1lg2/Pdl2* genes. Transcription factors activated by polarization signals show different binding patterns on regulatory elements in the coding regions of *Cd274/Pdl1* and *Pdcd1lg2/Pdl2*. STAT6 shows an intense binding pattern on enhancers closer to the *Pdcd1lg2/Pdl2* gene segment, while STAT1 peaks occurred more frequently on the regulatory element of *Cd274/Pdl1*. However, the opposite polarization signals IL-4 and IFNγ synergistically increase the expression of *Pdcd1lg2/Pdl2.* IL-4/IFNγ-co-treated cells were associated with more intense STAT1 transcription factor binding on the *Pdcd1lg2/Pdl2* promoter and enhancers, which may result by IL-4-mediated chromatin openness.

Our findings establish that macrophage polarization signals regulate differently the expression of PD-L1 and PD-L2, but IL-4/IFNγ co-treated murine macrophages show synergistic *Pdcd1lg2/Pdl2* induction.

F-KA5-5 - CARDIOTOXIC EFFECTS OF IMMUNE CHECKPOINT INHIBITORS

Zoltán Varga[1,2,3], Tamás Gergely[1,2,3]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary, [3]MTA-SE Momentum Cardio-Oncology and Cardioimmunology Research Group, Budapest, Hungary

Immune checkpoint molecules are physiological regulators of the adaptive immune response. Immune checkpoint inhibitors, such as anti-PD-1 or anti-CTLA-4 monoclonal antibodies, revolutionized cancer treatment and their clinical use is rising rapidly, reflected by thousands of ongoing clinical trials. However, they cause various immune-related adverse events, including acute and chronic cardiovascular toxicities. Of these, ICI-induced acute fulminant myocarditis is the most studied direct cardiotoxicity, although emerging clinical and preclinical data suggests the importance of other ICI-related chronic cardiac toxicities as well, such as accelerated atherosclerosis, heart failure and cardiac dysfunction. These chronic cardiovascular toxicities often remain hidden, as they may only appear in the presence of other co-morbidities. The mechanisms of ICI-induced myocarditis and cardiac dysfunction seem to have differences: myocarditis develops as a specific autoimmune response against cardiac cells, involving CD8+ T cells, while cardiac dysfunction after ICI therapy is related to disturbed cardiac transcriptomic and metabolic effects, pro-inflammatory cytokine expression and antibody-mediated ventricular dilation. The occurrence of these cardiac effects suggests a potential role of immune checkpoint molecules in maintaining cardiovascular homeostasis, and disruption of physiological immune checkpoint signaling may lead to cardiac pathologies, including heart failure.

F-KA5-6 - DROSOPHILA MODELS OF NEUROINFLAMMATION AND INFLAMMATORY BOWEL DISEASE

Gábor Juhász[1,2], Áron Szabó[1], Virág Vincze[1], Aishwarya Chhatre[1]
[1]Biological Research Centre, Szeged, [2]Eötvös Loránd University, Budapest

Drosophila is a popular animal model used for the study of not only cell and developmental biology, but also for understanding human disease mechanisms as well as for the screening of large drug libraries, which we are currently undertaking as part of the Biotechnology National Laboratory.

In my talk, I will introduce a Drosophila model of inflammatory bowel disease, which was inspired by the GWAS-based identification of variants in human Atg16L1 that predispose to Crohn's disease. Atg16L1 and its homologs are involved in autophagy, a lysosomal self-degradation pathway. We found that disrupting the C-terminal WD40 domain of Drosophila Atg16 does not affect autophagy but it leads to large-scale intestinal inflammation based on changes in gut morphology, intestinal stem cell signaling, and increased inflammatory cytokin and antimicrobial peptide production.

We next embarked on the analysis of the role of Atg16 and other autophagy genes after axon injury in the perpheral nervous system and the brain in Drosophila. Surprisingly, we found that a subset of Atg genes (including the WD40 domain of Atg16) is required in glia for the proper clearance of axon fragments. Our genetic, biochemical and microscopy analyses revealed that LC3-associated phagocytosis (LAP), a process that utilizes a subset of autophagy genes, is required for phagosome maturation in glia that is important for engulfed axon fragment clearance. Importantly, Atg16 WD40 domain mutant flies turned out to be more sensitive to traumatic brain injury (TBI), while their lifespan is similar to controls without TBI. Further studies are under way to understand the inflammatory consequences of glial autophagy and LAP defects in Drosophila.

F-KB5-1 - RAMAN SPECTRAL SIGNATURES OF PLASMA-DERIVED EXTRACELLULAR VESICLE-ENRICHED ISOLATES SUPPORT THE DIAGNOSIS OF DIFFERENT CANCEROUS DISEASES

Matyas Bukva[1,2,a,b], Edina Sebestyen-Gyukity[1,a], Timea Boroczky[1,2,12], Yasmin Ranjous[3], Maria Harmati[1], Gabriella Dobra[1,2], Adrienn Jenei[4], Laszlo Szivos[5], Katalin Hideghety[6,7], Krisztina Budai[8], Judit Olah[7], Peter Horvath[1], Gyorgy Lazar[9], Tamas Biro[10,11], Zoltan Konya[3], Pal Barzo[5], Almos Klekner[4], Krisztina Buzas[1,12,13,*]

[1]Laboratory of Microscopic Image Analysis and Machine Learning, Institute of Biochemistry, Biological Research Centre, Szeged, Hungary, [2]Doctoral School of Interdisciplinary Medicine, University of Szeged, Szeged, Hungary, [3]Department of Applied and Environmental Chemistry, University of Szeged, Szeged, Hungary, [4]Department of Neurosurgery, Clinical Centre, University of Debrecen, Debrecen, Hungary, [5]Department of Neurosurgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [6]ELI-ALPS, ELI-HU Non-Profit Ltd., Szeged, Hungary, [7]University of Szeged, Faculty of Medicine, Department of Oncotherapy, Szeged, Hungary, [8]Department of Surgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [9]Department of Surgery, Faculty of Medicine, University of Szeged, Szeged, Hungary, [10]Department of Immunology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [11]Monasterium Laboratory, Münster, Germany, [12]Department of Immunology, Faculty of Medicine, University of Szeged, Szeged, Hungary, [13]Department of Immunology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary

a Co-first authors
b Presenting author
* Corresponding author

Introduction

Spectroscopic analysis of the molecular composition of small extracellular vesicles (sEVs) is a promising but underexplored method for diagnosing cancerous diseases, particularly central nervous system tumors. Using a sufficient number of clinical samples and Raman spectroscopic analyses, we attempt to elucidate the potential role of plasma-derived sEVs in diagnosing seven distinct patient groups.

Methods

The study is conducted in accordance with the Declaration of Helsinki, informed consent forms are collected and the study was approved by national ethics committee. Up to 490 plasma samples will be obtained from seven patient groups (glioblastoma multiforme, meningioma, melanoma and non-melanoma brain metastasis, colorectal tumors, melanoma and a control group). SEV isolation is performed through differential centrifugation. The isolates are characterized by Western Blot, transmission electron microscopy and nanoparticle tracking analysis. Principal Component Analysis–Support Vector Machine algorithm is performed on the Raman spectra for classifications. Classification accuracy, sensitivity, specificity and the Area Under the Curve (AUC) value are used to evaluate the performance of classification.

Results

According to preliminary results, the patient groups are distinguishable with 80–95% sensitivity and 80–90% specificity. AUC scores of 0.82–0.9 suggest excellent classification performance.

Conclusion

Our findings indicate that Raman spectroscopic analysis of sEV-enriched plasma isolates is a promising strategy for the development of noninvasive, cost-effective methods for the clinical diagnosis of various cancers.

Funding

GINOP-2.2.1–15-2017–00052; ÚNKP-22-3-New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund; University of Szeged, Faculty of Medicine, Szent-Györgyi Albert Research Fund (2021)

F-KB5-2 - CIRCULATING EXTRACELLULAR VESICLE LEVELS ARE INVERSELY CORRELATED TO CHOLESTEROL LEVELS IN HYPERCHOLESTEROLEMIA MOUSE MODELS

Brachyahu Meir Kestecher*[1, 2,3], Sayam Ghosal[1, 2], Bernadett Réka Bodnár[1, 2], Adrienn Szabó[1, 2], Nabil Sayour[4], Tamás Gergely[4], Krisztina Németh[2,3], Zoltán Giricz[4], Zoltán Varga[4], Péter Ferdinandy[4], Edit I Buzás[1, 2,3], Xabier Osteikoetxea[1, 2]
*Presenting Author
[1]HCEMM-SU Extracellular Vesicles Research Group, Budapest, Hungary Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [2]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, [3]ELKH-SE Translational Extracellular Vesicle Research Group, [4]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Abstract:

Introduction:

Atherosclerotic Cardiovascular Disease (ACVD) contributes to over 40 % of total deaths in Western society. Low-density lipoprotein (LDL) is a major player in the genesis of ACVD. Earlier, our laboratory demonstrated that LDL and extracellular vesicles (EVs) associate in blood plasma: allowing us to hypothesise that EVs may play a role in atherogenesis.

Methods:

Here, 11-week-old male C57BL-6 PCSK9-KO (n=6) and LDLR-KO mice (n=6) were used to study the effects these proteins play in atherogenesis, cardiac function, body mass (BM), blood plasma EV-, as well as cholesterol levels. 11-week old male C57BL-6 mice were used as controls (n=6). Mice were fed high fat diet (HFD) for 12 weeks. Cardiac function was assessed using a Vevo F2 LAZR-X Ultra-high to Low-Frequency Ultrasound. Blood was obtained from the retrobulbar venous plexus, or the vena cava and atherosclerotic plaque was analysed in post-mortem using Oil-Red-O staining of the aortic arch. EV levels were measured by flowcytometry in accordance with MISEV2018 and MIFlowCyt-EV guidelines.

Results:

We found that LDL and total cholesterol levels were significantly reduced in the PCSK9-KO group (p=0.0022 and p=0.0006, respectively), whereas LDLR-KO showed significant increase (p=0.0003 and p=0.0004, respectively). High-density lipoprotein (HDL) showed no significant changes. Based on Annexin V binding and CD63 expression, a significant increase in EV levels was observed in the PCSK9-KO (p=0.0184 and p=0.0411, respectively) and LDLR-KO groups (p=0.0003 and p=0.0411, respectively). CD81 expression did not change in the PCSK9-KO group. However, a significant reduction was observed in the LDLR-KO group (p=0.0026). Oil-Red-O staining showed no change in arterial plaque build-up. The PCSK9-KO group had a significantly higher BM (p=0.0405). PCSK9-KO mice showed significantly improved cardiac output (p= 0.0005) and ejection fraction (p=0.0022), and LDLR KO only showed significant increase(p=0.0087) in the ejection fraction. Fractional shortening was also shown to be prognostically favourable only in the PCSK9-KO group (p=0.0007).

Conclusions:

PCSK9-KO mice show an overall prognostically favourable cardiovascular function after HFD. With increased BM and adipose tissue, however, maintenance of HFD with PCSK9-KO may lead to other obesogenic health determinants. Despite high cholesterol levels, LDLR-KO mice do not exhibit signs of decreased cardiovascular function. It was also observed that within

each group, as cholesterol levels increased, the EV levels were reduced indicative of an inverse relationship between circulating cholesterol and EVs.

Keywords:
Atherosclerosis, extracellular vesicles, low density lipoproteins, cardiac function

Funding:
This work was funded the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No 739593, as well as RRF-2.3.1-21-2022-00003, 2019-2.1.7-ERA-NET-2021-00015 and 2019-2.1.7-ERA-NET-2021-00015.

F-KB5-3 - THE ROLE OF THE ENHANCER REGULATION

Ildikó Miklya
Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

According to Knoll's definition: „Enhancer-sensitive neurons are capable of changing excitability and work on a higher activity level due to natural or synthetic enhancer substances."

Selegiline/(-)-deprenyl, the β-phenylethylamine (PEA)-derivative, now a worldwide used selective MAO-B inhibitor drug to treat Parkinson's disease (PD) and major depression disorder (MDD), catalyzed the discovery of the catecholaminergic activity enhancer (CAE) effect, which is unrelated to MAO-B inhibition. This finding clarified that PEA, a highly potent releaser of catecholamines from their intraneuronal pools, is still classified as the prototype of the indirectly acting sympathomimetics. However, under natural conditions PEA acts as a selective CAE substance, but we cannot use therapeutically because of the very short duration effect of the compound. The catecholamine releasing effect of PEA is exerted in much higher than the physiological concentrations of this trace-amine. This property of PEA completely concealed its CAE effect, which remained unidentified. Selegiline/(-)-deprenyl, the unique PEA-derivative which is devoid of the catecholamine-releasing property, is a highly selective long-acting CAE substance in nanomolar concentration range.

Knoll realized later that tryptamine, the well-known serotonin releaser, in low concentration is a peculiar serotonergic activity enhancer substance, and BPAP (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine, was developed as a synthetic selective enhancer compound, which is hundred times more potent enhancer substance than selegiline/(-)-deprenyl, and now is the best experimental tool to detect the enhancer-sensitive regulations in the mammalian brain.

Selegiline/(-)-deprenyl and (-)BPAP also played major role in the development of anti-aging research. These compounds can significantly improve the learning performance, extend the animal lifespan, and moreover suppress the tumor manifestation during the lifelong experiment, so improve the essential factors of healthy aging.

Besides that, in the recent study proved the enhancer-sensitivity of the glutamaterg transmitter system and described the enhancer-induced stabilization of the stress-hormone level. Furthermore, the synthetic enhancers increased the gene expression of BDNF in the hypocampus.

After that, question arises, what is the mechanism of the action of the enhancer substances?

The natural and the synthetic enhancer compounds can bind to the TAAR1 receptor, which has an important role in regulating of neurotransmission in the central nervous system and the neuroimmune system function, as well. The other component of the specific enhancer effect, that the synthetic enhancers can antagonize the tetrabenazine VMAT2 inhibitor effect, so we can call the enhancer substances as "vesicle loader compounds".

F-KB5-4 - THE ENHANCER COMPOUND (-)BPAP FACILITATES DOPAMINERGIC NEUROTRANSMISSION VIA ACTIVATION OF TAAR1 SIGNALING

László G. Hársing[1], Jr., Júlia Timár[1], Ildikó Miklya[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

Background. Trace amines (phenylethylamine, tyramine, tryptamine), classical amines (noradrenaline, dopamine, serotonin), and amphetamines were demonstrated to act as agonists on trace amine associated receptor 1 (TAAR1), a Gs protein-coupled intracellularly located metabotropic receptor present in the central nervous system. A series of trace amine analogues has been recently synthesized and investigated on neurotransmitter release in various rat brain areas[1]. These compounds are designated as enhancer drugs uniquely increase stimulation-induced neurotransmitter release in the low nanomolar concentration range without altering resting neurotransmitter efflux.

Aim. To search the mechanism of action of the enhancer compounds how they potentiate neurochemical transmission.

Methods. Slices of the striatum were prepared from rat brain, loaded with [^{3}H]dopamine, superfused, and the efflux of radioactivity was determined at rest and in response to electrical stimulation. Of the enhancer compounds, (-)BPAP ((*R*)-(-)-1-benzofuran-2-yl)-2-propylaminopentane) was selected for investigation in comparison with the releaser compound (±)methamphetamine. One-way ANOVA followed by the Dunnett's test and the Student paired *t*-test were used for statistical analysis.

Results. It was found that (-)BPAP (10^{-12}-10^{-11} mol/L) potentiated the electrical stimulation-induced [^{3}H]dopamine release and the TAAR1 antagonist EPPTB (10^{-8}-10^{-7} mol/L) suspended this effect. On the contrary, (±)methamphetamine (10^{-6}-10^{-5} mol/L) evoked increase of the resting [^{3}H]dopamine release in an EPPTB-dependent manner. Subsequent analyses indicated that the non-vesicular [^{3}H]dopamine release evoked by (±)methamphetamine and the (-)BPAP-induced vesicular [^{3}H]dopamine release were due to an increase in protein kinase C (PKC)-mediated phosphorylation. Furthermore, the (-)BPAP may primarily target vesicular [^{3}H]dopamine as was suggested by the finding that N-ethylmaleimide, which inhibits SNARE core complex disassembly, potentiated the enhancer effect.

Cunclusion. We have hypothesized that there are two binding sites present on TAAR1, one for the enhancer and one for the releaser compounds, and they activate two different PKC-mediated phosphorylation processes coupled to TAAR1 signaling. Releaser compounds may induce phosphorylation that targets plasma dopamine transporter resulting in reverse mode operation of the carrier and a consequent increase of resting dopamine release. On the contrary, the enhancer compound primarily affects PKC-mediated phosphorylation of proteins involved in exocytosis leading to increase the electrical stimulation-induced vesicular dopamine release. We have concluded that TAAR1 and its signaling possess a central governing role of presynaptic dopamine release originated from the vesicular and cytoplasmic neurotransmitter pools, respectively[2].

References. [1]Knoll, J. et al., Br. J. Pharmacol., 1999, 128, 1723-1732; [2]Harsing, L. G., Jr. et al., Int. J. Mol. Sci., 2022, 23, 8543.

F-KB5-5 - COMBINATION OF ENHANCER COMPOUNDS (-)DEPRENYL AND (-)BPAP WITH MAO-B INHIBITOR RASAGILINE WHICH FAILS TO SHOW ENHANCER ACTIVITY

Júlia Timár[1], László G. Hársing[1], Jr., Ildikó Miklya[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest

(-)Deprenyl (selegiline) was introduced as a selective MAO-B inhibitor for the treatment of Parkinsonian disorder in the 70's. Besides inhibiting B-type MAO enzyme, in lower doses it possesses a catecholaminergic activity enhancer effect, too. The other selective MAO-B inhibitor, rasagiline started to be used in the 90's, however, seems to lack the enhancer activity.

Aim of the present work was to further analyse the difference between the activity of enhancer compounds, like (-)deprenyl or (-)BPAP (benzofuranylpropylaminopentane) and the non-enhancer rasagiline, comparing their effects on learning and memory under difficult circumstances, when the cognitive capacity is impaired for example by age or by memory impairing drugs. In line with this the consequence of co-administration of the two drugs was also studied.

Method. Two types of behavioural tests were applied; the shuttle box test, where acquisition, retention, and consolidation processes are measured and the passive avoidance (PA) test where subjects have to learn to avoid an environment in which an aversive stimulus was previously delivered.

Results. In the shuttle box test there was no difference between the compounds in 3 months old animals who were treated with the drugs for one month - (-)deprenyl and rasagiline 0.001 mg/kg, (-)BPAP 0,0001 mg/kg – but in aged (12 months old) animals after 10 months treatment the acquisition ability of rasagiline-treated animals was significantly lower, than that of saline or enhancer treated ones. At the same time the tetrabenazine (TBZ) impaired learning capacity was restored by the enhancer drugs (5 days treatment), but not with rasagiline. When rasagiline was co-administered with (-)deprenyl or (-)BPAP, their restoring effect was completely abolished. In PA test, both scopolamine and morphine impair the memory, which effect can be antagonized by the enhancer compounds, but not by rasagiline.

Conclusion. The results support the possibility that rasagiline is not simply devoid of enhancer activity but it can affect the action of enhancer compounds, actually it can antagonize their effect. This results raise the possibility of differences between the mode of action of selegiline and rasagiline.

F-KB5-6 - EFFECT OF THE ENHANCER COMPOUND (-)BPAP ON THE AGE-RELATED DECLINE OF VARIOUS COGNITIVE FUNCTIONS

Aliz Judit Ernyey[1], Ferenc Kassai[1], Kata Kozma[1], Imola Plangár[1], Zsuzsa Somfai[1], Ildikó Miklya[1], István Gyertyán[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University Budapest

In the present study effect of the putative anti-aging compound (2R)-1-(1-benzofuran-2-yl)-N-propylpentane-2-amine ((-)BPAP, a deprenyl derivative) was investigated on cognitive decline in experienced, aged Long-Evans rats. During their lifetime, animals had acquired knowledge in various cognitive assays: five-choice serial reaction time task (5CSRTT, measuring attention), Morris water maze (MWM, paradigm of spatial learning), a cooperation task, carried out in pairs (modelling social cognition) and a motor skill-learning task „pot-jumping" (PJT). Their performance in these tests was then parallel followed from the age of 27 months until their death meanwhile half of them were treated with BPAP, the other half with saline.

Cognitive performance in various tasks showed different sensitivity/resistance to age-related impairment. Pot jumping performance started to impair first, at 21 months of age, followed by decreasing performance in 5CSRTT at 26 months. As third, navigation performance in Morris water-maze started to decline at 31 months. Performance in a cooperation task started to decline the latest, at 34 months of age.

Our findings suggest that in this process the primary factor was the level of motivation to be engaged with the task and not losing the acquired knowledge. The average lifespan of the whole tested rat population was 36 months. BPAP could not improve the cognitive performance, neither could it prolong lifespan. A possible reason might be that dietary restriction and lifelong cognitive engagement had beneficial effects on cognitive capabilities and lifespan ("healthy aging") creating a "ceiling effect" for further improvement. The results confirmed that experienced animals provide a translationally relevant model to study age-related cognitive decline and measure the effect of putative anti-aging compounds.

F-KB5-7 - ENHANCER COMPOUNDS AND STRESS CONDITIONS

Dóra Zelena
University of Pécs, Medical School, Institute of Physiology

Stress is without doubt promote the development of diseases and reducing stress can contribute to healthy aging. According to Selye, the father of stress concept, the major component of stress adaptation is the hypothalamic-pituitary-adrenocortical axis. In rodents its major effector molecule is the corticosterone.

We aimed to test whether the catecholaminerg enhancer drugs selegiline/(-)-deprenyl and BPAP (2*R*)-1-(1-benzofuran-2-yl)-*N*-propylpentane-2-amine in low, enhancer concentration (but not in monoamine oxidase effecting higher concentration) is able to reduce stress as a possible mechanism of their life-prolonging effect.

Using subchronic and chronic treatment we repeatedly confirmed that the enhancer doses of these compound were able to reduce resting corticosterone concentrations. We used adult as well as old (up to 2 year) male rats and the effect was always visible. The anxiety-like behavioural changes were not deeply influenced among normal conditions only when we tested the animals after sever stress (e.g. elevated plus maze (EPM) right after electric footshock).

These observations were in line with the known effect of these enhancer drugs on healthy aging and underline that they are not influence normal behaviour (i.e. fear from open spaces tested on EPM is normal), but can prevent stress-induced pathological alterations.

ACÉLLABOR

ROZSDAMENTES LABORATÓRIUMI BERENDEZÉSEK

Kísérleti állattartó rendszerek, szellőztetett állványok

Egyedi rozsdamentes bútorok, pocrendszerek

Sterilező kosarak, ipari mosogatók, kiegészítők

ACÉLLABOR KFT.
H–2220, Vecsés, Mátyás u. 2.
tel: +36 29 350 369
fax: +36 29 552 331
info@acellabor.hu
www.acellabor.hu

Biokasztel

www.kasztel.hu

Molecular Biology
Antibody /Reagent
Consumable
Filtration
Service/Synthesis
Plant Research
Veterinary
Rapid test

FAMÉ 2023

Mátraháza
7–9 June 2023

Poster abstracts

MCMXXXI
SOCIETAS
PHYSIOLOGICA
HUNGARICA
M.É.T

M M V B T

HUPHAR
Hungarian Society for Experimental
and Clinical Pharmacology

Youth Posters Physiology

INVESTIGATION OF ODONTOBLAST MECHANOSENSITIVITY

Márk Racskó[1,3], Árpád Kunka[1,2,3], Judit Bohács[4], Erika Lisztes[1], Gabriella Kovalecz[5], Etelka D. Tóth[6], Kinga Bágyi[4], Rita Marincsák[4], Balázs István Tóth[1]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen
[2]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, University of Debrecen,Debrecen, [3]Doctoral School of Molecular Medicine, University of Debrecen, Debrecen. [4]Department of Operative Dentistry and Endodontics, Faculty of Dentistry, University of Debrecen, Debrecen, [5]Department of Pediatric and Preventive Dentistry, Faculty of Dentistry, University of Debrecen, Debrecen, [6]Department of Dentoalveolar Surgery, Faculty of Dentistry, University of Debrecen, Debrecen

Odontoblasts are highly differentiated specialized cells that form the outermost layer of dental pulp tissue, lining the inner surface of dentin. They play a double role in dental homeostasis, responsible both for dentin production and the transduction of physical stimuli from the outside environment to nearby nerve endings. They can detect mechanical and thermal impulses applied to the tooth by sensing the movement of fluid in the dentinal tubule induced by these forces via mechanosensitive ion channels. However, several different mechanosensitive channels from the TRP and Piezo families have been reported to be expressed by these cells.

In our current research, we aim to investigate which of these channels are functionally present in human odontoblasts and how significant of a role they play in the marked mechanosensitivity exhibited by these cells.

We perform our experiments on odontoblast like cells differentiated from cultured primary dental pulp cells. Cultures were first established from pulps of healthy third molars removed from adult patients at the Faculty of Dentistry, then differentiation is induced by the addition of β-glycerophosphate to culture media. Odontoblast phenotype was confirmed by Alizarin Red staining. Ion channel functionality was investigated using the intracellular Ca^{2+} dye Fura-2 AM and pharmacological treatment using channel agonists and antagonists. Mechanical stimulation was simulated by application of a hypoosmotic solution or rapid fluid movement created by the pipetting function of the Flexstation III spectrometer used for measurements.

Agonists of Piezo1, TRPV2, TRPV4, TRPA1 were found to elicit significant increases in intracellular Ca^{2+} concentration, which were inhibited by specific antagonists of these channels. Previous studies on rodents have implicated TRPM8 in dental cold sensitivity as well, however we were unable to detect the presence of this channel in human odontoblasts, as menthol induced Ca^{2+} signals were abolished by a specific antagonist of TRPA1 and not by an antagonist specific to TRPM8. Increases in intracellular Ca^{2+} content induced by mechanical stimulation were also prevented by antagonists of the aforementioned channels to varying degrees, with Dooku1 and HC067047, antagonists of Piezo1 and TRPV4 respectively, showing the largest effect. In summary, while recent experiments in this field have mostly focused on Piezo1, our results indicate that several different ion channels may play a role in odontoblast mechanosensation.

FUNCTION OF A MUTANT RYANODINE RECEPTOR (T4709M) LINKED TO CONGENITAL MYOPATHY

Zsuzsanna Édua Magyar[1], János Almássy[2]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary,
[2]Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Physiological muscle contraction requires an intact ligand gating mechanism of the ryanodine receptor 1 (RyR1), the Ca^{2+}-release channel of the sarcoplasmic reticulum. Some mutations impair the gating and thus cause muscle disease. The mutation T4706M is linked to myopathy, characterized by severe muscle weakness. Although, the low expression of the T4706M RyR1 protein explain the symptoms, little is known about the function of the channel.

To learn if the channel function is affected so that it may account for the symptoms as well, we examined the mouse T4709M (TM) RyR1 at the single channel level. Ligands, including Ca^{2+}, ATP, Mg^{2+} and the RyR inhibitor dantrolene were tested.

The conductance of the TM channel was the same as wild type's (wt). New population of partial open (subconductive) states were not observed. Half of the TM channels exhibited high open probability and did not respond to the modifications of the $[Ca^{2+}]$ in the recording medium. The rest of the channels showed significantly lower activity at the physiologically relevant range of $[Ca^{2+}]$, compared to wt. The Mg^{2+}- and ATP-regulation of the mutant channels were intact, and they were inhibited by dantrolene.

These results suggest that these primary and/or secondary dysfunctions contribute to the severity of the disease.

THE ROLE OF INTRAAMYGDALOD OXYTOCIN IN THE REGULATION OF LEARNING-RELATED MECHANISMS

Dávid Vörös[1, 2], Orsolya Kiss[1,2], Tamás Ollman[1, 2,3,4], László Péczely[1, 3,4], Olga Zagorácz[1, 2,3,4], Erika Kertes[1, 2,3,4], Veronika Kállai[1, 2,3,4], Bettina László[1, 3], Beáta Berta[1, 2,3,4], Marcell Taigiszer[1, 2] , Hassan Moradi[1, 2] ,Kitti Mintál[1,3,5] , Attila Tóth[1,2,3,5] , Anita Kovács [1,2,3] László Lénárd[1, 2,3], Zoltán Karádi [1,3,5,6] Kristóf László[1,2,3]

[1] Medical School, Institute of Physiology, University of Pécs, Szigeti Str. 12, P.O. Box 99, 7602 Pécs, Hungary. [2] Neuropeptides, Cognition, Animal Models of Neuropsychiatric Disorders Research Group, Medical School, Institute of Physiology, University of Pécs, 7602 Pécs, Hungary. [3] Neuroscience Center, University of Pécs, 7602 Pécs, Hungary. [4] Learning in Biological and Artificial Systems Research Group, Medical School, Institute of Physiology, University of Pécs, 7602 Pécs, Hungary. [5] Cellular Bioimpedance Research Group, Szentágothai Research Center, University of Pécs, 7602 Pécs, Hungary. [6] Molecular Endocrinology and Neurophysiology Research Group, Szentágothai Center, University of Pécs, 7602 Pécs, Hungary

In addition to its peripheral effects, oxytocin is also known to have numerous central nervous system effects. Among others, it plays a role in social interaction, pair bonding and parenting behavior. We have showed recently the positive reinforcing and anxiolytic effects of oxytocin. The amygdala, part of the "social brain", receives oxytocinergic input from the hypothalamus and is relatively rich in oxytocin receptors. Our aim was to investigate the effects of oxytocin injected into the central nucleus of the amygdala in two different learning paradigms.

We investigated the effect of oxytocin on spatial learning in the Morris water maze test. Our results show that 10 ng of oxytocin injected into the central nucleus of the amygdala significantly reduced the time to locate the platform. Pretreatment with an oxytocin receptor antagonist blocked the effect of 10 ng oxytocin on place finding, while the antagonist alone did not affect target finding latency.

In the passive avoidance test, we investigated the role of oxytocin in avoidance learning. Injected into the central nucleus of the amygdala, 10 ng oxytocin significantly increased latency in the passive avoidance test, but 100 ng oxytocin had no significant effect in either test. Oxytocin receptor antagonist pretreatment inhibited the latency-enhancing effect of 10 ng oxytocin, whereas oxytocin receptor antagonist alone did not affect latency time.

Our results suggest that intraamygdaloid oxytocin acts in a dose-dependent manner on learning and memory processes and that these effects are oxytocin receptor specific.

EFFECT OF CB-1 RECEPTOR DEFICIENCY ON MORPHOLOGICAL AND FUNCTIONAL CHANGES IN RELAXATION MECHANISMS OF THE FEMALE MOUSE AORTIC WALL

Bálint Bányai[1]; Stella Kiss[1,2]; Gabriella Dörnyei[2]; Mária Szekeres[1,2]; Eszter Mária Horváth[1];
[1]Institute of Physiology Semmelweis University, Budapest, [2]Morphological and physiological institute Faculty of Health Sciences Semmelweis University, Budapest

Introduction: The endocannabinoid system (ECS) is well known for its psychoactive effects, but it also act on the female reproductive system. Cannabinoids are involved in the regulation of the hypothalamo-pituitary-ovarian axis (HPO) and estrogen production. Estrogens have also been shown to significantly modulate cardiovascular function. In our previous experiments, we have shown increased estrogen-induced relaxation of the abdominal aorta in cannabinoid receptor 1 (CB-1) knock out mice. Therefore, we aimed to investigate the effect of ECS and the role of endothelial factors in the estrogen-induced vascular response.

Aim: To investigate the possible morphological and signaling changes underlying the enhanced estrogen-dependent relaxation observed in CB-1 receptor-deficient animals.

Methods: Our experiments were performed in CB-1 receptor knock out and wild type female mice. After anaesthesia (pentobarbital 50mg/kg ip.), abdominal aortic segments were isolated from the animals for previous myographic and histological as well as immunohistochemical (IHC) measurements in our study. The structure of the vessels investigated by hematoxylin-eosin (HE) and resorcin-fuchsin (RF) stained sections, and proteins involved in the signaling process were examined by immunohistochemical labeling (estrogen receptor (ER), thromboxane receptor (TP) and endothelial nitric oxide synthase (eNOS) and cyclooxygenase 2 (COX-2).

RESULTS: In HE staining, the intima-media ratio of CB-1 KO group was significantly lower compared to the control group ($p<0.05$). No significant difference in ER receptor and TP receptor density was found between the two groups. COX-2 density was significantly lower in the CB-1 KO group compared to the control group ($p<0.05$). eNOS density was significantly higher in the CB-1 KO group ($p<0.001$).

CONCLUSION: CB-1-KO mice are characterized by increased estrogen-induced vasorelaxation, which our results suggest may be associated with increased production of endothelial NO (nitric oxide); presumably associated with decreased levels of the constrictor prostanoids.

THE INVESTIGATION OF A NEW BRET SYSTEM TO STUDY PROTEIN INTERACTIONS

Kovács Orsolya Tünde[1], Soltész-Katona Eszter[2], Barsi Szilvia[1], Misák Ádám[1], Tóth András[1,2,3], Tóth Dániel[1], Várnai Péter[1], Hunyady László[1,2], Turu Gábor[1,2]
[1]Department of Physiology, Semmelweis University, Budapest, [2]Institute of Enzymology, Eötvös Loránd Research Network, Research Centre for Natural Sciences, Budapest,
[3]Department of Internal Medicine and Haematology, Semmelweis University, Budapest

Titration BRET measurements are commonly used to study dynamic interactions. The disadvantage of this system is that it is cumbersome and time-consuming. Our aim was to develop a system in which static interactions can be studied under dynamic conditions in a simple way.

We investigated the interaction of HTSF1 protein and β-arrestin protein. β-arrestin proteins terminate the G-protein signaling after activation of GPCRs (G-protein coupled receptors) and also orchestrate a new wave of signal transduction steps. They regulate a broad range of cellular processes e.g. migration, proliferation, differentiation, and apoptosis.

A rapamycin system was used in this new method, which binds FRB and FKBP molecular tags upon stimulation. HEKT cells were transfected with; L10-FRB-mVenus, FKBP-Barr2 or FKBP-Barr2-384del (pre-activated form), and HTSF1-RLuc8 or HTSF1mut-RLuc8 (β-arrestin binding S-T pattern is mutated). In our experiments, we also cotransfected various GRKs (G protein-coupled receptor kinases) to promote protein phosphorylation.

Our results show that the static interaction of HTSF1 with β-arrestin2 can be detected with good sensitivity in our BRET system and this system can be used to rapidly investigate interactions that are otherwise difficult to manipulate dynamically.

Supported by the ÚNKP-22-4-I New National Excellence Program of the Ministry for Culture and Innovation from the source of the National Research, Development, and Innovation Fund.

ROLE OF THE TREK-1 POTASSIUM CHANNEL IN PULMONARY ARTERIAL RESTING TONE

Réka Csáki[1], Chandran Nagaraj[2], Miklós Lengyel[1], Frigyes Mészáros[1], János Almássy[1], Andrea Olschewski[2,3], Péter Enyedi[1]
[1]Department of Physiology, Faculty of Medicine, Semmelweis University, Budapest, Hungary [2]Ludwig Boltzmann Institute for Lung Vascular Research, Graz, Austria, [3]Experimental Anaesthesiology, Department of Anaesthesiology and Intensive Care Medicine, Medical University of Graz, Graz, Austria

Various potassium channels are expressed in the vessels of the pulmonary circulation, including the smooth muscle cells of the small pulmonary arteries (PASMCs). They hyperpolarize and relax the SMCs thereby contribute to the maintenance of the reduced/low vascular tone. Depolarization from the normal resting membrane potential of PASMCs leads to vasoconstriction, vascular remodelling and increased pulmonary vascular resistance. As a consequence, pulmonary arterial hypertension (PAH) and eventually right heart failure develops, which are serious and often fatal diseases. Downregulation of potassium channel activity in PAH patients may significantly contribute to the observed electrophysiological changes of PASMCs. One of the background (K2P) potassium channels, TASK-1, has become the focus of interest as its dominant negative mutation has been associated with familial form of PAH.

Our aim was to identify further potassium channel/s which might be important in the maintenance of the hyperpolarized resting membrane potential of PASMCs in healthy individuals, and to investigate whether (and to what extent) their altered activity could be a pathogenic factor in PAH. In this process we also tested pharmacological agents known to act on the relevant potassium channel considering also the possibility that a channel activating, cell hyperpolarizing drug might have beneficial effect in PAH. RT-qPCR analysis revealed TREK-1 to be the most abundantly expressed K2P channel in both donor and IPAH PASMCs, with moderately reduced expression level in the IPAH group. The functional significance of TREK-1 was addressed by patch clamping; measuring the TREK1 derived leak potassium current of PASMCs and the effect of its modulation on the membrane potential. The effect of TREK-1 activity on the intracellular Ca2+ signalling was examined by Ca2+ imaging.

As expected, the resting membrane potential of IPAH PASMCs was depolarized compared to donor cells. We found a significant activation of the background potassium current both in the donor and IPAH patients by the specific TREK activator, ML-335. Pharmacological inhibition or siRNA silencing of TREK-1 in donor PASMCs depolarized their membrane potential. Application of ML-335 hyperpolarized donor PASMCs and normalized the membrane potential of IPAH PASMCs. Consistent with the effects on the resting membrane potential, inhibition of TREK-1 augmented, while activation of the channel attenuated the calcium response to extracellular acidification.

Our results suggest that TREK-1 might represent a new pharmacological target for the treatment of IPAH

A MORPHOLOGICAL, HEMODYNAMIC AND HEMORHEOLOGICAL COMPARISON OF ARTERIO-VENOUS SHUNTS IN DIFFERENT LOCATIONS AS HEART FAILURE MODELS IN RATS.

Fazekas László Á.[1], Bernát Brigitta[2], Baráth Barbara[1], Varga Ádám[1], Mátrai Ádám A.[1], Shaked Or[1], Fiebai Owen[1], Al-Smadi Mohammad W.[1], Fillér Csaba[3], Juhász Tamás[3], Priksz Dániel[2], Juhász Béla[2], Szilvássy Zoltán[2], Németh Norbert[1]
[1]Department of Operative Techniques and Surgical Research, [2]Department of Pharmacology and Pharmacotherapy, [3]Department of Anatomy, Histology and Embryology, Faculty of Medicine, University of Debrecen, Debrecen

To study heart failure, numerous small animal models are known in the literature. However, their design, standardization, and reproducibility vary widely. Different localisation and size of arterio-venous shunts could affect the hemodynamics and hemorheology which can cause modified volume overload. We investigated the development of volume-overload heart failure models using aorto-caval and femoral localized arterio-venous shunts.

In this study, we involved 28 Sprague-Dawley rats (permission reg. nr.: 25/2022/UDCAW). In general anaesthesia, after median laparotomy, caudal caval vein and abdominal aorta dissection, and wound closure happened in the control group (C, n=8). In the aorto-caval group (AC, n=10), an 18G-needle was used to puncture the caudal caval vein through the abdominal aorta, above the bifurcation. The femoral vein and artery were joined end-to-side in the femoral arterio-venous group (FAV, n=10). The animals were followed-up for 15 weeks. Hematological, and hemorheological laboratory tests, hemodynamic, echocardiography and histological examinations were performed.

The AC group's intervention was quick but had more serious complications (bleeding), while the FAV anastomosis needed more surgical skill. Compared to control, echocardiography (LA/Ao, E wave, and E/e' ratio characteristics) clearly showed a higher pressure load in the anastomosed group with the largest amplitude in the AC group, earliest on the 3rd postoperative week. The weight of the hearts was the highest in the AC group (1.05±0.1g) compared to the femoral (1.81±0.29g) and to the C group (1.05±0.1g). The highest flow rates were also measured in the AC group's aortas (72.8±16.26ml/min vs FAV:24.71±9.52ml/min; C: 3.79±0.73ml/min). The circulatory of the central nervous system (common carotid arteries flow rate) was affected mostly in the AC group. Haematological and hemorheological (red blood cell deformability and aggregation) values also showed a worsening most notably in the AC group.

Both models resulted in hematological and micro-rheological parameters worsening, depending on the size and localisation and can be used to study heart failure associated with volume overload. A milder and more standardised surgical design and progression is desirable in the point of view of animal welfare, surgical implementation and clinical extrapolability. In this aspect, the femoral group was found to be more suitable, as confirmed by flow dynamics and echocardiographic measurement. (ÚNKP-22-3-I-DE-344, "OTKA"-139184)

DOSE-DEPENDENT EFFECTS OF THE REGULAR PHYSICAL EXERCISE ON THE RETINAL AND COGNITIVE FUNCTIONS IN AN AGING RAT MODEL

Réka Szekeres[1], Anna Szilágyi[1], Barbara Takács[1], Vera Tarjányi[1], Nóra Lampé[1], Dániel Priksz[1], Mariann Bombicz[1], Balázs Varga[1], Rudolf Gesztelyi[1], Zoltán Szilvássy[1], Béla Juhász[1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of General Medicine, University of Debrecen

Introduction: the benefits of regular physical activity have been reported in numerous studies over the past decades. With increasing age, the regenerative capacity of the cells deteriorates, with a concomitant increase in free radical production and consequent oxidative stress, leading to cellular damage and loss of function. As a consequence, the prevalence of several diseases such as ophthalmological and neurodegenerative diseases increase in the ageing population. In this study, we aimed to investigate the effects of the long-term recreational and forced exercise on age-related eye and neurodegenerative diseases in an ageing rat model.

Materials and methods: 18 months old male Sprague Dawley rats were divided into 3 treatment groups: (I) control group: they modelled the ageing, physically inactive population; (II) recreational group, in which cages standardized running wheels were placed and they used the wheels according to their needs for 6 months; (III) forced running group, who ran 6 days a week for 6 months at a fixed speed for a fixed duration. At the endpoint, Morris Water Maze (MWM) test was performed to assess the animals' cognitive function, followed by electroretinography (ERG) measurements under ketamine-xylazine anaesthesia. Finally, Western blot technique was used to determine the molecular changes underlying functional differences.

Results: The ERG outcomes showed that recreational physical activity significantly improved the retinal function, however, based on the MWM test, animals in the forced running group had the best spatial memory and learning abilities. Our western blot analysis revealed that the recreational group had significantly lower levels of the oxidative stress enhancer MAO-B, the inflammatory marker GFAP, but higher expressions of the anti-aging SIRT6 were detected, compared to the aged control and forced running groups. We hypothesize that the improved cognitive function is due to the upregulation of the CREB/BDNF pathway in the forced running group.

Conclusions: Our results suggest that the beneficial effects of exercise vary depending on the intensity and frequency chosen. The type of exercise to be selected is determined by the goal to be achieved within the body.

Supported by: TKP2021-EGA-18, ÚNKP-22-3-II-DE-363

INVESTIGATING TISSUE DAMAGE INDUCED CALCIUM SIGNALING IN ZEBRAFISH

László Fazekas[1,2,3], Nada Al-Sheraji[1], Fabian Dehne[1,3], Benoit Roux[1,3], Diána Kaszás[1,2,3], Ágnes Enyedi[4], Balázs Enyedi[1,2,3]
[1]Department of Physiology, Semmelweis University, Budapest,[2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, [3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest, [4]Department of Transfusiology, Semmelweis University, Budapest

Calcium signals are among the earliest signaling events triggered by epithelial wounding. They are essential in mediating the very first tissue-protective responses including leukocyte recruitment and wound closure. Despite the importance of these signaling events, little is known about their molecular regulation. In a zebrafish tail fin wounding model, we have previously identified wound margin localized persistent, tissue-penetrating wave-like, and cell swelling-dependent oscillatory forms of Ca^{2+} signals. In seeking regulators of these Ca^{2+} signaling patterns, we turned our attention to plasma membrane Ca^{2+}-ATPases (PMCA).

Using Western blot and immunostaining we first identified zebrafish PMCA4 as the major PMCA isoform in the tail fin of the larvae. Zebrafish PMCA4 is orthologous to human PMCA4, but its function has not yet been described in detail. To investigate the role of this ATPase, we expressed the zebrafish PMCA4 in HEK293A cells. Our measurements showed that fish PMCA4 is capable of similar cellular Ca^{2+} export as its human counterpart. We then moved to *in vivo* experiments in zebrafish larvae. In order to measure Ca^{2+} level changes, we created transgenic fish lines ubiquitously expressing a green fluorescent Ca^{2+} sensor, GCaMP7s. Using spinning disk confocal microscopy, we could then follow in real-time the rapid Ca^{2+} level changes after amputating the tip of the tail fin or by using a pulsed UV-laser ablation, which allows us to visualize signaling events during the moments of wounding.

To delineate the role of PMCA4 in wound-induced Ca^{2+} signaling, we used morpholino oligonucleotides to transiently knock it down. We observed that this resulted in diminished wound-induced oscillatory Ca^{2+}-signaling and an increase in the Ca^{2+} levels measured at the wound margin. Furthermore, we also measured altered leukocyte wound migration in the knockdown animals with decreased speed of cellular motion and impaired wound orientation. We are currently working on finding a mechanistic explanation for the later results which we also confirmed by Crispr-mediated F0 knockout tools. In these experiments, 3 different guide RNAs are used to eliminate the expression of the gene of interest directly in the injected larvae. Further experiments on CRISPR/Cas9-mediated full PMCA4 knockout animals are also in progress.

Our results so far provide opportunities for a more detailed understanding of tissue-injury induced Ca^{2+} signaling, and for mapping the role of the PMCA4 protein in the process.

THE ADVENTITIAL LAYER PLAYS A CRUCIAL ROLE IN THE SIGNAL TRANSDUCTION OF VASOCONSTRICTION INDUCED BY 18:2 LYSOPHOSPHATIDIC ACID

Krisztina Vén[1], Balázs Besztercei[1], Mónika Kosztelnik[1], Gábor Tigyi[1,2], Zoltán Benyó[1] and Éva Ruisanchez[1]
[1]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary, [2]Department of Physiology, University of Tennessee Health Science Center, Memphis, USA

Lysophosphatidic acid (LPA) is a bioactive lipid mediator, which exerts its effects via its six specific GPCRs mediating important physiological and pathophysiological responses, like platelet activation or cell-migration. During vascular injury or rupture of an atherosclerotic plaque, the consequential platelet activation leads to the release of mainly polyunsaturated LPAs, especially 18:2 LPA. In hypertension and acute myocardial infarction, the level of these polyunsaturated LPAs is also elevated. Based on the previous results of our research group, LPA_1 receptor activation by the 18:2 LPA evokes cyclooxygenase 1 activation with consequential thromboxane A_2 (TXA_2) release, and vasoconstriction.

In this study we further analyzed the signaling mechanisms underlying the constrictor effect of 18:2 LPA.

We did experiments in aortas isolated from Bl6 mice. Our main method was the measurement of the vascular tone with myography. The endothelium, and in some cases the adventitia as well, was mechanically removed and the 18:2 LPA was administered on the resting tone. TXA_2 release of the vessels was measured by ELISA. Primary vascular cells were isolated from different layers of the aorta and their intracellular Ca^{2+} signal upon LPA administration was determined. qPCR was used to measure the expression of the LPA_1 receptor in the lamina media and adventitia.

According to our results, removal of the adventitia significantly decreases the elicited vasoconstriction, and results in a complete loss of thromboxane release. In agreement with this, LPA_1 receptor is predominantly expressed in the adventitial layer, as removal of the adventitia resulted in diminished expression of Lpar1. Staining of the LPA_1 receptor was only present in the adventitial layer, but not in the lamina media. Primary isolated vascular smooth muscle cells exhibited negligible Ca^{2+} signal upon administration of 18:2 LPA, however a subpopulation of adventitial cells showed marked intracellular Ca^{2+} increase when stimulated by 18:2 LPA.

In conclusion, LPA 18:2 has strong vasoconstrictor activity, due to its ability to increase LPAR1–Gi–COX mediated thromboxane release from the adventitia, highlighting the significance of this layer in the regulation of vasomotion.

THE ROLE OF NEUTROPHIL EXTRACELLULAR TRAPS IN THE DEVELOPMENT OF ACUTE KIDNEY INJURY DURING EXPERIMENTAL VENO-VENOUS EXTRACORPOREAL MEMBRANE OXYGENATION

Noémi Vida[1], Ámos Gajda[1], Ádám Hodoniczki[1], Gyöngyvér Vigyikán[1], Zoltán Varga[1], Attila, Rutai[1], Mihály Boros[1], Dániel Érces[1], Gabriella Varga[1]
[1]University of Szeged

Introduction In our previous studies, we demonstrated signs of an inflammatory reaction in the kidneys accompanying experimental veno-venous extracorporeal membrane oxygenation (vvECMO) even before hemodynamic changes occurred. We hypothesized that humoral factors might play a key role in inducing this process, so our aim was to investigate the changes in inflammatory factors that play a crucial role in the formation of neutrophil extracellular traps (NET) during acute kidney injury resulting from experimental vvECMO in anesthetized and mechanically ventilated dwarf pigs.

Methods We established an extracorporeal circulation loop using jugular and femoral vein cannulation. The vvECMO group (n=6) underwent 24 hours of continuous ECMO treatment followed by a 6-hour post-ECMO period. The anesthetized group (n=6) underwent veno-venous cannulation without ECMO therapy, and observation lasted for 30 hours. During the intervention, we performed hemodynamic monitoring, blood gas analysis, measured hourly urine output, and took plasma samples every 6 hours to determine inflammatory mediators (Il-1β, Il-8) that affect NET formation. At the end of the protocol, we performed histological examination (periodic acid-Schiff reaction) of collected kidney samples and measured the extent of leukocyte activation using myeloperoxidase (MPO) enzyme activity.

Results There was no difference in mean arterial pressure or cardiac output, but heart rate significantly increased in the vvECMO group from the 25th hour. The histological examination of the kidneys in the vvECMO group confirmed ischemic damage, and we found elevated levels of Il-1β (97.5±24.3 vs. 161.9±39.8 pg/ml) and Il-8 (45.2±11.8 vs. 83.6±14.3 pg/ml) in plasma samples from the 12th hour of the experiment. We also found significantly higher MPO enzyme activity (4.34±1.7 vs. 11.15±2.4 mU/mg protein) in tissue samples collected at the end of the experiment.

Conclusion Based on the observed increase in Il-1β and Il-8 at the beginning of experimental vvECMO and the leukocyte activation detected in kidney tissue, NET formation can be confirmed. This process may play an important role in the development of acute kidney injury, even in the early stages of vvECMO treatment.

Támogatók: NKFIH-K120232
"This research work was conducted with the support of the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture and Innovation."

RELATIONSHIP BETWEEN ION CURRENTS AND MEMBRANE CAPACITANCE IN CANINE VENTRICULAR MYOCYTES

Csaba Dienes[1], Balázs Horváth[1,2], Zsigmond Kovács[1], Norbert Szentandrássy[1,3], János Magyar[1,4], Tamás Bányász[1], Péter P. Nánási[1,5]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2]Faculty of Pharmacy, University of Debrecen, [3]Department of Basic Medical Sciences, Faculty of Dentistry, University of Debrecen, [4]Division of Sport Physiology, Department of Physiology, Faculty of Medicine, University of Debrecen, [5]Department of Dental Physiology and Pharmacology, Faculty of Dentistry, University of Debrecen

Ion current densities, derived by normalizing current amplitudes to membrane capacitance (C_m), are conventionally used in cellular cardiac electrophysiology. This convention assumes that these variables are linearly related for any given ion current. Therefore, we aimed to investigate the relationship between amplitudes and integrals of the major cardiac ionic currents and C_m, using correlation and linear regression.

Comparisons were made based on data derived from conventional voltage clamp (CVC) and action potential voltage clamp (APVC) experiments. Normal distribution of data was tested with Shapiro-Wilk test. In case of normal distribution, correlations were characterized with the Pearson's correlation coefficient (r), and the significance of correlation (p). If the distribution was not normal, the Spearman's correlation coefficient was calculated.

Under APVC conditions good correlation was observed between C_m and current amplitudes or current integrals in the case of I_{K1}, I_{Kr} and $I_{Ca\text{-}L}$. For I_{NCX} the correlation was moderate and for $I_{Na\text{-}late}$ and I_{Ks} the correlation was weak to moderate. Under CVC conditions the correlation was good for I_{K1}, moderate for I_{Kr} and $I_{Ca\text{-}L}$, while weak for I_{Ks}. In the case of I_{to1} the correlation between the peak amplitude and C_m was negligible (r=0.21) when analyzing all cells together, however, good correlations (r>0.7) were obtained when the cells were analyzed separately for cells of subepicardial, subendocardial and midmyocardial origin. In all cases, the 95% confidence intervals of y intercepts contained y=0.

In conclusion, there is a good correlation in general between ion current amplitudes or integrals and C_m. Limited correlations likely originate from spatial inhomogeneity of ion currents and/or non-ideal experimental conditions. This must be considered when interpreting ion current measurements in cardiac cells.

EXPERIMENTAL MODELING OF NEONATAL HYPOTHERMIA IN THE RAT

Kata Fekete[1], Zsolt Fehér[1,2], Krisztián Klonfár[1,] Bíbor Gárdos[1], János Radó[3], Eszter Pákai[1], András Garami[1]
[1]Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary, [2]Department of Anaesthesiology and Intensive Therapy, Medical School, University of Pécs, Pécs, Hungary, [3]Institute of Physiology, Medical School, University of Pécs, Pécs, Hungary

Newborns, especially premature infants, have limited ability to maintain core temperature compared to adults. Brown adipose tissue develops most intensely shortly before birth and plays a key role in thermoregulation. We aimed to investigate the role of brown adipose tissue in protecting the core temperature of mature neonates and preterm infants by developing and using a rat model.

We wanted to test a new animal model, which was preceded by an extensive literature review. In our studies, we used neonatal Wistar rats as a model for full-term newborns (7-day-old) and for preterm infants (2-day-old). Each age group was exposed to a thermally neutral condition or to cold environment. In separate experiments, thermogenesis was induced by a $ß_3$ receptor agonist. Temperature was measured simultaneously at different locations on the animals' body surface using thermocouples.

No significant difference was found between the temperatures of body parts in the thermoneutral environment in the full-term group ($p>0.05$). In the cold, the interscapular area (brown adipose tissue localization) had higher temperature (~23,5°C) than the brain (~22,5°C). During cold exposure of the preterm group, the interscapular temperature was also higher (~21°C) than that of the brain (~20°C). However, the body temperatures in all regions were lower in the preterm than in the full-term cold-exposed rats. We also found weaker thermogenesis in the preterm group in response to the $ß_3$ agonist.

As conclusions, activation of brown adipose tissue could be demonstrated in our model, thus it may be suitable for subsequent applied physiology research. The difference between brown adipose tissue activity in the full-term and preterm neonates highlights the vulnerability of preterm infants to cold. Our results may help to understand the thermoregulation of preterm and full-term infants and prevent them from developing hypothermia.

Funding: This work was supported by the New National Excellence Program of the Hungarian Ministry for Innovation and Technology Grants UNKP-22-3-I-PTE-1558 (to K. F.) and National Research, Development and Innovation Office Grant FK 138722 (to A. G.).

Keywords: hypothermia, thermoregulation, brown adipose tissue, newborn, thermogenesis

LIVE VISUALIZATION OF LTB_4 GRADIENTS WITH A NOVEL FLUORESCENT BIOSENSOR

Szimonetta Tamás[1,2,3], Benoit Roux[1,3], Boldizsár Vámosi[1], Balázs Enyedi[1,2,3]
[1]Department of Physiology, Semmelweis University, Budapest, [2]MTA-SE Lendület Tissue Damage Research Group, Hungarian Academy of Sciences and Semmelweis University, Budapest, [3]HCEMM-SE Inflammatory Signaling Research Group, Department of Physiology, Semmelweis University, Budapest

Background: Leukotriene B_4 (LTB_4) plays a prominent role in launching and propagating inflammatory responses. Acting as a signal relay molecule, LTB_4 creates local and long-range gradients to orchestrate leukocyte recruitment and neutrophil swarming during tissue damage. Despite its profound biological relevance, we lack tools to directly measure and visualize these chemoattractant gradients *in vivo*.

Aims: Create a genetically encoded biosensor to detect and monitor levels of LTB_4 *ex vivo* and *in vivo* in zebrafish larvae during tissue damage induced neutrophil swarming.

Methods & Results: We developed GEM-LTB_4, a GPCR-based fluorescent indicator by inserting a circularly permuted EGFP (cpEGFP) module into the third intracellular loop of the high-affinity LTB_4 receptor, BLT1. We optimized and increased the sensitivity of our sensor in HEK293 cells by screening different length and amino acid composition linkers flanking the cpEGFP module. The best version, namely GEM-LTB_4, shows precise membrane localization, high affinity (Kd ~ 19.8 nM) and specificity towards LTB_4, and a robust shift in fluorescence upon LTB_4 exposure (1 to 1.5 fold increase). To measure whether GEM-LTB_4 could detect physiological concentrations of LTB_4, we isolated murine neutrophils and seeded them on GEM-LTB_4 expressing cells. When activated with fMLP, we detected an overall increase in LTB_4 production and also captured LTB_4 signals derived from individual neutrophils.

We then created a transgenic GEM-LTB_4 expressing zebrafish line and found that our sensor responded similarly in 4-day old larvae to increasing doses of exogenous LTB_4 as it did in HEK293 cells. To determine whether we could detect endogenous LTB_4 release in zebrafish larvae, we first recruited neutrophils (known to produce LTB_4) to an open wound and then activated them using the Ca^{2+} ionophore A23187. With our sensor, we were able to visualize the generation of endogenous LTB_4 gradients, which was completely abolished by adding zileuton, a strong inhibitor of LTB_4 production.

Conclusions: In summary, we developed a fluorescent biosensor with high sensitivity and specificity for LTB_4. With GEM-LTB_4 we could monitor the live release of LTB_4 from murine neutrophils and were able to detect and visualize LTB_4 gradients likely derived from single cells. When expressed in zebrafish, we could not only detect LTB_4 applied exogenously, but also neutrophil-induced endogenous LTB_4 production and gradients. Since LTB_4 plays a central role in inflammatory processes as a main driver of chemotaxis and neutrophil swarming, we believe that GEM-LTB_4 will further help to understand this complex response.

VITAMIN D DEFICIENCY ASSOCIATED WITH AGE-DEPENDENT VASCULAR DYSFUNCTION IN MICE

Csillag Virág Tóth[1], Nóra Melinda Kerkovits[1], Margit Kerék[1], Imre Babay[1], Dorina Nagy[1], Éva Pál[1,2], Mónika Kosztelnik[1,2], Zoltán Benyó[1,2]
[1]Institute of Translational Medicine, Semmelweis University, Budapest, Hungary, [2]Eötvös Loránd Research Network and Semmelweis University (ELKH-SU) Cerebrovascular and Neurocognitive Disorders Research Group, Budapest, Hungary

Introduction: Approximately 40% of the European population is vitamin D deficient, and the prevalence increases with age. In recent years, several studies have demonstrated that vitamin D deficiency leads to a deterioration of the cardiovascular system, including vascular function, but the precise pathomechanism of this decline, particularly in the elderly, is not fully understood.

Aim: We aimed to investigate the vascular effects of vitamin D deficiency in young and aged mice and to identify the molecular and cellular mechanisms involved in the alteration of vascular reactivity.

Methods: Segments of the thoracic aorta isolated from wild-type (WT) and vitamin D receptor gene deficient (VDR KO) young and aged (3 and 11 months) male mice were examined under isometric conditions using myograph. The vascular responses to phenylephrine were normalized to 124 mM K^+-induced contraction, and acetylcholine-induced endothelium-dependent relaxation was normalized to the pre-contraction induced by phenylephrine. In addition, we determined the expression levels of relaxation-associated M3 muscarinic receptor (*Chrm3*), endothelial nitric oxide NO synthase (*Nos3*), soluble guanylate cyclase (*Gucy1a1* and *Gucy1b1*) and adventitial macrophage marker (*F4/80*) genes from myographically studied vessel segments. The presence of the macrophages was examined using immunhistochemistry.

Results: While no differences were observed between contractions to phenylephrine, the relaxant effect of acetylcholine was significantly reduced in aortas of aged VDR KO mice compared to young WT, young KO and aged WT vessels (with 27.5; 25.7 and 25.7 %). No significant differences were observed in the expression of *Chrm3, Nos3, Gucy1a1 and Gucy1b1* genes between experimental groups, but VDR deficient animals had higher levels of *F4/80* mRNA regardless of age. There was a negative correlation between the expression of macrophage marker and the relaxation induced by acetylcholine ($r=-0.2955$, $p<0.0106$).

Conclusion: Our results suggest that vitamin D deficiency impairs endothelium-dependent relaxation of blood vessels in older age. This is not due to impaired NO production or smooth muscle signalling, but is probably due to reactive oxygen free radicals produced by macrophages in the vascular adventitia, which react with the NO produced, thereby reducing its dilator capacity. These findings suggest that vitamin D deficiency induces a pro-inflammatory state with increased myeloid infiltration in the vascular wall, which is exacerbated with age. The resulting endothelial dysfunction may increase the risk of developing cardiovascular disease.

Funding: ÚNKP-22-2-I-SE-26; ÚNKP-22-4-II-SE-17; TKP2021-EGA-25; PD-143327; NKFIH K-135683, K-139230

DO THE MEASUREMENT RESULTS REFLECT REALITY? - CHALLENGES IN HEART FAILURE BIOMARKER RESEARCH...

Pintér Tamás Bence[1], Dr. Szabó Attila Ádám[1], Dr. Fagyas Miklós[1]
[1]DE ÁOK Kardiológiai Intézet, Klinikai Fiziológiai Tanszék

Nowadays, the neprilysin inhibitor sacubitril in combination with an angiotensin II receptor antagonist is successfully used in the treatment of heart failure patients, which illustrates the prominent role of neprilysin (NEP) in the pathogenesis of cardiovascular diseases. By inhibiting the NEP enzyme, sacubitril significantly reduces the number of hospitalisations and mortality from cardiovascular causes. There are significant individual differences in the efficacy of the medication and the response to therapy, and we wanted to study the mechanisms underlying this.

Serum NEP concentration was determined by commercially available ELISA kits and serum NEP activity by end-point fluorescence assay. The NEP gene was analysed by Sanger sequencing and the detected genetic variations by restriction fragment length polymorphism analysis.

Serum NEP concentrations in cardiovascular patients showed significant interindividual variation (mean: 35.41 pg/mL [min-max: 0.38-10765 pg/mL; n= 75), which was suggested to be due to genetic polymorphisms. Sequencing of the NEP gene of the individual with the highest NEP concentration (NEP= 10765pg/mL) identified a mutation at position chr3:155180618 of exon 23, which increases NEP concentration. Its presence was tested in several patients, but high serum NEP concentrations were measured even in the absence of the mutation. Investigation of the relationship between NEP concentration and activity suggested the presence of an endogenous NEP inhibitor in serum. Fourfold diluted serum reduced recombinant NEP activity by more than 60%. Removal of the two most abundant serum proteins (IgG, albumin) from the sample did not increase the activity of serum endogenous NEP, but abolished its inhibitory effect on recombinant NEP. IgG had no effect, whereas albumin significantly reduced recombinant NEP activity with an IC50 of 15.03 g/L.

Albumin is an endogenous inhibitor of serum NEP, which, in addition to reducing NEP activity, may affect the results of ELISA-based NEP concentration measurements. This sheds new light on the findings of previously published studies, as determined by the methods we used. In conclusion, the physiological regulation of NEP and the mechanism of action of NEP inhibitors hold many surprises for the times to come.

INVESTIGATION OF PARTNER PROTEINS OF CYTOSKELETAL SEPTIN7 IN MYOGENIC CELL LINE AND SKELETAL MUSCLE

Ágnes Viktória Bana[1], Brigitta Tillmann[1], László Szabó[1,2], Zsolt Ráduly[1,2], Beatrix Dienes[1], László Csernoch[1,2], Mónika Gönczi[1,2]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, [2]ELKH-DE Cell Physiology Research Group, Debrecen

The cytoskeletal system plays an important role in determining the localization of intracellular and membrane-bound proteins within skeletal muscle fibers. Besides the three member of the above mentioned system, septins are accepted as the fourth members of the cytoskeleton. These 30-65 kDa highly conserved GTP-biding proteins form hetero-oligomeric complexes and higher-order structures like filaments and rings. They are involved in several intracellular processes, such as cell mobility and endocytosis. The number of septin isoforms encoded is variable among different organisms. In humans 13 different isoforms were identified, which were classified into 4 groups (Septin 2, 3, 6 and 7) on the basis of sequence homology. Septin7 being the sole member of its group has a prominent role in the formation of hetero-oligomeric complexes and also in the connection of the septin oligomers with other proteins.

The aim of our research was to determine the interaction partners of Septin7 in skeletal muscle and muscle-derived cell lines. Therefore we performed immunoprecipitation on muscle samples isolated from *m.pectoralis*, *m.glutealis*, *m.quadriceps* and *m.tibials anterior* of C75/Bl6 mice, on individual *flexor digitorum brevis* (FDB) muscle fibers, and on undifferentiated and differentiated C2C12 cell cultures. The expression of cytoskeletal partner proteins identified by mass spectrometry in the sample were further analyzed by western blotting, while their intracellular localization was investigated by high-resolution confocal microscopy (Airyscan LSM 880) following immunofluorescent staining of the different samples.

We demonstrated that Septin7 forms complexes with several other septin isoforms; Septin2 was identified in all samples, while Septin5 was present only in cell cultures. Analyzing the intracellular localization and structural appearance of these two septin isoforms revealed that Septin5 also forms filaments mostly co-localized with Septin7, whereas the pattern of Septin2 exhibited a double band on isolated FDB fibers indicating its localization close to the T-tubule. The cytoskeletal protein actin was present in every sample, α-actinin was detected in muscle samples, while tubulin-α1B was identified in the IP samples of C2C12 cultures. Actin showed a high degree of co-localization with Septin7 in myoblasts, similar specific pattern was observed between α-actinin and Septin7 in FDB fibers. Although tubulin forms a complex with Septin7, the overlap between these two proteins was not complete.

Our results suggest that the Septin7 presumably forms a functional complex with other cytoskeletal elements both in cell culture and in skeletal muscle fibers. Septin7 could significantly contributes to the construction of the cytoskeletal system and thus play a key role in the normal development and functioning of skeletal muscle.

INTEGRATING AN ELECTROCHEMICAL METHOD INTO PRECLINICAL RESEARCH: GLUCOSE-LEVEL MEASUREMENTS AT THE PREFRONTAL CORTEX IN RATS

Evelin Szabó[1, 2], Anita Kovács[2], Lívia Nagy[1], Géza Nagy[1], László Lénárd[2], Dóra Zelena[2]
[1]Institute of Chemistry, Faculty of Sciences, University of Pécs, Pécs, Hungary, [2]Institute of Physiology, Medical School, Pécs University, Pécs, Hungary

INTRODUCTION: Brain glucose metabolism, especially in the prefrontal cortical area (PFC), could play a crucial role in the development of posttraumatic stress disorder (PTSD). For measuring metabolic processes in vivo we need adequate temporal resolution. Despite glucose sensors are available commercially, these are not built for measuring inside the brain. In a collaboration we are developing an enzyme-based biosensor with the aim of measuring glucose-levels at the prefrontal cortex of rats.

MATERIALS AND METHODS: In an enzyme reaction glucose-oxydase generates hydrogen-peroxide, the concentration of which can be measured by periodically interrupted amperometry. The challenge was to generate a reliably measuring miniature electrode.

RESULTS: Our sensor can exclude other potentially interfering electroactive species such as ascorbic acid, but measures glucose in an adequate concentration-range. Its lifespan has been extended to two weeks with a new design for enzyme immobilization. The modified measuring method improves the sensitivity and lowers the limit of detection. In pilot experiments we proved that the peripheral modulation of glucose levels by glucose or insulin administration has a measurable effect centrally, in the PFC.

CONCLUSION: In this collaboration we established a replicable protocol for electrode design. Our further research using this biosensor can contribute to the understanding of the metabolic aspects of PTSD, therefore, to improve the efficacy of its therapy. The knowledge gained during development of the biosensor has opened a new window for applying this electrochemical method to other projects and animal models.

AN UNEXPECTED ENZYME IN VASCULAR SMOOTH MUSCLE CELLS: ANGIOTENSIN II UPREGULATES CHOLESTEROL-25-HYDROXYLASE GENE EXPRESSION

Kinga Bernadett Kovács[1], Janka Borbála Gém[1], Szilvia Barsi[1], Bernadett Perey-Simon[1], László Hunyady[1,2,3], András Balla[1,2]
[1]Department of Physiology, Semmelweis University, Budapest, [2]ELKH-SE Laboratory of Molecular Physiology, Eötvös Loránd Research Network and Semmelweis University, Budapest, [3]Research Centre for Natural Sciences, Institute of Enzymology, Budapest

Angiotensin II (AngII) is a vasoactive peptide hormone and the effector of the reninangiotensin-aldosterone system. It exerts its main physiological effects through type 1 angiotensin II receptor (AT1R), but it can also contribute to the development of cardiovascular diseases. Likewise, oxysterols such as 25-hydroxycholesterol (25-HC), the product of cholesterol-25-hydroxylase (CH25H), can have harmful effects on the vasculature since they affect vascular smooth muscle cells (VSMCs) negatively. However, there is no established connection between AngII and 25-HC production in VSMCs.

Our study aims to explore how AngII affects Ch25h gene expression in rat primary VSMCs. RNA-sequencing was used to determine which genes are differentially expressed in VSMCs stimulated with AngII compared to vehicle-stimulated cells. We performed qRT-PCR measurements to asses Ch25h mRNA levels in our VSMC samples. VSMCs were stimulated with AngII for various time spans to determine Ch25h expression changes over time. We used multiple inhibitors to specify which signaling pathways are involved in the AngII-induced Ch25h expression changes. LC-MS/MS was used to measure 25-HC levels in the supernatant of AngII-stimulated VSMCs.

Our data show that Ch25h expression was robustly upregulated in response to AngII stimulus, Ch25h mRNA levels were at a peak one hour after stimulation. The AT1R, Gq/11, and p38 MAP kinase inhibitors prevented the AngII-induced Ch25h upregulation. LC-MS/MS results demonstrated that 25-HC is present in the supernatant of AngII-stimulated VSMCs. 25- HC concentration was highest (on average 8.2 ng/ml) four hours after AngII stimulation.

Based on our results we conclude that AngII-induced Ch25h upregulation is AT1R, Gq/11 and p38 MAP kinase signaling dependent in primary rat VSMCs. The elevation of 25-HC levels in AngII-stimulated VSMC supernatants means that CH25H enzyme is active and functional in primary rat VSMCs. Our study shows that AngII stimulus induces Ch25h upregulation and subsequently 25-HC production in VSMCs. Our findings can lead to the better understanding of mechanisms in the pathogenesis of cardiovascular diseases.

SYSTEMIC INFLAMMATION INDUCED BY ANGIOTENSIN II INCREASES C3a-MEDIATED VASOCONSTRICTION IN MICE

Imre Babay[1], Nóra Melinda Kerkovits[1], Csillag Virág Tóth[1], Gábor Szénási[1], Mónika Kosztelnik[1,2], Zoltán Benyó[1,2]
[1]Institute of Translational Medicine, Semmelweis University, Budapest, [2]Eötvös Loránd Research Network and Semmelweis University (ELKH-SU) Cerebrovascular and Neurocognitive Disorders Research Group, Budapest

Introduction: Cardiovascular diseases are known to be associated with activation of the complement system, however, the precise relationship between the two processes is partly unclear. The complement component C3a receptor (C3aR) has been identified in various cell types within the mouse arterial wall and the vasoconstrictor effect of C3a may be mediated by resident macrophages in the adventitia via thromboxane A_2 (TXA_2) release, as found by our workgroup in previous research.

Aims: The primary objective of this study was to determine the effects of chronic angiotensin II (Ang II) administration on C3a-induced vasoconstriction in mice, along with demonstrating the presence of macrophages in the adventitia using alternative methods.

Methods: Changes in the isometric tension of the vascular segments were measured using myography after isolating thoracic and abdominal aortic segments of adult male C57BL/6 mice. The mice were infused with either Ang II (520 ng/kg/min) or saline for 14 days via micro-osmotic pumps implanted in the subscapular region. qPCR assays were performed to measure the expression level of C3aR and the mouse macrophage marker F4/80. The expression of other genes involved in the signaling pathway, such as Cyclooxygenase 1 and 2 (*Cox1* and *Cox2*), thromboxane A synthase 1 (*Tbxas1*), and thromboxane A2 receptor (*Tbxa2r*), were investigated. The localization of C3AR1 was confirmed using immunohistochemistry. The mechanism of the contraction was examined using the cyclooxygenase (COX) inhibitor indomethacin and SQ29548, a thromboxane prostanoid (TP) receptor antagonist.

Results: An increased response to C3a (63–77) was observed in the vascular segments due to the increased expression of C3aR caused by Ang II treatment compared to controls that received saline infusion. The qPCR results revealed an increase in the expression of F4/80 and C3aR in the aortas, particularly in the adventitia, due to Ang II infusion. After removing the endothelium, the vasoconstriction increased, while the vessels became unresponsive to C3a after the administration of COX and TP receptor inhibitors and the removal of the adventitia.

Conclusion: It can be concluded from our results that chronic Ang II administration can lead to an enhanced vasoconstrictor response to C3a and an increased expression of C3aR in the aortic wall, particularly in the adventitia where macrophages accumulate. Our findings propose that the vasoconstriction induced by C3a in the mouse aorta is facilitated by macrophages that have gathered in the adventitia. During the development of various cardiovascular diseases, the effects of anaphylatoxins could be amplified and may contribute to the progression of the diseases.

Funding: NKFIH K-125174, K-135683, K-139230, 2020-1.1.6-JÖVŐ-2021-00010, EFOP-3.6.3-VEKOP-16-2017-00009, TKP2021-EGA-25.

DEVELOPMENT OF AN INDUCED PLURIPOTENT STEM CELL-BASED ORGANOID MODEL FOR INVESTIGATING THE MOLECULAR MECHANISMS OF CEREBELLAR ATAXIA

Alexandra Sándor[1], Kornélia Szebényi[1]
[1]Institute of Enzimology, Research Centre for Natural Sciences

The cerebellum is important for processing motor and sensory information. Due to its continoued development during the post-natal period, it is vulnerable to a number of pathological processes, for example to different type of ataxias. Spinocerebellar ataxias (SCA) represent a group of ataxias with a diverse range of neurological disorders, mainly characterised by the loss of motor co-ordination. Recently it has been discovered that pathological pathways of different genetic sub-types of SCA can overlap and they affect not only Purkinje cells, but also other cell types of the cerebellum, such as Bergmann glia cells. Since the development of the human cerebellum is completely different from that of the mouse cerebellum, it is essential to use human model systems to study both developmental biological and neurodegenerative changes. The human induced pluripotent stem cell (hiPSC)-derived cerebellar organoid system contains the disease relevant cell types in a tissue-like organization and therefore can provide a relevant model for investigating the molecular mechanisms of ataxias affecting the human cerebellum.

First we developed a reproducible differentiation protocol for the generation of a hiPSC-derived organoid model of the cerebellum, which also enables the production of cerebellar organoids from hiPSC lines created from patients with SCA. The cell types found in the organoids on days 21, 35 and 50 of the differentiation were characterized by immunocytochemical methods, using markers specific to the developmental stages of the cerebellum. We showed that Kirrel2-positive Purkinje progenitors appear already on day 35, from which Calbindin-positive Purkinje neurons develop by day 50. By optimizing the culture conditions, the later developing astroglial cells were already detectable on the 50th day. In order to prove that the model is also suitable for detecting pathological changes, we treated the organoids with IL-1β, capable of inducing ataxia in mice. IL-1β treatment increased the expression level of the autophagy marker P62, as detected by western blot. In order to find out whether this change occurred in a cell-specific manner, we examined P62 in organoid sections together with neuronal (MAP2) and astroglial (GFAP) markers.

Overall, it can be concluded that we have created an organoid model of the human cerebellum, which is suitable for examining cell-specific pathological changes and can serve as a platform for the development of therapies targeting them.

ARRESTICK MOTIF CONFERS STABILITY AND ENABLES NON-RECEPTOR PROTEIN BINDING TO β-ARRESTINS

Eszter Soltész-Katona[1,2,£], Katalin Kis[2,], András Dávid Tóth[1,2,3], Viktor Guti[2], Sharon Gilzer[2], Susanne Prokop[2], Roxána Boros[2], Ádám Misák[2], András Balla[2,4], Péter Várnai[2,4], Lilla Turiák[5], András Ács[5], László Drahos[5], László Hunyady[1,2], Gábor Turu[1,2]
[1]Institute of Enzymology, Centre of Excellence of the Hungarian Academy of Sciences, Research Centre for Natural Sciences, Eötvös Loránd Research Network Budapest, [2]Department of Physiology, Semmelweis University Budapest, [3]Department of Internal Medicine and Haematology, Semmelweis University Budapest, [4]ELKH-SE Laboratory of Molecular Physiology, Eötvös Loránd Research Network and Semmelweis University Budapest, [5]Institute of Organic Chemistry, Eötvös Loránd Research Network, Research Centre for Natural Sciences Budapest
[£]These authors contributed equally to this work.

The phosphorylation motifs of G protein-coupled receptors affect the binding and function of β-arrestins. However, the exact pattern of phosphorylated amino acids responsible for sustained interaction remains unclear. To address this, we utilized 1D sequence convolution trained on a database consisting of GPRCs with previously determined β-arrestin binding properties. With our approach, we identified the amino acid pattern necessary for GPCRs to stably interact with β-arrestins, which motif we named arreSTick. Our model accurately predicts the stability of coupling between GPCRs and β-arrestins, as well as the location of the tight interaction in the receptor sequence. Additionally, we show that the arreSTick pattern is not only found in GPCRs but also in many non-receptor proteins. Using proximity biotinylation assay and mass spectrometry analysis, we demonstrate that the arreSTick motif controls the interaction of several non-receptor proteins with β-arrestins. For instance, the HIV-1 Tat Specific Factor 1 (HTSF1), a nuclear transcription factor, contains the arreSTick pattern, and its subcellular localization is affected through coupling to cytoplasmic β-arrestin2. Our findings reveal a broader regulatory role for β-arrestins in phosphorylation-dependent interactions, not only with GPCRs but also with non-receptor proteins.

Youth Posters Pharmacology

A HIV-ELLENI ANTIVIRÁLIS SZER (DELAVIRDINE) GÁTOLJA A HERG ÁRAMOT

József Óvári[1], Zsigmond Máté Kovács[1], Csaba Bálint Dienes[1], Zalán Barta[1], Ádám Fehér[2], Zoltán Varga[2], János Magyar[3], Tamás Bányász[1], Péter Pál Nánási[4], Balázs Horváth[1], Norbert Szentandrássy[5]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, [2]Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, [3]Division of Sport Physiology, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, [4]Department of Dental Physiology and Pharmacology, Faculty of Dentistry, University of Debrecen, Debrecen, [5]Department of Basic Medical Sciences, Faculty of Dentistry, University of Debrecen, Debrecen

Delavirdine is an anti-HIV 1 reverse transcriptase inhibitor. The molecule contains a methanesulfonamide group similarly to some blockers of the rapid component of the delayed rectifier potassium current (I_{Kr}). I_{Kr} is an important current of the ventricular myocardium responsible for initiating late repolarization. Its pore forming channel protein is hERG, targeted by class III antiarrhythmic drugs. Inhibition of I_{Kr} prolongs the action potential (AP) and can cause early afterdepolarizations thereby increasing the risk of cardiac arrhythmias such as sudden cardiac death.

Our goal was to investigate the effects of delavirdine on cardiomyocytes and expressed hERG channels.

APs were recorded with sharp microelectrode technique at 37 °C in enzymatically isolated canine left ventricular cardiomyocytes. Ion currents were measured with whole-cell voltage-clamp technique on hERG channels expressed in HEK (human embryonic kidney) cells.

Delavirdine decreased hERG current in a concentration-dependent manner with a half-inhibitory concentration of 11 µM. 10 µM delavirdine increased the action potential duration at 90 % of repolarization (APD_{90}) and reduced the APD_{50}/APD_{90} value. Maximal rates of depolarization ($V+_{max}$), early repolarization ($V_{Ph1}max$), and late repolarization ($V-_{max}$) were all decreased. The membrane potential difference between resting value and that recorded at 20% duration of APD_{90} (Plateau20 amplitude) slightly increased. The effects of delavirdine were reversible after 20 minutes of washout with the exception of $V+_{max}$ value.

Based on our results the effect of delavirdine on action potential caused by inhibiting potassium currents. The APD_{90} increase likely due to the inhibition of I_{Kr} this was confirmed on hERG channels. Reduction of APD_{50}/APD_{90} and $V-_{max}$ can be caused by the inhibition of inward rectifier potassium current. $V_{Ph1}max$ reduction and Plateau20 amplitude increase can be the result of the inhibition of transient outward potassium current.

HEMOKININ-1 IS INVOLVED IN LEARNING AND MEMORY FUNCTIONS IN MICE

Eszter Kepe[1], Valéria Tékus[1], Zsuzsanna Helyes[1,2,3,4], Éva Borbély[1,2]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs, Hungary; [2]National Laboratory for Drug Research and Development, Magyar tudósok krt. 2. H-1117 Budapest, Hungary, [3]Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, Hungary, [4]PharmInVivo Hungary Ltd, Pécs

The tachykinin hemokinin-1 (HK-1) is expressed in several brain regions such the frontal cortex, hippocampus, amygdala both is mice and humans. It acts via the NK1 tachykinin receptor, but other targets are also suggested. The roles of HK-1 in pain and mood regulation are established, but little is known about its involvement in learning and memory. One study demonstrated stimulatory action of HK-1 on cholinergic neurons, and the related tachykinin Substance P was shown to improve learning. Therefore, we investigated the role of HK-1 in cognitive functions in mice under normal and pathological conditions.

Different memory parameters of young (3-5 months) and old (18 months) hemokinin-1-deficient (HK-1 KO) and C57Bl/6 wildtype (WT) female and male mice were tested in the Y and radial arm maze (YM and RAM) as well as in the novel object recognition test (NOR). During the 5-min-long experimental periods spontaneous alternations and arm entries in YM, reference and working memory errors in RAM and discrimination as well as recognition indices in NOR were determined. The muscarinic receptor antagonist scopolamine (1 mg/kg) was injected i.p. to impair cognitive functions in young mice.

In the YM test, male WT animals showed higher alternation index than females, and scopolamine reduced this parameter in males, but not in females. This alternation decrease was also observed in aged male WT animals, but no changes were observed in females neither by scopolamine nor aging. In RAM, female HK-1 KO mice treated with both saline and scopolamine showed worse memory with more errors than their WTs, but this difference was not detected in males. Interestingly, old male HK-1-deficient mice found less rewards in this test compared to the WTs. Scopolamine treatment did not significantly affect RAM parameters in either group and did not cause significant changes in the NOR test in WT animals. However, scopolamine-treated HK-1-deficient mice of both sexes showed reduced recognition index compared to WTs. Impaired memory observed in HK-1-deficient mice also occurred in old animals compared to young ones.

We provide here the first data for the involvement of HK-1 in learning and memory function. Identifying the target and mechanisms of action of HK-1 might help to understand its roles in cognitive functions and provide perspectives for drug development.

Funding: Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, National Brain Research Program 3.0, National Research, Development and Innovation Office - OTKA K138046 and OTKA FK137951, TKP2021-EGA-16, János Bolyai Research Scholarship of the Hungarian Academy of Sciences ÚNKP-21-5 new National Excellence Program of the Ministry for Innovation and Technology, Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.

OPTIMIZATION AND VALIDATION OF THE ENDOTOXIN-INDUCED ACUTE AIRWAY INFLAMMATION MOUSE MODEL

Emese Ritter[1], Kata Csekő[1,2], Zsuzsanna Helyes[1,2,3,4]
[1]Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School; [2]National Laboratory for Drug Research and Development, Budapest; [3]ELKH-PTE Chronic Pain Research Group; [4]PharmInVivo Hungary Ltd, Pécs

Airway inflammation has recently become in the focus of drug research, with a large number of drug development and repositioning studies being launched. In preclinical research, the endotoxin (lipopolysaccharide:LPS)–induced acute interstitial pneumonitis in rodents is the most commonly used mechanism model. However, studies often apply different LPS serotypes, doses, routes of administration and reference compounds, therefore comparisons are difficult. Here we aimed to optimize and validate this model with the reference compound dexamethasone in mice.

Pulmonary inflammation was induced by intratracheal administration of 100, 50, 20, 5 µg LPS (E. coli O111:B4; in 60 µl phosphate buffered saline (PBS)) in C57BL/6J mice, the intact control group received PBS. The groups with different doses of LPS were further divided into: i) LPS+vehicle (physiological saline ip.), ii) LPS+dexamethasone (5mg/kg ip. glucocorticoid). Respiratory functions were measured by restrained plethysmography in conscious mice 24 h later, then lungs were excised under anaesthesia and weighed. Inflammatory parameters were evaluated on hematoxylin-eosin stained lung sections.

LPS-evoked body weight loss, lung swelling and airway function alterations were not dose-dependent. Dexamethasone treatment counteracted the approximately 10-15% weight loss in the 50, 20 and 5 µg LPS groups, and significantly reduced lung oedema, increased breathing frequency and peak expiratory flow rate, as well as decreased tidal volume, minute ventilation and expiratory time in the 5 µg LPS group. LPS-induced inflammatory histopathological alterations such as edema formation, neutrophil granulocyte and eosinophil macrophage infiltration were dose-dependent, however, quantitative analysis is required to more accurately determine the effect of dexamethasone.

These results demonstrate dose-independent functional and morphological changes in response to LPS, which can be reduced by the glucocorticoid positive control only in case of 5 µg endotoxin. Since standardized, well-characterized and validated preclinical models are essential for pharmacological research, we suggest to use of this LPS dose in order to be able to compare the results with the reference compound.

Acknowledgements: TKP2021-EGA-13, Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, Hungary; RRF-2.3.1-21-2022-00015

ALPHA-2 ADRENOCEPTOR AGONISTS CAN AGGRAVATE RATHER THAN AMELIORATE INDOMETHACIN-INDUCED SMALL BOWEL DAMAGE IN RATS

Tóth András[1], Hutka Barbara[1§], László Szilvia[1], Pálnok Patrik[1], Kovács Krisztián[2], Gyires Klára[1], Zádori Zoltán[1]
[1] Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2] Department of Laboratory Medicine, Semmelweis University, Budapest, Hungary, [§] Current address: Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary

Introduction: It is increasingly recognized that non-steroidal anti-inflammatory drugs (NSAIDs) induce significant damage to the small intestine, with the prevalence rate of mucosal breaks of around 50% in chronic users. The development of small intestinal injury does not depend on acid secretion, but, instead, on other factors, including enteric bacteria, bile acids, prostaglandin deficiency and topical damaging effects. Since there is no well-established prophylactic approach, there is an urgent need to discover agents, that could prevent NSAID enteropathy. Besides others, our previous studies have also demonstrated that α2 adrenoceptor agonists induce protective effect in the stomach by multiple mechanisms. However, little is known about the effect of these medications on NSAID enteropathy.

Aims: To assess the effects of the α2 adrenoceptor agonist (clonidine and dexmedetomidine) on NSAID-induced enteropathy in an animal model.

Methods: In the first experiment male Wistar rats (180-220 g) were treated with clonidine (10 and 100 μg/kg) or dexmedetomidine (5 and 50 μg/kg) or their vehicle (distilled water) given by gavage twice daily for 3 days. NSAID enteropathy was induced by a single dose of indomethacin (20 mg/kg, suspended in 1% hydroxyethylcellulose solution), administered by gavage on day 2. On day 4, rats were euthanized, and the severity of enteropathy was evaluated by histological analyses; by measuring inflammatory marker levels in the bowel wall; and by assessing the extent of blood and serum protein loss. The gut microbiota composition was analysed by qPCR.

In the second experiment we assessed dexmedetomidine's effect on the blood pressure and on the blood flow of the small intestine in anesthetized rats.

Results: Indomethacin-induced enteropathy was associated with shortening of the small bowel (as a result of inflammation), elevation of inflammatory markers (myeloperoxidase, pentraxin-3), in the intestinal wall, and with severe blood loss, serum protein loss, and changes in the gut microbiota composition. Neither clonidine nor dexmedetomidine treatment could alleviate the observed pathology, moreover the higher dose of dexmedetomidine deteriorated some parameters.

Besides, enteropathy was accompanied with lower blood pressure, and oral dexmedetomidine reduced it further, and moderately reduced the blood flow of the small intestine.

Conclusion: Our results suggest that pharmacological stimulation of α2 adrenoceptors cannot prevent NSAID-induced enteropathy, moreover, it seems, they can aggravate it, despite their well-established gastroprotective effect. This difference may be due to the different pathogenesis of gastro- and enteropathy, or partly because α2 adrenoceptors can reduce the blood flow of the small intestine. Nevertheless, further investigations are needed to completely elucidate the mechanisms by which they affect enteropathy.

Funding: NKFI FK 138842, and EFOP-3.6.3-VEKOP-16-2017-00009

ASSOCIATION BETWEEN HYPOTHALAMUS CONNECTIVITY AND CIRCADIAN VARIATION OF MIGRAINE ATTACK ONSET

Anna Németh[1,2], Kinga Gecse[1,2], Dániel Baksa[1,2,3], Dóra Dobos[1,2], Csaba Aranyi[4], Miklós Emri[4], György Bagdy[1,2], Gabriella Juhász[1,2]
[1]Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, [2] NAP3.0-SE Neuropsychopharmacology Research Group, Hungarian Brain Research Program, Semmelweis University, Budapest, [3]Department of Personality and Clinical Psychology, Institute of Psychology, Faculty of Humanities and Social Sciences, Pazmany Peter Catholic University, Budapest, Hungary, [4] Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen, Debrecen

Introduction: Migraine attacks can be triggered by a myriad of factors, for instance changes in sleeping pattern and stress. Hypothalamus as a key regulatory region of the circadian rhythm and stress response, may affect the onset of migraine attacks that usually show diurnal variations. Previous studies demonstrated periodic hypothalamic functional connectivity alterations during migraine cycle. Therefore, hypothalamic functional connectivity may play a role in circadian variation of migraine attack onset.

Aim: The aim of this study was to investigate the functional connectivity alterations of hypothalamus regarding the circadian variation of migraine attack onset.

Methods: The study population included 46 patients with episodic migraine without aura divided into two groups based on their migraines chronobiologic characteristics. Morning start group (n=18) showed typical circadian attack onset before noon, meanwhile evening start group (n=28) between noon and midnight. Participants underwent a resting-state functional magnetic resonance imaging session in the afternoon. They were headache- and medication-free. Seed-to-voxel functional connectivity analysis was conducted with hypothalamus (MNI coordinates: x=-6; y=-6; z=12) as seed region. Two-sample t-test was performed to compare hypothalamic functional connectivity pattern between morning and evening start groups using SPM12. All analysis was corrected for age, sex and motions.

Results: Increased functional connectivity was found between hypothalamus and cerebellar Crus I (p_{FWE}=0.007) in morning start group compared to evening start group. No significantly increased hypothalamic connectivity was found in evening start group compared to morning start group.

Conclusions: Increased functional connectivity between hypothalamus and cerebellum characterizes migraine patients in interictal period compared to healthy controls. Previous study hypothesized a “reset-model” theory explaining low hypothalamic-cerebellar connectivity during migraine attack, which increases towards the next attack. Moreover, clock genes are highly expressed in cerebellum indicating its circadian oscillator effect. In line with previous studies, our results indicate that hypothalamic-cerebellar connectivity might affect the diurnal variation of migraine attack onset. However, the exact mechanism should be further investigated.

Funding: Development of scientific workshops of medical, health sciences and pharmaceutical educations (EFOP-3.6.3-VEKOP-16-2017-00009); ÚNKP-22-3-II-SE-27; 2017-1.2.1-NKP-2017-00002, NAP2022-I-4/2022; TKP2021-EGA-25; OTKA (K143391); ERA PerMed (2019-2.1.7-ERA-NET-2020-00005).

DIET-INDUCED MODERATE OBESITY DISTURBS CARDIAC MITOCHONDRIAL HOMEOSTASIS AND SERCA2A, WHICH ARE NOT RESTORED BY MAO-B INHIBITION.

Szabolcs Hambalkó [1], Csilla Pelyhe [1], Csenger Kovácsházi [1], Bence Kenyeres [1], Bernadett Kiss [1,2], Bence Ágg [1,2,3], Tamás G. Gergely[1,4], Bennet Weber[1], András Makkos[1,2], Gábor B. Brenner[1], Tímea Komlódi[5], László Tretter[5], Csaba Horváth[6], Izabela Jarabicová[6], Adriana Adameová[6,7], Anikó Görbe [1,2,8], Paola Poggi[9], Alexandros Chatgilialoglu[9], Attila Oláh[3], Tamás Radovits[3], Zoltán V. Varga[1,4], Béla Merkely[3], Péter Ferdinandy[1,2,8] and Zoltán Giricz [1,8]
[1]Semmelweis University, Department of Pharmacology and Pharmacotherapy, 1085 Budapest, Hungary, [2]MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, 1089 Budapest, Hungary, [3]Heart and Vascular Center, Semmelweis University, Budapest, Hungary, [4]HCEMM-SU Cardiometabolic Immunology Research Group, Semmelweis University, 1089 Budapest, Hungary, [5]Department of Medical Biochemistry, Faculty of Medicine, Semmelweis University, 1085 Budapest, Hungary, [6]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovak Republic, [7]Centre of Experimental Medicine, Institute for Heart Research, Slovak Academy of Sciences, Bratislava, Slovak Republic, [8] Pharmahungary Group, 6722 Szeged, Hungary, [9]Remembrane S.r.L., Imola, Italy

Obesity is a major risk factor for the development of cardiovascular diseases, which is associated with oxidative stress and chronic inflammation. Monoamine oxidases (MAOs) are major sources of mitochondrial reactive oxygen species. We have shown previously that MAO-B selective inhibitor selegiline reduces visceral adiposity in obesity, however, it has not been assessed if selegiline can alleviate cardiac oxidative stress. Therefore, we investigated the effects of selegiline on cardiac redox homeostasis and cellular damage in a high-fat high-sucrose diet (HFD)-induced obesity model of rat. We demonstrate that specific MAO-B inhibition by selegiline reduces cardiac mitochondrial ROS production in healthy, but not in HFD obese rats. Although HFD did not affect pro-survival, pro-death and oxidative stress-related mechanisms, it decreased sequestosome-1 level and B-cell lymphoma 2-associated X protein/B-cell lymphoma 2 (Bax/Bcl-2) ratio, and increased TNF and NF-kB expressions. Selegiline did not affect any of these HFD-induced alterations. Simulated hypercholesterolemic treatment disrupted mitophagy in H9c2 cardiomyocytes which was not restored by selegiline. Both SERCA2a and its upstream modulators were affected by HFD and selegiline, however it did not manifest in altered cytosolic calcium dynamics. In addition, we identified a previously unknown cardiac signaling molecule, forkhead box P2 gene (Foxp2), which was decreased in obesity, but not restored by selegiline. In conclusion, MAO-B inhibition is of no significant therapeutic value to alleviate cardiac consequences of obesity.

THERAPEUTIC PROPERTIES OF THE ANTHOCYANIN RICH PRUNUS CERASUS EXTRACT ON HYPERCHOLESTEROLAEMIA-ASSOCIATED CARDIAC DYSFUNCTION

Barbara Takács[1], Anna Szilágyi[1], Réka Szekeres[1], Vera Tarjányi[1], Dániel Priksz[1], Mariann Bombicz[1], Rudolf Gesztelyi[1], Zoltán Szilvássy[1], Béla Juhász[1], Balázs Varga[1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of General Medicine, University of Debrecen

The present study evaluates the potential therapeutic effects of anthocyanin rich Prunus cerasus (sour cherry) extract (PCE) on atherosclerosis-associated cardiac dysfunction. This condition is strongly associated with the impairment of both the nitric oxide – protein kinase G (NO-PKG) pathway and the antioxidant capacity. After creating the rabbit model of atherosclerotic cardiomyopathy with cholesterol rich diet, for 12 weeks the animals were divided into 4 groups: untreated control (C) group; healthy rabbits with 9 g/kg PCE treatment (C+PCE); hypercholesterolemic (HC) group kept on atherogenic diet; 9 g/kg PCE treated HC group (HC+PCE). After echocardiographic measurements along with serum analysis and ex vivo vascular studies, western blot technique and histological stains were performed. Dyslipidaemia, diminished endothelial function and marked signs of diastolic dysfunction along with myocardial hypertrophy and decreased cardiac expression of eNOS, PKG, SERCA2a and hsp70 were observed in HC rabbits. The PCE treatment significantly improved both the lipid profile and the cardiac function, moreover, significant PCE-associated increases were detected in the myocardial protein levels, but the vascular status was unaffected. Western blot analysis further revealed hypercholesterolaemia-associated increases in HO-1 expression. Although further investigations are required, anthocyanins through their effects on the myocardium may complement the therapy of atherosclerosis-associated diastolic dysfunction.

Supported by: New National Excellence Programme (ÚNKP-22-3) and Thematic Excellence Programme (TKP-2021-EGA).

CYCLODEXTRIN DERIVATIVES DECREASE TRPV1 AND TRPA1 ION CHANNEL ACTIVATION *IN VITRO*

Andrea Nehr-Majoros[1], Zsuzsanna Helyes[1], Éva Szőke[1]
[1]University of Pécs Medical School Department of Pharmacology and Pharmacotherapy, Pécs

Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) are expressed on primary sensory neurons and peripheral nerve endings. These non-selective ion channels are involved in pain integration and neurogenic inflammation. In the cell membrane they are embedded in specialized cholesterol and sphingolipid containing microdomains and these so-called lipid rafts facilitate TRP activation. Targeting protein-lipid interactions is a promising way of analgesia by novel mechanism of action. Cyclodextrins (CDs) are cyclic oligosaccharides forming inclusion complexes with cholesterol. Our research group revealed that lipid raft disruption by Methyl-β-cyclodextrin (MCD) leads to decreased TRP ion channel function with analgesic effect in different mouse models of inflammation and pain.
CD derivatives (Randomly methylated β-CD: RAMEB; (2-Hydroxypropyl)-γ-cyclodextrin: HPGCD; (2-Hydroxyproyil-β-cyclodextrin: HPBCD; Sulfobutylated β-cyclodextrin: SBECD; (2-Hydroxy-3-N,N,N-trimethylamino)propyl-β cyclodextrin: QABCD; isomeric mixtures of Heptakis(2,6)-di-O-methyl β-CD: DIMEB-50 and DIMEB-95; Heptakis(2,3,6)-tri-O-methyl β-CD: TRIMEB) were tested in respect of their cytotoxicity (24-hour treatment, chinese hamster ovary (CHO) cells) with CellTiter-Glo® Luminescent Cell Viability Assay.
To reveal the effect of CD treatment on mitochondrial function of CHO cells MitoTracker™ Red CMXRos fluorescent dye was used in laser scanning confocal microscopic experiments.
To detect alterations of TRPV1 and TRPA1 receptor activation after CD treatment radioactive 45Ca-uptake measurements were performed on CHO cells expressing TRPA1/V1 receptors.
In CellTiter-Glo® Assay methylated CD derivatives showed significant cytotoxic effect in lower concentrations (1-3 mM), non-methylated CDs did not decrease viability up to 10 mM. Mitochondrial function significantly decreased detected by MitoTracker™ Red labeling after 24-hour CD treatment in case of all methylated derivatives, except DIMEB.
All investigated CDs were able to inhibit 45Ca-uptake in TRPA1 and TRPV1 receptor-expressing CHO cells, presumably via lipid raft disruption.

LOW DOSE DOXYCYCLINE INHIBITS CHRONIC SMOKE-INDUCED CARDIOPULMONARY COMORBIDITIES IN A MOUSE MODEL: ROLE OF MATRIX METALLOPROTEINASES?

Kata Csekő[1,2], Ágnes Kemény[1,3], Tamás Kiss[1], Éva Szőke[1,2], László Deres[4,5], Krisztián Erős[6], Róbert Halmosi[5], Csilla Terézia Nagy[7], Krisztina Kiss[8,9], Ibolya Kiss[10], Attila Felinger[10], Péter Ferdinandy[7,9], Péter Bencsik[9,11], Zsuzsanna Helyes[1,2,12,13]
[1]Dept. of Pharmacology and Pharmacotherapy, University of Pécs Medical School, Pécs; [2]National Laboratory for Drug Research and Development, Budapest; [3]Dept. of Medical Biology, University of Pécs Medical School, Pécs; [4]Szentágothai Research Centre, University of Pécs; [5]I[st] Dept. of Internal Medicine, University of Pécs Medical School, Pécs; [6]Dept. of Biochemistry and Medical Chemistry, University of Pécs Medical School, Pécs; [7]Cardiometabolic Research Group, Dept. of Pharmacology and Pharmacotherapy, Semmelweis University, Faculty of Medicine, Budapest; [8]II[nd] Dept. of Pediatrics, Faculty of Medicine, Semmelweis University, Budapest; [9]Pharmahungary Group, Szeged; [10]Dept. of Analytical and Environmental Chemistry, University of Pécs, Faculty of Sciences, Pécs; [11]Cardiovascular Research Group, Dept. of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Szeged, Szeged; [12]Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs; [13]PharmInVivo Ltd, Pécs

Cigarette smoke-induced inflammatory processes and consequent tissue damage are the main causes of chronic obstructive pulmonary disease (COPD). We previously demonstrated significantly elevated matrix metalloproteinase-2 and -9 (MMP-2/-9) activities in the mouse lung after 6 months of smoke exposure (CSE). Since the involvement of MMPs in smoking/COPD-related cardiopulmonary alterations is not known, we aimed to elucidate their roles and the therapeutic potential of MMP inhibition in a well-characteried, chronic CSE-induced cardiopulmonary comorbidity mouse model.

COPD-like changes were induced by whole-body CSE for 30 min twice daily, 10 times/week for 6 months in male C57Bl/6J mice. Animals were treated orally with subantimicrobial dose of doxycycline (0.5 mg/mL, 80 mg/kg) as a non-selective MMP inhibitor. MMP-2/-9 activities were measured by gelatin zymography and expressions by qPCR. Respiratory functions were assessed by whole body pletysmography and cardiac functions by echocardiography. The effect of doxycyclin on MMP-2/-9 activities were analysed in tissue homogenates after incubation with the measured *in vivo* plasma concentration of doxycyclin.

MMP-9, but not MMP-2 mRNA expression was upregulated in the lung after 6 months of CSE. Although cardiac MMP-2 mRNA expression significantly upregulated already at 3 month and remained elevated compared to the intact condition, its activity did not change. MMP-9 Six months of CSE significantly decreased the left ventricular ejection fraction, deceleration time and tricuspidal annular plane discursion time indicating both systolic and diastolic dysfunctions, which were significantly improved by doxycyclin treatment. Despite in vitro inhibition of MMP activity by doxycycline based on its in vivo plasma levels significantly decreased MMP-9 activity in lung samples but not MMP-2 activity either in lung or in cardiac homogenates. Regarding respiratory function parameters only mid-expiratory flow showed CSE-iduced deterioration, which was not altered by doxycyclin.

Systemic treatment with subantimicrobial dose of doxycycline has beneficial effects on COPD-related chronic cardiac dysfunctions, but it seems not to be directly related to the inhibition of cardiac MMP-2 activity.

Acknowledgements: TKP2021-EGA-13, Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, Hungary; RRF-2.3.1-21-2022-00015

SMALL INTESTINAL ISCHEMIA/REPERFUSION INJURY CAN BE ALLEVIATED BY CHRONIC ADMINISTRATION OF CELECOXIB BUT NOT ROFECOXIB IN RATS

Szilvia B. László[1], Barbara Hutka[1§], Tamás Hegyes[2], András S. Tóth[1], Klára Gyires[1], Zoltán S. Zádori[1]

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; [2]Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary;

[§]Current address: Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary

Introduction: The occlusion of superior mesenterial artery (SMA) leads to extended small intestinal mucosal damage. However, there is no drug available on the market which is capable to ameliorate the mesenterial ischemia/reperfusion (I/R) injury. The role of cyclooxygenase-2 (COX-2) is still not clear in the literature. Acute administration of selective COX-2 inhibitors is likely to be protective in mesenterial ischemia/reperfusion model in rats, although the mucosal damage was aggravated by knocking-out of the COX-2 gene in mice.

Method: Male Wistar rats (220-350 g) were treated orally with two selective COX-2 inhibitors, celecoxib (10, 100 mg/kg) or rofecoxib (5, 50 mg/kg) dissolved in hydroxyethycellulose, once daily for 7 days. On the 8th day, SMA was occluded for 30 minutes after midline laporotomy under pentobarbital anesthesia (intraperitoneally 60 mg/kg). Occlusion was followed by 120 minutes of reperfusion. At the end of protocol, animals were terminated and distal jejunal samples were taken for further investigations (histology, Western blot, ELISA, qRT-PCR).

Results: The chronic administration of celecoxib and rofecoxib did not affect the body weight and small intestinal length. The jejunal myloperoxidase (MPO), interleukin-1β (IL-1β) and pentraxin-3 (PTX3) levels were significantly elevated by I/R in the vehicle-treated group. Celecoxib significantly and dose-dependently reduced the jejunal level of these inflammatory mediators. The mucosal amount of claudin-1 and occludin significantly decreased in the vehicle-treated I/R group, while the chronic celecoxib treatment significantly and dose-dependently prevented these alterations. According to histological analysis, the I/R provoked mucosal injury in the small intestine, which could be reduced by chronic administration of celecoxib. Interestingly, rofecoxib did not influence the I/R-induced alterations of MPO, claudin-1, occludin and PTX3.

Conclusion: The chronic treatment with celecoxib significantly and dose-dependently diminished the intestinal damage after I/R, while rofecoxib had no effect. Therefore, further investigations are needed to explain the different results obtained with the two drugs.

NEW MOLECULES IN DEPRESSION MAY DEPEND ON THE CONSTELLATION OF ITS TRADITIONAL RISK FACTORS – IS IT A WAY FROM GENOMICS TO PRECISION MEDICINE?

Eszlári Nóra[1], Hullám Gábor[1], Gál Zsófia[1], Török Dóra[1], Nagy Tamás[1], Millinghoffer András[1], Baksa Dániel[1], Gonda Xénia[1], Antal Péter[1], Bagdy György[1], Juhász Gabriella[1]
[1]Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary; and NAP3.0-SE Neuropsychopharmacology Research Group, Hungarian Brain Research Program, Semmelweis University, Budapest, Hungary

Precision medicine is an emerging approach in depression treatment. However, present strategies aimed at patient stratification for an optimal treatment choice mainly rely on biomarkers that need a specific (e.g., genetic or imaging) expertise to assess. Therefore, forming subgroups based on traditional risk factors of depression and then identification of biomarkers specific to each subgroup may contribute to an easier application of precision medicine strategies.

Our present aim was to explore the genomic background of depressive symptoms within specific clusters characterized by distinct patterns of five traditional depression risk factors. They are sex, neuroticism (a propensity to experience negative emotions), body fat percentage, years spent in education, and number of stressful events in the last two years.

323,081 UK Biobank participants (application 1602) were included in the study. Two-step cluster analysis was run separately in the two sexes and including the other four depression risk factors as input variables. A genome-wide association study for depressive symptoms was run within each resulting cluster. Summary data-based Mendelian randomization (SMR) was used to explore whether gene expression mediates genetic effects on depressive symptoms. For this, the PsychENCODE transcriptome database was used, with RNA-seq data of frontal and temporal human brain cortices.

We could identify eight clusters with distinct patterns of the input variables, but only two of them showed suggestive results in the SMR analyses. In a female cluster with high neuroticism and body fat levels but low stress and education levels, a higher expression level of long noncoding RNA *AC009404.2* mediated between rs17047412 C allele and higher depression level ($p_{SMR}=1.52x10^{-5}$). In contrast, in males with a protective constellation of traditional risk factors (high level of education, and low levels of neuroticism, stress, and body fat), decreased expression of two actin cytoskeleton-linked genes mediated the risk for depression. Particularly, rs2696295 T allele may increase depression level via decreased *SGCA* expression ($p_{SMR}=1.93x10^{-5}$), and rs10861966 G allele via decreased *CORO1C* expression ($p_{SMR}=3.58x10^{-5}$). These two genes reside in two different chromosomes.

The potential role of lncRNAs in depression has already been discussed in the literature, but our results corroborate it only in a well-specified female subgroup. Involvement of actin cytoskeleton in the emergence of psychiatric symptoms has been discussed e.g., in our previous work with catenin alpha-2. However, our present results underscore it only in the absence of traditional depression risk factors in males. A limitation of our present results is that they do not survive correction for multiple testing. However, they may represent a way from depression genomics towards the application of precision medicine strategies.

Supported by: 2019-2.1.7-ERA-NET-2020-00005; NAP2022-I-4/2022; ÚNKP-22-4-II-SE-1.

SEVERITY OF IMMUNE CHECKPOINT INHIBITION-INDUCED CARDIOTOXICITY DEPENDS ON SEX AND COMORBIDITIES: A MOUSE STUDY

Nabil V. Sayour[1,2], Dániel Kucsera[1,2], Péter Ferdinandy[1,3], Zoltán V. Varga[1,2]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, Hungary, [3]Pharmahungary Group, Szeged, Hungary

Immune checkpoint inhibitors (ICI) have revolutionized cancer therapy by enhancing the cytotoxic effects of T cells against tumors, however, the cardiotoxic - and other immune-related adverse - effects of ICI therapies are increasingly recognized. Despite the accumulating data on the underlying mechanisms, the influence of sex and cardiometabolic comorbidities on ICI-induced cardiotoxicity still remains to be investigated. Therefore, here we aimed to assess how ICI-induced cardiotoxicity is influenced by sex and cardiometabolic comorbidities in an aging mouse model.

To this end, 17 months old male or female C57BL6/J mice were randomized into control diet (CON) or high fat diet plus L-NAME (HFD) groups (n=20-22). 15 weeks after initiation of diet, each group was randomized to receive PBS (VEH) or 200 ug anti-PD-1 mAb (ICI) 3-times a week for 2 weeks (i.p., in 200 uL), followed by termination of mice. Echocardiography was performed 1 day before initiation of VEH or ICI treatment (baseline - BL), and at termination (TRM). Organs were sampled for histology and western blot experiments. Cardiac histology was performed for cell-surface-area (CSA) and microvascular density (MVD) measurements. Western blots on spleens for T-cell exhaustion markers were performed. All data were evaluated in a blinded fashion. Data are calculated as mean ± standard error of the mean. When TRM was compared to TRM, two-way ANOVA with Tukey's post hoc test was used; when TRM was compared to BL, two-way ANOVA, repeated measures, Sidak's post hoc test was used.

At BL, HFD caused no change in ejection fraction (EF), however, E/e´ was significantly increased in male HFD (29.28±1.43 in CON vs. 37.54±2.33 in HFD, $p<0.05$), but not in female HFD mice, compared to the corresponding CON groups. At TRM, ICI treatment led to a significantly decreased EF compared to BL in the CON diet groups of both sexes (for males: 63.68±2.60 at BL vs. 53.59±2.49 at TRM, $p<0.05$; for females: 69.76±1.77 at BL vs. 58.15±3.69 at TRM, $p<0.05$), but not in the HFD groups. CSA was significantly increased and MVD was significantly reduced in male HFD mice receiving ICI treatment, compared to corresponding CON and/or VEH groups. ICI treatment had no effect on CSA or MVD in female mice independently of HFD. Western blot analyses show an increase in T-cell exhaustion markers in male HFD vs. CON spleens, but not in female mice.

In conclusion, this is the first demonstration of measuring ICI-induced cardiotoxicity in aged mice of both sexes with cardiometabolic comorbidities. In male, but not in female mice, HFD caused diastolic dysfunction. ICI treatment of CON, but not of HFD mice of both sexes caused systolic dysfunction. Only in male HFD mice, ICI caused a significant cardiac hypertrophy with no change in cardiac function. The causes of sex-, and diet-related differences in ICI-related cardiotoxicity will be investigated in the future, with a special emphasis on immune functions.

MITOCHONDRIAL EFFECTS OF INHALED METHANE THERAPY AT DIFFERENT STAGES OF 24-H SEPSIS PROGRESSION IN RATS

Levente Gulácsi[1], Bálint Czakó[1], László Juhász[1], Attila Rutai[1], Szabolcs Péter Tallósy[1], Mihály Boros[1], József Kaszaki[1]
[1]Institute of Surgical Research, Albert Szent-Györgyi Medical School, University of Szeged, Szeged

Introduction: Changes in oxygen extraction (OER) during sepsis determine the development of tissue oxygen debt, and thus the progression of multi-organ failure. Methane (CH_4) is a non-toxic biological gas, and several studies established that low concentrations of inhalation treatments exert anti-inflammatory effects in acute ischaemia/hypoxia-associated pathologies. Our main goal was to investigate the effects of CH_4 therapy applied at different time intervals during sepsis progression on organ, cellular (leukocyte) and subcellular (mitochondrial) levels in an intraabdominal rat sepsis model.

Methods: Male Sprague-Dawley rats (350-400g) were subjected to faecal peritonitis (0.6 g/kg i.p.; n=24) or sham-operation (n=7). Septic animals were divided into untreated and CH_4-treated (2% CH_4 in normoxic air) subgroups (n=8, each) with different starting time but the same duration of treatments between 3-6 h, 16-20 h and 20-22 h of sepsis progression, respectively. The animals were anaesthetised after 22 h for invasive monitoring with haemodynamic, respiratory and biochemical monitoring, the severity of organ dysfunctions was evaluated with a rat-specific scoring system (ROFA). OER was calculated from arterial and venous blood gas values, myeloperoxidase (MPO) levels, a marker a neutrophil activation, were determined from plasma, kidney and cerebellar samples. Mitochondrial complex I-II-linked oxygen consumption (CI-CII OXPHOS) were measured with high-resolution respirometry (Oroboros O2k, Austria).

Results: Sepsis resulted in elevation in organ-dysfunction parameters, the MPO levels increased, OXPHOS values decreased significantly. All CH_4 treatments reduced the OER but did not affect significantly the global ROFA scores as compared to the untreated septic group. However, kidney and cerebellum MPO concentrations were markedly decreased in all treated animals, earlier (3-6 h and 16-20 h) CH_4 treatments significantly improved cerebellar CII OXHOS, while all the CH_4 treated groups showed significantly higher CII OXPHOS values in the kidney.

Conclusion: We demonstrated that exogenous CH_4 administration can influence the sepsis-induced neutrophil activation and OER-linked renal mitochondrial dysfunction. Additionally, CH_4 treatments applied in earlier phase of sepsis exerted beneficial effects on cerebellar mitochondrial respiration too. We assume that a timely initiation of a prolonged inhalation therapy regimen may alleviate the organ damage caused by sepsis in the longer run.

"This research work was conducted with the support of the National Academy of Scientist Education Program of the National Biomedical Foundation under the sponsorship of the Hungarian Ministry of Culture and Innovation."

THE TOPICALLY APPLIED HYDROXYLAMINE DERIVATIVE, BGP-15 SHOWED RETINOPROTECTIVE EFFECTS IN ANIMAL MODEL OF EYE ISCHEMIA

Anna Szilágyi[1], Barbara Takács[1], Balázs Varga[1], Béla Juhász[1], Zoltán Szilvássy[1]
[1]Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecent

Introduction: Diabetic retinopathy is one of the most common causes of blindness in Hungary and because of the ever-increasing incidence of diabetes mellitus, this microangiopathic complication has become more and more prevalent. Treatment of diabetic retinopathy includes good serum glucose control, intraocular anti-VEGF therapy, photocoagulation and surgical treatments, such as vitrectomy. The hydroxylamine derivative, BGP-15 is a relatively new drug candidate, which has been tested in systemic administration in nephrotoxicity, neuropathy, myopathy, ischaemic-reperfusion injury of the heart and especially insulin resistency. Based on the latter two mechanisms of effect, our workgroup investigated the compound in type 2 diabetic animals as a potential candidate in the treatment and prevention of the disease, and found it to be retinoprotective.

Methods: In the present study, taking into consideration all the beneficial effects of BGP-15 in the many different pathological conditions via systemic administration, including diabetes, we wanted to test the efficacy of a local formulation in a rat model of eye ischemia, a mechanism seriously involved in diabetic retinopathy as well. We administered an eye drop twice a day for a week and injected BGP-15 solution intravitreally once in the beginning of the reperfusion phase. Electroretinography was carried out 7 days later. Animals were then sacrificed and the eyeballs were removed for further histological examinations.

Result: After the topical BGP-15 treatment, electroretinogramms, elicited by 3000 mcd*s/m2 and high-intensity 10 000 mcd*s/m2 light stimuli in scotopic settings, showed significant differences in both a- and b-waves amplitudes and in their respective implicit times. Retinal thickness in histological sections was significantly greater in the topical BGP-15 treated group and the healthy, untreated group's results were not significantly different from the other groups' results.
Based on the electroretingraphical results, the intravitreal injection was so harmful that we had to exlude these groups from further investigations, unfortunately.

Conclusion: Topical BGP-15 formulation has been shown to be promising and could be valuable for research into future treatments for diabetic retinopathy.

Our study was supported by: New National Excellence Programme (ÚNKP-22-3) and Thematic Excellence Programme (TKP-2021-EGA).

TRANSCRIPTOMIC ANALYSIS OF DEEP-INFILTRATING COLONIC ENDOMETRIOSIS SAMPLES SUGGESTS PATHOPHYSIOLOGICAL ROLES OF HORMONE, GROWTH FACTOR AND CYTOKINE SIGNALLING

Norbert Tóth[1,2*], József Kun[1,3*], Réka Brubel[4], Attila Bokor[4], Péter Urbán[3], Krisztina Pohóczky[1,2,5#], Zsuzsanna Helyes[1,2,6#]

[1]University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy (Pécs, Hungary), [2]National Laboratory for Drug Research and Development (Budapest, Hungary), [3]University of Pécs, Szentágothai János Research Center, Genomics and Bioinformatics Core Facility (Pécs, Hungary) , [4] Semmelweis University, 1st Department of Obstetrics and Gynecology (Budapest, Hungary), [5]University of Pécs, Faculty of Pharmacy, Department of Pharmacology (Pécs, Hungary), [6]Eötvös Loránd Research Network (Chronic Pain Research Group) (Pécs, Hungary)

[*,#]These authors contributed equally to the present work.

Endometriosis is an estrogen-dependent common disease (10% of fertile women) represented by ectopic endometrium outside the uterus most commonly in the colon, which results in infertility and pain. The pathophysiological mechanisms are unclear, but complex sensory-vascular-immune interactions are likely to be involved. Unbiased transcriptomic analysis of tissue samples is a useful tool to determine key mediators, signaling processes and pathways and to identify potential novel drug targets. Therefore, here we analyzed the mRNA expression profile of deep-infiltrating colonic endometriosis samples in comparison with normal tissues.

In total 9 bowel endometriosis, 13 control bowel and 9 control endometrium samplex were examined by next generation sequencing (Illumina NovaSeq 6000) following QuantSeq 3’ mRNA library preparation. After filtering and trimming, reads were aligned to the human reference genome GRCh37 using the STAR aligner and counted using HTseq. Data were normalized and differential expression analysis was run by the limma-voom approach. Functional enrichment analysis was performed by the KEGG database.

33 genes were significantly upregulated including leptin receptor, insulin-like growth factor binding protein 5, transforming growth factor beta 3, collagen VIII, XIV and XVI. Meanwhile, compared to control bowel tissues 238 genes were significantly upregulated such as estrogen-receptor 1, progesterone receptor and voltage-dependent Ca^{2+} channel 1G, while 107 genes were downregulated like calcium/calmodulin-dependent protein kinase and vasoactive intestinal peptide. In comparison with control endometrium samples 488 genes were significantly upregulated, while 69 downregulated (e.g. interleukin-2 and 20 receptors). The KEGG pathway analysis revealed potential roles of the altered receptors, ion channels, growth factors and cytokines in promoting cell motility, survival and proliferation, cell cycle progression, creation of stress fibers, inflammatory reactions and decreasing apoptosis.

Pharmacological interventions to modify these targets and pathways might provide novel perspectives for endometriosis therapy.

Grants: TKP2021-EGA-13, RRF-2.3.1-21-2022-00015, Eötvös Loránd Research Network, János Bolyai Research Scholarship, ÚNKP-22-5-PTE-1731, PTE ÁOK KA-2022-11

ALTERED AMINOACID, MONOAMINE AND GLYCEROPHOSPHOLIPID METABOLITE PROFILE IN THE RAT PLASMA IN AN OROFACIAL INFLAMMATORY PAIN MODEL

Krisztina Takács-Lovász [1], Tímea Aczél[1], Kata Bölcskei[1], József Kun[1,6], Mihal Ciborowski[5], Witold Bauer[5], Barna Vásárhelyi[7], Gellért B. Karvaly[7] and Zsuzsanna Helyes[1,2,3,4]
[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, [2]PharmInVivo Ltd., [3]National Laboratory for Drug Research and Development, Magyar tudósok krt. 2. H-1117 Budapest, Hungary, [4]Eötvös Lorand Research Network, Chronic Pain Research Group, University of Pécs, [5]Univeristy of Byalistok, Byalistok, [6]Bioinformatics Research Group; Bioinformatics and Genomics Core Facility, Szentágothai Research Center, University of Pécs, [7]Department of Laboratory Medicine, Semmelweis University, Hungary

Orofacial pain and allodynia result from the activation and sensitization of trigeminal primary afferents similarly to migraine, it often occurs in interictal migraine periods. The mechanisms are not clearly understood, and the therapy is often unsatisfactory. Unbiased metabolomic analysis is a useful tool to investigate pathophysiological mechanisms and pathways. Here we analyzed the metabolomic changes in the rat plasma in an orofacial inflammation model.

Inflammation was induced by s.c. injection of 50 µl complete Freund's adjuvant (CFA) into the right whisker pad of male Wistar rats. Facial touch sensitivity was measured with von Frey filaments and blood samples were collected 3 days later. Control rats received the same volume of saline (1). Plasma metabolic fingerprinting was performed by LC-Q-TOF-MS, targeted analysis was conduced the by MxP® Quant 500 kit (Biocrates). Samples with >20 % CV were filtered out, MetaboAnalyst 4.0 and Reactome platforms were used for analysis.

Approximately 30% facial allodynia developed 3 days after CFA injection in the affected area. Increased plasma serotonin, carnosine, decreased sarcosine and methionine sulfoxide levels were detected in CFA-treated rats compared to saline. Based on the list of significantly changed metabolites between the two groups, Reactome analysis demonstrated the enrichment of Na^+/Cl^--dependent neurotransmitter transporters, amino acid transport across the plasma membrane, tryptophan catabolism, metabolism of amino acids and derivatives. Metaboanalyst revealed altered phenylalanine, tyrosine and tryptophane biosynthesis, as well as tryptophan metabolism.

These results suggest that metabolomic analysis gives valuable insight into the mechanisms of trigeminovascular activation related to pain, and it might provide a useful platform for the investigation of trigeminal pain conditions including migraine.

This research was supported by National Research Development and Innovation Office grant OTKA FK132587 and FK138046. TKP2021-EGA-16 has been implemented with the support provided from National Research, Development and Innovation Fund of Hungary financed under EGA 16 funding scheme. RRF-2.3.1-21-2022-00015 has been implemented with the support by the European Union.

(1) Aczél, T., Kun, J., Szőke, É., Rauch, T., Junttila, S., Gyenesei, A., Bölcskei, K., & Helyes, Z. (2018). Transcriptional Alterations in the Trigeminal Ganglia, Nucleus and Peripheral Blood Mononuclear Cells in a Rat Orofacial Pain Model. Frontiers in molecular neuroscience, 11, 219. https://doi.org/10.3389/fnmol.2018.00219

CARDIOTOXICITY OF ANTI-PD-1 IMMUNE CHECKPOINT INHIBITOR IN MICE WITH PRIOR CARDIAC ISCHEMIC INJURY

Tamás G. Gergely[1,2,3], Tamás Kovács[1,2,3], Nabil V. Sayour[1,2,3], Viktória E. Tóth[1,2,3], Dániel Kucsera[1,2,3], Zsófia Onódi[1,2,3], Imre Vörös[1,2,3], Márton Kocsis[1,2,3], Gábor M. Mórotz[1,2,3], Péter Ferdinandy[1,4], Zoltán V. Varga[1,2,3]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, [3]MTA-SE Momentum Cardio-oncology and Cardioimmunology Research Group, Budapest, [4]Pharmahungary Group, Szeged

Introduction: Immune checkpoint inhibitors (ICI), such as monoclonal antibodies targeting programmed death ligand-1 (PD-1), revolutionized cancer treatment. However, they can lead to several cardiovascular adverse effects, ranging from mild cardiac dysfunction to fulminant, lethal myocarditis. Nevertheless, the mechanisms and risk factors behind the diverse forms of ICI-induced cardiotoxicity are not understood currently.

Hypothesis: In this study, we hypothesized that a prior cardiac ischemic injury, leading to acute immune cell infiltration and activation, but without subsequent heart failure, can exacerbate the cardiotoxicity and cardiac inflammation caused by anti-PD-1 monoclonal antibodies. Furthermore, we aimed to investigate in our mouse model whether abatacept, an inhibitor of T-cell co-stimulation, can ameliorate the ICI-induced cardiac effects.

Methods: First, we treated 8 weeks-old C57BL/6J mice with isoprenaline (ISOP group, 160 mg/kg, n = 43) or with its solvent (CON group, n = 38), to induce reversible cardiac ischemia. Validation of the ischemic injury was performed in 6 randomly selected animals from each group two days after the treatment with histology and echocardiography. After this, the animals underwent 16 weeks of recovery period, followed by echocardiography to confirm cardiac functional recovery. Here, mice from both groups were randomized to three further treatment groups: isotype control, anti-PD-1 alone, or anti-PD-1 combined with abatacept and were treated for two weeks, with three weekly intraperitoneal injections (immune checkpoint inhibition phase). Echocardiography, qRT-PCR and histology was performed to evaluate cardiac function and inflammation.

Results: Two days after the initial ISOP treatment, mice displayed significant reduction in ejection fraction and infiltration of inflammatory cells were seen on histology. During the recovery period, 8 mice from the ISOP group and one mouse from the CON died. After the immune checkpoint inhibition phase, mice with prior ischemic injury and anti-PD-1 treatment (ISOP + anti-PD-1 alone) showed significant cardiac dysfunction on echocardiography, while animals with abatacept treatment (ISOP+anti-PD-1+abatacept) showed normal cardiac function. With qRT-PCR and histology, increased infiltration of T-cells and macrophages was seen in the myocardium of the ISOP+anti-PD-1 treated group compared to CON animals, with increased expression of pro-inflammatory cytokines, including *Il17a*, *Il23* and *Ifng*. However, no cardiac infiltration was seen in mice without prior ischemic injury and the pro-inflammatory cytokine response was less pronounced as well.

Conclusion: Prior cardiac ischemic injury without overt cardiac dysfunction exacerbates cardiac inflammation and cardiotoxicity induced by anti-PD-1 immune checkpoint inhibition therapy. Patients with pre-existing ischemic heart disease may be at greater risk for developing ICI-induced severe cardiac adverse events.

Posters Other Topics

THE EFFECTS OF GHRELIN AND [D-LYS3]-GHRP-6 ON ACUTE NICOTINE WITHDRAWAL FOLLOWING CHRONIC NICOTINE ADMINISTRATION

Jázmin Ayman[1], Megyesi Kíra[2], Roberta Dochnal[3], Krisztina Csabafi[4], Zsolt Bagosi[4]
[1]Department of Obstetrics and Gynecology, Szent-Györgyi Albert School of Medicine, University of Szeged, [2]Interdisciplinary Center for Excellence, Clinical Research Competence Center, Szent-Györgyi Albert School of Medicine, University of Szeged, Interdisciplinary Center for Excellence, Clinical Research Competence Center, Szent-Györgyi Albert School of Medicine, University of Szeged, [3]Department of Pediatrics and Pedriatic Health Center, Szent-Györgyi Albert School of Medicine, University of Szeged, [4]Department of Pathophysiology, Szent-Györgyi Albert School of Medicine, University of Szeged

Ghrelin is a natural orexigenic neuropeptide and [D-Lys3]-GHRP-6 is a synthetic Met-enkephalin analogue. Both act through the GHSR-1a receptor, although it has been unclear whether [D-Lys3]-GHRP-6 is an agonist or an antagonist. Our previous study has suggested that ghrelin may play role in the development of nicotine dependence. In our present experiments, we investigated the effects of ghrelin and [D-Lys3]-GHRP-6 on acute nicotine withdrawal following chronic nicotine administration. In order to do so, male Wistar rats were used. They were treated intraperitoneally (ip) with nicotine for 7 days, twice a day (in the morning and evening) and intracerebroventricularly (icv) with ghrelin and/or GHRP-6 for the same period, once a day (in the evening). After 7 days of nicotine administration and 1 day of withdrawal, rats were monitored in a conducta system based on the principles of the open-field test. A previous study demonstrated that nicotine at lower doses stimulates, whereas at higher doses inhibits locomotion, and that ghrelin enhances the biphasic, stimulatory-inhibitory effect induced by nicotine. In the present study, horizontal and vertical activity increased after chronic nicotine treatment, but both decreased after acute nicotine withdrawal. Ghrelin administration did not affect the hyperactivity induced by chronic nicotine treatment, but prevented the hypoactivity produced by acute nicotine withdrawal. The effects of [D-Lys3]-GHRP-6 regarding locomotion were very similar to those of ghrelin. Our results suggest that, just like ghrelin, [D-Lys3]-GHRP-6 acts through the GHSR-1a receptor as an agonist, and not as an antagonist, and both peptides attenuate the symptoms of acute nicotine withdrawal.

Acknowledgements: The present study was sponsored by SZAOK-SZGYA: 2023.02.01.-2025.01.30.

THE EFFECTS OF UROCORTIN FRAGMENTS ON ANXIETY AND DEPRESSION

Megyesi Kíra[1], Jázmin Ayman[2], Zsolt Bozsó[3], Krisztina Csabafi[4], Zsolt Bagosi[4]
[1]Interdisciplinary Center for Excellence, Clinical Research Competence Center, Szent-Györgyi Albert School of Medicine, University of Szeged, [2]Department of Obstetrics and Gynecology, Szent-Györgyi Albert School of Medicine, University of Szeged, [3]Department of Medical Chemistry, Szent-Györgyi Albert School of Medicine, University of Szeged, [4]Department of Pathophysiology, Szent-Györgyi Albert School of Medicine, University of Szeged

The urocortins (Ucn1, Ucn2 and Ucn3) are corticotropin-releasing factor (CRF)-like neuropeptides with similar amino acid sequences, but different receptor affinities compared to CRF. CRF binds with higher affinity to the CRF1 receptor, whereas Ucn2 and Ucn3 bind with much higher affinity to the CRF2 receptor and, therefore, they are considered to be selective CRF2 receptor agonists. Our previous study has shown that Ucn2 and Ucn3 ameliorate anxiety- and depression-like symptoms in mice. The aim of the present study was to investigate the effects of Ucn2 and Ucn3 fragments on anxiety and depression. For this purpose, male C57BL/6 mice were used. They were injected intracerebroventricularly (icv) with different urocortin fragments. After thirty minutes, the mice were tested in elevated plus-maze test and forced swim test. Ucn2(1-21) was the only effective fragment of Ucn2 that induced both anxiolytic and antidepressant effects. Several fragments of Ucn3 were effective, but the most significant anxiolytic and antidepressant effects were observed with Ucn3(36-38). Our results suggest that the full sequence of selective CRF2 receptor agonists is not required to exert anxiolytic and antidepressant effects. We also found that the biologically active site is located at the N-terminal site for Ucn2 and at the C-terminal site for Ucn3. Our next study will aim to find the biologically active amino acid sequence of Ucn1, binding with equal affinity to both CRF receptors.

Acknowledgements: SZAOK-SZGYA: 2023.02.01.-2025.01.30.

NETWORK-BASED ANALYSIS OF ADVERSE EVENT REPORTS TO IMPROVE SIGNAL DETECTION IN PHARMACOVIGILANCE

Pétervári Mátyás[1,2], Benczik Bettina[1,3], Balogh Olivér[1], Petrovich Balázs[1], Ágg Bence[1,3], Ferdinandy Péter[1,3]
[1]Cardiometabolic and MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér 4., 1089 Budapest, Hungary, [2]Sanovigado Kft, Budapest, Hungary, [3]Pharmahungary Group, Szeged, Hungary

Introduction: Signal detection in pharmacovigilance is the process of detecting potential causal associations between the consumed or administered drugs and the experienced adverse events from individual case safety reports (ICSRs). Signal detection methods provide positive results in only around 2.1% of cases, creating a demand for more complex and more efficient approaches in the pursuit of drug safety.

Aim: The aim of this study was to develop a novel network-based method for the evaluation of ICSRs, which could outperform the reporting odds ratio (ROR) statistical signal detection method used by the European Medicines Agency (EMA) in their pharmacovigilance system (EudraVigilance).

Methods: We developed a web-based application, called Vigilace™, that creates a network representation of the provided ICSRs, in which nodes represent drugs and adverse events, and edges are their associations. Edge weights are calculated by taking the co-occurrence count of the source and target nodes in the data, divided by the number of 3-node subgraphs (triangles) the edge was part of in the network. Consequently, edges without triangles are excluded. Given the type of the third node in the considered triangles, we defined three separate scores (full, drug, adverse event), and termed the weights as normalized edge weight for signals (NEWS) scores. For evaluation, the ICSRs of five cardiovascular adverse events (acute myocardial infarction, arrhythmia, pulmonary hypertension, QT prolongation, postural orthostatic tachycardia syndrome) were requested from the EudraVigilance database, amounting to an overall 72 475 reports. We created our ground truth reference dataset by merging the SIDER and IMI Protect drug adverse event databases, which contain known drug-event associations, mostly from text mined drug labels. The merged set was reduced to only contain entries of the five investigated adverse events.

Results: Evaluation was performed by comparing the performance of the calculated NEWS scores against the ROR method, using its 95% confidence interval lower bound values, ROR(−). The different score types showed varying levels of improvements, but the general tendency was close to or significantly better than the ROR values. In a particular example, AUROC analysis for the acute myocardial infarction resulted in 0.863 ($NEWS_{Full}$), 0.856 ($NEWS_{D}$) and 0.739 ($NEWS_{AE}$) against 0.720 for the control ROR(−) method. Overall, the $NEWS_{D}$ variant was the best performing across the investigated adverse events.

Conclusion: Our work is the first demonstration on the viability of network-based representations, especially the $NEWS_{D}$ score, in pharmacovigilance for the purpose of signal detection.

NETWORK THEORETICAL MICRORNA-TARGET PREDICTION IN PRESSURE OVERLOAD-INDUCED LEFT VENTRICULAR HYPERTROPHY AND SYSTOLIC HEART FAILURE

Mihály Ruppert[1], Sevil Korkmaz-Icöz[2], Bettina Benczik[3,4], Bence Ágg[3,4], Dávid Nagy[1], Tímea Bálint[1], Alex Ali Sayour[1], Attila Oláh[1], Bálint András Barta[1], Kálmán Benke[1], Péter Ferdinandy[3], Béla Merkely[1], Mathias Karck[2], Tamás Radovits[1*], Gábor Szabó[2,5*]
[1]Heart and Vascular Center, Semmelweis University, Budapest, [2]Department of Cardiac Surgery, University of Heidelberg, Heidelberg, [3]Pharmahungary Group, Szeged, [4]Cardiometabolic and MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, [5]Department of Cardiology, Angiology and Pulmonology, University Hospital Heidelberg, Heidelberg

Although systolic function characteristically shows gradual impairment in pressure overload (PO)-evoked left ventricular (LV) hypertrophy (LVH), rapid progression to congestive systolic heart failure (HF) occurs in distinct cases. The molecular mechanisms of the transition between adaptive and maladaptive changes in case of PO-evoked LVH to HF are less known.

Here, we aimed to examine microRNA (miRNA) expression and miRNA-driven posttranscriptional gene regulation in the two forms of PO-induced LVH (with/without systolic HF) and identify the differences between the two groups and the sham group.

PO was induced by aortic banding (AB) in rats. Sham-operated animals served as controls. The majority of AB animals demonstrated concentric LVH and slightly decreased systolic function (termed as AB_{LVH}). Contrarily, in some AB rats, severely reduced ejection fraction, LV dilatation and increased lung weight-to-tibial length was noted (referred to as AB_{HF}). To understand the underlying molecular regulation, miRNA expression profiles were assessed by small RNA-sequencing. To reveal genes affected by the changes identified in the miRNA expression profiles, unbiased miRNA-target prediction was performed using the network theoretical miRNAtarget software.

Global LV miRNA profiling revealed 50 differentially regulated miRNAs based on p-value and fold change threshold in AB_{HF} compared to AB_{LVH}. In the AB_{LVH} group when compared to the sham group 14 differentially expressed miRNAs were identified based on p-value and fold change threshold. In case of the AB_{HF} group, even under stricter conditions (q-value and fold change value based selection), 62 miRNAs showed differential expression compared to the sham group. Network theoretical miRNA-target analysis predicted more than 3000 genes with miRNA-driven dysregulation between AB_{LVH} and AB_{HF}. Target validation revealed 5 genes (*Fmr1*, *Zfpm2*, *Wasl*, *Ets1, Atg16l1*) predicted with high node strength (≥absolute value of 4), which showed decreased mRNA expression in AB_{HF}. When comparing AB_{LVH} and AB_{HF} to sham miRNA-target prediction identified 957 and 3353 possible targets, respectively. In case of the comparison of AB_{LVH} with sham, no genes with high node strength were identified, while in case of the comparison of AB_{HF} with sham 7 predicted genes (*Fmr1*, *Zfpm2*, *Mier3, Clock*, *Ets1, Tle4*, *Atg16l1*) with high node strength were successfully validated.

PO-evoked systolic HF is associated with a unique miRNA signature, which negatively regulates the mRNA expression of *Fmr1*, *Zfmp2*, *Wasl*, *Ets1* and *Atg16l1*. The network theoretical miRNA-target prediction also revealed that besides the dysregulation of the above mentioned genes, *Mier3, Clock* and *Tle4* genes might also play an important role in the development of the differences between HF and healthy conditions.

Project no. RRF-2.3.1-21-2022-00003 has been implemented with the support provided by the European Union. TKP2021-EGA-23 has been implemented with the support provided by the Ministry of Innovation and Technology of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme. The study was supported by the National Research, Development and Innovation Office of Hungary (National Heart Program; NVKP-16-1-2016- 0017), by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (to M.R.), by a grant from the National Research, Development and Innovation Office (NKFIH) of Hungary (K134939 to T.R.) and by the Medical Faculty of the University of Heidelberg Germany (to S. Korkmaz-Icöz). BÁ was supported by the New National Excellence Program of the Ministry for Innovation and Technology from the National Research, Development and Innovation Fund source (ÚNKP-20-4-I-SE-7, ÚNKP-21-4-II-SE-18).

COMPERATIVE ANALYSIS OF PATTERN RECOGNITION RECEPTORS IN VARIOUS ORGANS OF POSTMORTEM SARS-COV-2 PATIENTS

Eduárd Bíró[1], Rebeka Fekete[2], Ádám Dénes[2#], Szilvia Benkő[1#]
[1]Inflammationphysiology Research Group, Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary.' [2]Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary.
joint senior authors

Introduction: The COVID-19 pandemic had enormous socio-economic impact due to the rapid spread of the SARS-CoV-2 virus and the large number of patients affected. In addition to the detrimental effects of acute infection, many people experience post-covid syndrome (PCS) after severe or even mild COVID-19 illness. While the acute phase of the disease is characterized by high proinflammatory cytokine levels and prolonged multiorgan inflammation, symptoms including extreme fatigue, pain, sleep disturbance, cognitive impairment and neurological abnormalities may occur even months following infection, and have also been associated with pro-inflammatory markers. Host cells recognize microbial- and viral danger-associated molecular patterns via germ line-encoded pattern recognition receptors (PRRs), which regulate cell division, cell death or cytokine production among many other roles. Though studies have shown the involvement of various PRRs in SARS-CoV-2, no comprehensive study has yet been performed to investigate the expression of different PRRs in correlation with viral copy numbers and cytokine expression in vital organs.

Objective: Our aim was to determine the extent of viral load, pattern recognition receptor- and inflammatory cytokine expression in tissue samples from different organs (brain, lung, spleen, liver) of patients who died from COVID-19 disease.

Materials and methods: Following tissue homogenization of postmortem samples, RNA was isolated using TRIzol reagent. The viral load was determined by TaqMan™ 2019nCoV Assay Kit v1 (Thermofisher). Gene expression was determined using qRT-PCR method.

Results: Viral nucleic acid was detectable not only in the peripheral tissues (lung, liver, spleen) but also in various brain regions, like *gyrus rectus, temporal cortex, hypothalamus and medulla oblongata.* The viral load showed significant correlation with the antiviral IFNB1 gene expression in the lung and *medulla oblongata*. In peripheral tissues we found elevated levels of NLRC5, RIG-I, TLR9, AIM2 and NOD1 in the spleen; increased TLR3 in the liver; and upregulated expression of NLRP3 and IL-1β in the lung. We also found that from the different brain regions the *medulla oblongata* showed increased expression of RIG-I, MDA5, NLRC5, TLR3 and TLR7.

Supported by NKFIH OTKA K131844 (SB) and the ÚNKP-22-3 New National Excellence Program (EB) of the Ministry for Culture and Innovation from the source of the National Research, Development and Innovation Fund, Momentum program (AD) and MTA post-COVID PC20222-4/2022 (AD).

DRUG CANDIDATE BGP-15 EXERTS ANTIARRHYTHMIC EFFECTS AND ALTERS HEART RATE VARIABILITY IN A FREELY MOVING RAT MODEL

Bernát Brigitta[1], Erdélyi Rita[1,2], Fazekas László[3], Garami Gréta[1], Szilágyi Anna[1], Szekeres Réka[1], Takács Barbara[1], Bombicz Mariann[1], Varga Balázs[1], Sárkány Fruzsina[4], Ráduly Arnold[4], Papp Zoltán[4], Tóth Attila[4], Szilvássy Zoltán[1], Juhász Béla[1], Priksz Dániel[1]
[1]Department of Pharmacology and Pharmacotherapy, Debrecen, [2]Department of Dentoalveolar Surgery, Debrecen, [3]Department of Operative Techniques and Surgical Research, Debrecen, [4]Division of Clinical Physiology, Department of Cardiology, Debrecen

Ventricular arrhythmias, which incidence is still rising globally, are accounted for a significant proportion of cardiovascular-disease deaths. Despite this, many antiarrhythmic drugs fail to show a clear benefit in reducing cardiovascular mortality; therefore, there is still a need for the development of new antiarrhythmic treatments. Publications suggest that the drug candidate BGP-15 may exert cardioprotective effects, although its main mode of action has remained elusive. The concept of measuring heart rate variability (HRV) as a quantitative indicator of autonomic activity has been inspired by experimental findings that link the susceptibility to lethal arrhythmias either to increased sympathetic or decreased vagal tone.

Based on the above, we investigated the effects of BGP-15 on the electrocardiogram (ECG), isoproterenol (ISO)-induced cardiac arrhythmias, HRV, and echocardiographic parameters in a telemetry-implanted rat model. We aimed to build up a radiotelemetry system as anesthetics have many effects on the cardiovascular system.

Sprague-Dawley rats (n=32) underwent telemetry-implantation and the recovery period was followed by a 14-day treatment. First, a single oral BGP-15 bolus (40 mg/kg) was administered, followed by a 24-hour ECG recording to assess the effects of BGP-15 on HRV parameters. After, rats were divided into 4 groups: Control (vehicle-treated), BGP-15 (40 mg/kg), ISO (1 mg/kg), and ISO+BGP-15 (1 mg/kg and 40 mg/kg, respectively). ISO was administered i.p. on the 1st, 4th, 8th, and 12th days. On the 13th day, short-term HRV parameters were determined, and arrhythmic events were counted. On the last day, echocardiography was performed, and rats were sacrificed. In protocol II rats (n=8) were subjected to 0.1 mg/kg i.p. ISO treatment and then BGP-15 dose escalation (40, 80, and 160 mg/kg, i.p.) under echocardiographic monitoring (under ketamine/xylazine anesthesia). In addition, isolated canine cardiomyocyte experiments were carried out to further evaluate BGP-15-ISO interactions.

In this study BGP-15 was found to significantly reduce arrhythmogenesis, heart rate and prevent ECG changes resulting from beta-adrenergic overstimulation. The drug candidate increased vagally mediated HRV parameters and enhanced left ventricle relaxation in telemetry-implanted rats. At cellular level, BGP-15 pre-treatment (100 µM) before ISO application completely prevented ISO-induced aftercontractions and the reactivation of Ca^{2+} transients in isolated cardiomyocytes.

As the substance is proven to be well-tolerated in human studies, we suggest that the drug candidate might have an increasingly promising clinical value in preventing lethal arrhythmias.

Project no. TKP2021-EGA-18 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme; GINOP-2.3.4-15-2020-00008.

STRUCTURAL ELUCIDATION OF LIGAND BINDING MECHANISMS TO SOMATOSTATIN RECEPTORS

Rita Börzsei[1], Dóra Biskup[1], Csaba Hetényi[1], Balázs Zoltán Zsidó[1], Erika Pintér[1], Zsuzsanna Helyes[1]
[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Szigeti út 12, Pécs 7624, Hungary

Somatostatin is an endogenous cyclic neuropeptide, which is widely expressed in the body. It plays an important role in regulation of the endocrine system, neurotransmission, cell proliferation, relieving pain and inflammation. The therapeutic application of somatostatin is limited due to its diverse biological effects and rapid degradation. The design of metabolically stable, highly potent and selective analogues necessitates the knowledge of atomic resolution binding pattern of somatostatin to its receptors. There are five sub-types of somatostatin receptors, of which the fourth sub-type is the most promising for development of a new analgetics.

In the present study, we feature our recent work on structural elucidation of binding pattern of active ligands to somatostatin receptors at atomic resolution. Our findings show good agreement with available experimental results and provide a new pathway for the design of new, subtype four-selective somatostatin analogues.

Acknowledgements. This work was supported by European Union (RRF-2.3.1-21-2022-00015), Hungarian National Research, Development and Innovation Office (K-134214, K-138046), Gedeon Richter Talentum Foundation (D. Biskup). We acknowledge KIFÜ for awarding us access to resource based in Hungary.

MICROGLIA DEPLETION PREVENTS LACTATION BY INHIBITION OF PROLACTIN SECRETION

Csikós Vivien[1], Oláh Szilvia[1], Dóra Fanni[1], Arrasz Nikolett[1], Cservenák Melinda[1], Dobolyi Árpád[1]
[1]Eötvös Loránd University,Budapest

Microglial cells were eliminated from the brain with sustained 3-4 weeks long inhibition of colony stimulating factor 1 receptor by Pexidartinib 3397 (PLX3397). The prepartum treated mice mothers did not feed their pups after parturition. The pups of mothers treated orally only in the postpartum period starting immediately after parturition showed reduced body weight by 15.5 ± 0.22 postnatal days as the treatment progressed without the mothers showing altered caring behaviors. The apparent weight gain of foster pups during a suckling bout was reduced in mother mice fed by PLX3397-containing diet and also in rat dams following sustained intracerebroventricular infusion of PLX3397 in a separate experiment suggesting that lactation was affected by the reduced number of microglia. Prolactin secretion and signaling were markedly reduced in PLX3397-treated mothers. The results suggest that microglial cells are required for prolactin secretion and lactation while maternal motivation may not be directly affected by microglia.

Grant support: NAP3 project of the Hungarian Academy of Sciences (NAP2022-I-3/2022), the NKFIH OTKA K134221 research grant, ÚNKP-21-3, and the Joseph Cours Scholarship, ELTE Eötvös Loránd University, Budapest, Hungary.

POSITIVE INOTROPIC RESPONSE TO ACETYLCHOLINE IN THE RIGHT AND LEFT VENTRICULAR MYOCARDIUM ISOLATED FROM CANNABIDIOL-TREATED ZDF RATS

Teleki Vanda[1], Óvári Ignác[1], Viczján Gábor[1], Varga Balázs[1], Szilágyi Anna[1], Takács Barbara[1], Szekeres Réka[1], Lampé Nóra[1], Juhász Béla[1], Szilvássy Zoltán[1], Gesztelyi Rudolf[1], Erdei Tamás[1]
[1]University of Debrecen, Faculty of Medicine, Department of Pharmacology and Pharmacotherapy, Debrecen

Our research group have started developing an ex vivo method enabling the examination of the ventricular myocardium and the potential cardioprotective effects of drug candidates against various noxae. In the present pilot study, we quantified the inotropic effect exerted by norepinephrine and acetylcholine, the two most significant neural regulators of the heart, on isolated, paced right and left ventricular trabeculae.

Metabolically intact (lean) and diseased (obese) types of Zucker Diabetic Fatty (ZDF) rats were used. The lean rats, fed with conventional rat chow, formed the negative control group (Lean group). The obese rats were maintained on diabetogenic rat chow, furthermore they received 60 mg/kg/day CBD or vehicle via gavage for four weeks, forming the treated group (Obese+CBD group) and the positive control one (Obese group), respectively. Right and left ventricular trabeculae were isolated from the animals, and concentration-effect (E/c) curves were generated with noradrenaline and acetylcholine, recording the inotropic response.

Noradrenaline was found to induce a positive inotropic effect in all the three groups (that showed the viability of the ventricular samples). The weakened ventricular response of obese animals, in comparison with the lean ones, seemed to be slightly enhanced by the CBD treatment in the samples from both sides. In the case of acetylcholine, CBD treatment produced a clearly visible (although statistically not significant) positive inotropic effect as compared to a minimal negative inotropic effect (or no response) observed in the Lean and Obese groups (also in samples from both sides). It may be hypothesized that this effect of acetylcholine was not mediated by the cardiac M_2 receptor. Instead of or in addition to this, the acetylcholinesterase inhibitory effect of CBD may also be associated with this phenomenon.

Project no. TKP2021-EGA-18 has been implemented with the support provided from the National Research, Development and Innovatidon Fund of Hungary, financed under the TKP2021-EGA funding scheme, GINOP-2.3.4.-15-2016-00002.

TOLPERISONE-PREGABALIN COMBINATION PROVIDES ANTIALLODYNIC EFFECT DEVOID OF MOTOR DYSFUNCTION IN THE MODEL OF PERIPHERAL MONONEUROPATHY IN RATS

Nariman Essmat[1], Anna R. Galambos[1], Péter P. Lakatos[2], David Á. Karádi[1], Sarah K. Abbood[1], Zoltán S Zádori[1], Kornél Király[1], Éva Szökő[2], Tamás Tábi[2], Mahmoud Al-Khrasani[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, 4 Nagyvárad tér, Budapest, H-1089, Hungary, [2]Department of Pharmacodynamics, Semmelweis University, 4 Nagyvárad tér, Budapest, H-1089, Hungary.

Background: Neuropathic pain (NP) is a debilitating chronic condition that results from a disease or damage affecting the somatosensory neurons. Treatment of painful neuropathy can be difficult due to the diversity of etiologies and the intricacy of the underlying mechanisms. Monotherapy-based treatment failed to produce optimal pain management, the combination of drugs with various modes of action is being applied in clinical settings in hope of achieving rapid onset, significant analgesia, and minimizing the adverse effects.

Aim: To investigate the acute antiallodynic effect of tolperisone and pregabalin alone or in low dose combination in partial sciatic nerve ligation (pSNL)-induced mono-neuropathic pain in rats. The impact of these treatments on motor function was also investigated.

Methods: The antiallodynic effect of oral tolperisone and pregabalin (both at 25, 50, and 100 mg/kg), was investigated in male Wistar rats with mono-neuropathy evoked by pSNL. The drugs were administered on day 7 after the operation and pain assessments were performed 1, 2, and 3 h thereafter. Next, chronic treatment continued for 2 weeks, and the pain threshold was determined again on days 14 and 21 following of operation. In another experiment setting, the effects of oral tolperisone and pregabalin (both at 25, 50, and 100 mg/kg) and their combination (both at 25 mg/kg), were investigated, after a single oral dose on day 14 after the operation. Allodynia was assessed by dynamic plantar aesthesiometer (DPA). Rotarod test was used to determine the motor coordination and balance after acute oral treatment with tolperisone (150 mg/kg), pregabalin (25 and 50 mg/kg) and their combination (both at 25 mg/kg).

Results: Neither tolperisone nor pregabalin showed antiallodynic effect after acute oral treatment with 25, 50, and 100 mg/kg. However, 100 mg/kg of tolperisone and 50 mg/kg of pregabalin produced a significant antiallodynic effect after 14 consecutive days of treatments. On the other hand, acute administration of the combination of oral tolperisone (25 mg/kg) and pregabalin (25 mg/kg) significantly elevated the paw withdrawal threshold of the operated paw after unilateral pSNL, indicating acute onset of antiallodynia. In the rotarod test, acute oral tolperisone at a dose of 150 mg/kg failed to induce motor dysfunction. Pregabalin produced motor dysfunction following 50 mg/kg administration. Interestingly, the combination of 25 mg/kg tolperisone and 25 mg/kg pregabalin did not induce motor dysfunction.

Conclusions: Tolperisone or pregabalin alone produces analgesia only after chronic treatment. As a novel finding, the combination composed of lower doses of both tolperisone and pregabalin provides antiallodynic effect of fast onset and is devoid of motor dysfunction. Thus, this finding implies that this combination might create future satisfaction with respect to NP therapy.

Funding: The present work was supported by 2018-1.3.1-VKE-2018-00030 project.

EVALUATION OF THE ANTIOXIDANT EFFECT OF KD15 AND KD36 NEWLY SYNTHETIZED MOLECULES

Adina Fésüs[1,2], Anita Kónya-Ábrahám[3], Éva Sipos[1,2], Kitti Szőke[2], István Bak[2], Attila Kiss-Szikszai[3], István Lekli[2]
[1]Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Debrecen, Hungary, [2]Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, Hungary, [3]Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, University of Debrecen, Hungary

KD molecules are national developed halogen-containing chromone (back-bone structure of flavonoids) derivatives with antioxidant effect.

In this study our aim was to investigate whether the pretreatment with KD15 and KD36 molecules have a protective effect against hypoxia-reoxygenation (H/R) induced acute injury on cardiac myocytes.

In our series of experiments H9c2 rat cardiomyoblast cells were treated for 24 hours and the Ic50 value of the molecules was determined. After that, we investigated the cells viability in the presence of H_2O_2 using MTT assay. The concentrations of 3 and 10 µM proved to be the most effective. Subsequently, these concentrations were used to pretreat cardiomyocytes, followed by four/three hours of H/R. Cell viability was determined by trypan blue (dye) exclusion test. Furthermore, lactate dehydrogenase (LDH) assay was used to confirm these results. Western blot analysis was used to examine the expression levels of the proteins involved in apoptosis and autophagy.

Our findings demonstrate that the pretreatment with both molecules significantly improved the cardiomyocytes viability after H/R. However, the 10 µM concentration proved to be more effective for both molecules. Moreover, the KD36 molecule was less cytotoxic. Furthermore, we found a significant decrease in the level of the oxidative stress caused by H/R, leading to an increased release of LDH. At the same time, the activities of cytoprotective antioxidant enzymes, heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and catalase (CAT) were also increased.

Overall, we found that KD15 and KD36 molecules may have a beneficial effect in prevention of H/R-induced cytotoxicity through the reduction of oxidative stress.

Acknowledgments: NKFI-143360, TKP2021-EGA-18

GLYCINE TRANSPORTER-1 INHIBITORS DELAY THE ONSET OF MORPHINE ANTINOCICEPTIVE TOLERANCE IN RATS

Galambos Anna Rita[1], Nariman Essmat[1], Lakatos Péter Pál[2], Sarah Kadhim Abbood[1], Geda Orsolya[2], Riba Pál[1], Király Kornél[1], Szökő Éva[2], Tábi Tamás[2], Harsing László Gábor[1], Zádor Ferenc[3], Al-Khrasani Mahmoud[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, 4 Nagyvárad tér, Budapest, H-1089, Hungary., [2]Department of Pharmacodynamics, Semmelweis University, 4 Nagyvárad tér, Budapest, H-1089, Hungary., [3]Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary

BACKGROUND: Opioid analgesics are used to manage mild-severe pain; however, their long-term use is hampered by the development of central and peripheral side effects such as analgesic tolerance, addiction, respiratory depression, and constipation. Recent data reveal that glycine transporter-1 (GlyT-1) inhibitors could produce anti-allodynic effects in animal-developed neuropathic pain, which shares overlapping spinal mechanisms with opioid tolerance. Thus, GlyT-1 inhibitors might provide novel therapeutic possibilities to prevent or at least delay the development of opioid analgesic tolerance.

AIMS: To elucidate the impact of GlyT-1 inhibitors on opioid antinociceptive tolerance developed by chronic morphine treatment in rats.

METHODS: Thermal pain model, the rat tail-flick assay was carried out on male Wistar rats (180-250g) to assess the antinociceptive effects of test compounds and combinations after acute and chronic administrations. The antinociceptive effect of morphine hydrochloride (10 mg/kg) alone or in combination with the GlyT-1 inhibitor NFPS was determined on days 1 and 10 after subcutaneous (sc.) treatments. Morphine and NFPS (0.3, 0.6mg/kg) were administered twice and once daily, respectively. On days 1 and 10, the antinociceptive effect of morphine, NFPS, or their combination was measured at 30, 60, 120, and 180 min after sc. treatments. As control, vehicle (10% DMSO or saline) was used. Cerebrospinal fluid (CSF) was collected from treated groups at the end of the treatment period, and glycine level was measured by capillary electrophoresis. Rat motor coordination and balance were also measured by the rotarod test on days 1 and 10. Area under curve (AUC) values of the antinociceptive time-effect course was calculated from individual animals. Statistics: one-way ANOVA followed by Newman-Keuls multiple comparison was used.

RESULTS: Acute treatment with sc. morphine or its combination with NFPS produced significant antinociceptive effects, yet NFPS alone failed to show effect. After chronic treatment, the combination of morphine and 0.6 mg/kg NFPS but not morphine alone showed significant antinociception, indicating the development of antinociceptive tolerance to the later treatment. Vehicle treated groups failed to show antinociception either after acute or chronic treatments. Next, treatment with 0.3 mg/kg or 0.6 mg/kg NFPS alone or in combination with morphine showed significant increase in the CSF glycine level. Finally, chronic treatment with 0.3 mg/kg NFPS did not show any effect on the motor function of the animals at the tested time points.

CONCLUSIONS: GlyT-1 inhibition results in an enhanced spinal glycinergic system which in turn, might contribute to the delayed development of morphine antinociceptive tolerance. The observed effect may be a result of the activation of the post-synaptic glycinergic receptors or receptors on glial cells which have been reported to be implicated in opioid analgesic tolerance.

THE BIOMARKER ROLES OF MACROPHAGE MIGRATION INHIBITORY FACTOR IN SEPTIC PATIENTS

András Garami[1], János Toldi[1,2], Zsolt Fehér[1,2], Leonardo Kelava[1], Kata Fekete[1], Bíbor Zsófia Gárdos[1], Krisztián Klonfár[1], Eszter Pákai[1]
[1]Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary, [2]Department of Anesthesiology and Intensive Care, Medical School, University of Pécs, Pécs, Hungary

Macrophage migration inhibitory factor (MIF) was considered as a biomarker in sepsis, but its diagnostic and prognostic value has remained unclear in human studies. We used a dual approach to clarify the value of MIF as a sepsis biomarker: 1) meta-analysis of clinical trials and 2) a single-center prospective, observational study.

For the meta-analysis, 21 eligible studies were identified, including data from 1876 subjects (of which 1206 had sepsis). In the septic patients, blood MIF levels were significantly higher than in healthy controls with a standardized mean difference (SMD) of 1.47 ($p < 0.001$). Importantly, in sepsis serum MIF was also higher than in patient groups with nonseptic systemic inflammation (SMD = 0.94; $p < 0.001$). Markedly greater elevation in blood MIF level was found in nonsurvivors than in survivors with SMDs of 0.75 ($p < 0.001$).

In our clinical study, repeated measurements of MIF in serum and urine were performed on days 0, 2, and 4 from admission to the intensive care unit (ICU) in 50 adult septic patients. In nonsurvivors, there was an increase in serum MIF level from day 0 to 4, whereas in the survivors there was rather a decrease ($p = 0.018$). Furthermore, urine MIF was markedly lower in patients who died than in survivors of sepsis ($p < 0.050$). In contrast with serum MIF, urine MIF levels did not show temporal changes: there was no meaningful difference between day 0 and 4.

In conclusion, blood MIF level is more elevated in systemic inflammation caused by infection (i.e., sepsis) compared to noninfectious causes. In more severe forms of sepsis, including fatal outcome, MIF levels are higher than in less severe forms. Our results also suggest that kinetics of serum MIF during the initial days from ICU admission can predict death, while lower urine MIF levels can also indicate death without showing meaningful temporal kinetics. These results suggest that MIF can be a valuable diagnostic and prognostic biomarker in sepsis.

Funding: NKFIH FK 138722

PREDICTIVE VALUE OF BODY TEMPERATURE ON THE OUTCOME OF ACUTE PANCREATITIS

Eszter Pákai[1], Krisztián Klonfár[1], Bíbor Zsófia Gárdos[1], Kata Fekete[1], Alíz Fazekas[1], Nelli Farkas[1,2], András Garami[1]
[1]Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pécs, Pécs, Hungary, [2]Institute of Bioanalysis, Medical School, University of Pécs, Pécs, Hungary

Acute pancreatitis (AP) is one of the most frequent gastrointestinal causes of hospitalization with an incidence of 34 per 100,000 people, which continuously increasing worldwide, imposing a huge burden for healthcare. The disease presents with varying degrees of severity, ranging from a mild disease with excellent recovery rates to severe pancreatitis with mortality rates of up to 35%. The early development of systemic inflammation is often accompanied by changes in the regulation of deep body temperature (Tb), which can be manifested as fever or hypothermia.

We conducted the collection and the analysis of published human data in order to reveal an association between Tb on hospital admission and the severity and outcome of AP. Our extensive literature search was successful, 9369 studies has been identified. After removal of duplicates and irrelevant papers based on title and abstract, the full text of 132 articles was checked, then 26 studies remained, from which the suitable data of 40629 patients could be extracted. After structuring the extracted data, we used a multiple approach to identify any potential association between Tb and disease progression.

With meta-regression, we did not find any significant association between Tb and amylase or C-reactive protein levels in the serum, while there was tendency for positive correlation with APACHE II and SOFA clinical severity scores. However, there was significantly positive correlation between Tb and the length of hospitalization. Importantly, we found a very strong positive correlation between Tb and mortality, indicating substantial increase in death rate for every 1°C elevation in Tb. Similar results were found in forest plots, indicating higher mortality rates in the patients with elevated Tb.

Our findings highlight the importance of proper Tb measurements in AP and identify Tb as a predictive biomarker of the severity and fatality of the disease.

Funding: NKFIH FK 138722

ROLE OF TYROSINE-KINASES IN THE ANGIOTENSIN II-INDUCED GENE EXPRESSIONAL CHANGES IN VASCULAR SMOOTH MUSCLE CELLS

Janka Gém[1], Kinga Kovács[1], László Hunyady[1,2], András Balla[1,3]
[1]Department of Physiology, Semmelweis University, Budapest, Hungary, [2]Institute of Enzymology, Research Centre for Natural Sciences, Budapest, Hungary, [3]MTA-SE Laboratory of Molecular Physiology, Budapest, Hungary

Type 1 angiotensin II receptor (AT1R) is a member of G protein-coupled receptor family (GPCR) that initiates various intracellular signaling pathways after binding its ligand, the angiotensin II (AngII). The AT1R activation in vascular smooth muscle cells (VSMCs) results in fast vasoconstriction leading to elevation of systemic blood pressure. Moreover, AT1R also conveys numerous long-term effects, such as increasing cell proliferation or migration via AngII-induced gene expression changes. These mechanisms play important role in the development of AngII related pathological conditions, like vascular remodeling, hypertension and atherosclerosis. It is already known, that the transactivation of epidermal growth factor receptor (EGFR) and other receptor tyrosine-kinases by activated AT1R have important role in these pathologic long-term effects.

Our aim was to investigate the role of AT1R mediated activation non-receptor and receptor tyrosine-kinases in AngII-induced physiological and pathophysiological long-term effects.

In this study we used primary VSMC cultures, isolated from the aorta thoracalis of young, male Wistar rats. Cells were exposed to different tyrosine-kinase inhibitor treatments before hormonal stimulations to examine which of these enzymes are involved in AngII induced long-term effects. Our previous results revealed that genes of certain dual-specificity-phosphatases (DUSP) such as *DUSP5*, *DUSP6* and *DUSP10* show significant upregulation to AngII stimuli. The DUSP phosphatases are important regulators of mitogen activated protein kinase (MAPK) cascades. Our experiments revealed that dasatinib, a Bcr-Abl and Src kinase family inhibitor, mostly used in Philadelphia chromosome-positive chronic myeloid leukemia therapy, could effectively reduce AngII mediated upregulation of the examined DUSP isoforms. Furthermore, with Western-blot analysis, we demonstrated that dasatinib significantly reduces AngII caused p38 MAPK phosphorylation, which suggests that p38 MAPK plays a crucial role in the formation of long-term effects induced by AngII. We demonstrated that the imatinib, a selective inhibitor of Bcr-Abl kinases, was not able to achieve the similar effect as the dasatinib suggesting that Src-family tyrosine kinase(s) play an important role in AngII-induced long-term cellular responses.

Our data can provide new insight into the physiology of VSMCs in response to AngII stimulation, and may lead to the development of novel types of drugs for the treatment of cardiovascular and other diseases.

Funding: Semmelweis University STIA-OTKA (STIAOTK0001)

INVOLVEMENT OF P2Y12 RECEPTORS IN A NITROGLYCERIN-INDUCED MODEL OF MIGRAINE

Gölöncsér Flóra[1], Baranyi Mária[1], Iring András[1], Hricisák László[2], Otrokocsi Lilla[1], Benyó Zoltán[2], Sperlágh Beáta[1]
[1]Laboratory of Molecular Pharmacology, Institute of Experimental Medicine, Budapest, [2]Institute of Translational Medicine, Semmelweis University, Budapest

P2Y12 receptors (P2Y12Rs) regulate various forms of pain and local and systemic inflammation. In this study, we investigated the involvement of centrally expressed and platelet P2Y12Rs in an animal model of migraine.

The effect of selective P2Y12 antagonists, centrally administered PSB-0739 and intraperitoneally administered clopidogrel, as well as P2Y12R gene (P2ry12-/-) deficiency, were investigated in mice treated with acute nitroglycerin (NTG). In addition, platelet depletion was used to investigate the role of platelet P2Y12Rs in migraine-like pain.

NTG induced sensory hypersensitivity in C57BL/6 wild-type (P2ry12+/+) mice, accompanied by increased c-fos and CGRP expression in the upper cervical spinal cord (C1–C2) and trigeminal nucleus caudalis. Similar changes were observed in P2ry12-/- mice. Prophylactic intrathecal administration of PSB-0739 reversed thermal hyperalgesia and head grooming time in wild-type mice but had no effect in P2ry12-/- mice. Additionally, PSB-0739 was effective when administered as a post-treatment. PSB-0739 administration suppressed c-fos expression in C1–C2 and trigeminal nucleus caudalis and reduced C1–C2 dopamine and 5-hydroxytryptamine levels in wild-type mice. NTG injection did not cause changes in serum cytokine levels in wild-type mice, nor did administration of PSB-0739. Furthermore, NTG treatment itself did not alter adenosine diphosphate (ADP)-induced platelet activation as measured by CD62P up-regulation in wild-type mice. Platelet depletion by anti-mouse CD41 antibody and clopidogrel reduced NTG-induced thermal hypersensitivity and head grooming time in mice.

Our results show that acute inhibition of P2Y12Rs alleviates migraine-like pain in mice by modulating c-fos expression and that platelet P2Y12Rs may contribute to this effect. Thus, blocking P2Y12Rs may have a therapeutic potential against migraine.

INVESTIGATION OF THE GADD34 INTERACTOME

Eszter Éva Kálmán[1], Aladár Pettkó-Szandtner[2], Orsolya Kapuy[1], Norbert Gyöngyösi [1]
[1]Department of Molecular Biology at the Institute of Biochemistry and Molecular Biology, Semmelweis University, Budapest, Hungary, [2]Laboratory of Proteomics Research, Biological Research Centre, Szeged, Hungary

GADD34 (growth arrest and DNA damage [GADD]-inducible34, protein phosphatase 1 regulatory subunit 15A) is one of the numerous regulatory subunits of protein phosphatase 1 (PP1). PP1 is one of the major eukaryotic phosphatases playing role in the control of circadian rhythm, glycogen metabolism, muscle contraction, cell progression, neuronal activities, splicing of RNA, cell division, apoptosis, regulation of membrane receptors and channels, translation and endoplasmic reticulum stress among others.

Our aim is to characterize the possible interactome of GADD34 in HEK293 cells by the so-called proximity-dependent biotin labeling method, using BirA* and Turbo-ID chimeras. The technique based on the aforementioned mutant prokaryotic biotin ligases fused to our protein of interest able to label possible interacting partners in close proximity in vivo. Since biotinylation is a rare protein modification in the cellular context, this method enables us to perform high-affinity purification and subsequent identification of the possible interactome by mass-spectrometry. In vivo proximity-dependent biotin labeling methods can be applied to insoluble proteins, weak and/or transient interactions and membrane proteins both.

Since GADD34 plays a well-characterized role in the recovery from ER stress response i.e. it dephosphorylates eIF2α-P, alleviating the translational inhibition to aid translational recovery and preserve energy sources of the cell, we investigated its interactome both in resting cells and during ER stress provoked by thapsigargin treatment. We could find nor ATF4 (Activating transcription factor 4) neither CHOP (DNA damage-inducible transcript 3, also known as C/EBP homologous protein) in our in vivo biotinylated samples, although GO (Gene Ontology) analysis showed significant enrichment of possible interacting partners in know functions of the GADD34 protein. While thapsigargin treatment showed significant effect on the GADD34 interactome, in our analysis we did not find any of the aforementioned proteins (i.e. ATF4 or CHOP) playing role in ER stress response. In addition, our data serve as a useful list of new putative partners and roles of GADD34. Our current experiments aim to see the difference between the interactomes of wild-type and mutant version of GADD34 lacking a conserved binding motif in stable cell lines.

This work was financed by GRANT FK 134267 (OK), Grant FK 132474 (NG), EU's Horizon 2020 research and innovation program under grant agreement No. 739593 (AP-S) and GINOP-2.3.2-15-2016-00032 (AP-S).

BASIC PHARMACOLOGICAL CHARACTERIZATION OF BM-112, A NEW H_2S-RELEASING ASPIRIN DERIVATIVE, IN ISOPROTERENOL-INDUCED CARDIAC HYPERTROPHY

Alexandra Gyöngyösi[1], Kitti Szőke[1], Ilona Bakai-Bereczki[2], Pál Herczeg[2], Anikó Borbás[2], István Lekli [1]
[1]University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen, [2]University of Debrecen, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Debrecen

Cardiac hypertrophy is a compensatory mechanism that occurs in conjunction with cardiovascular diseases. Hypertrophy and cardiac remodelling are potentially harmful to the heart and can result in arrhythmia and heart failure. Development of cardiac hypertrophy involves various pathophysiological signals, such as physical stimulation, chemical stimulation and hormone levels including, angiotensin II, norepinephrine and β-adrenoceptor agonists. Cardioprotective effects of H_2S and aspirin are being suggested by numerous studies. Since, H_2S plays a role in relaxation of vascular smooth muscle, protects against oxidative stress, myocardial hypertrophy and modulates inflammation. The aim of this study was to investigate the effects of a new H_2S-releasing aspirin derivative (BM-112) on isoproterenol (ISO)-induced cardiac hypertrophy giving special attention to modulating oxidative stress, apoptosis and autophagy on cardiomyocytes.

During our work, this newly synthesized compound BM-112 was characterized, H_2S-releasing ability was studied with a hydrogen sulfide sensor. H9c2 cardiomyocytes were treated with different concentrations of BM-112, in the presence or absence of ISO, for 24 hours and the effect on cell viability was examined using MTT assay. In order to get a broader picture of the degree of hypertrophy and the effects of BM-112, we determined the cell size with rhodamine-conjugated phalloidin dye. The level of mitochondrial oxidative stress after ISO+BM-112 treatment was measured with MitoSOX Red dye and compared to a slow releasing H_2S donor GYY4137. The alteration in autophagic and apoptosis' protein expressions were analyzed by Western blot analysis.

Based on our results, H_2S was successfully released from BM-112 in cell culture medium. No cytotoxic effect of BM-112 cells was observed on H9c2 at concentrations, which lower than 50 µM. During combined treatment with ISO, it was not showed significantly reduce cell viability after 24 hours. BM-112 was significantly reduced ISO-induced hypertrophy in cardiomyocytes. However, after MitoSOX Red staining, we observed increased mitochondrial ROS formation in the ISO+BM-112 group. Thus, further studies are needed to determine the precise signaling effects of BM-112 in ISO-induced cardiac hypertrophy.

Acknowledgments: NKFI-143360, TKP2021-EGA-18

ALTERED INTESTINAL EXPRESSION OF PATTERN RECOGNITION RECEPTORS AND DISTINCT ANTIMICROBIAL PEPTIDES IN NON-STEROIDAL ANTI-INFLAMMATORY DRUG-INDUCED ENTEROPATHY

Arezoo Haghighi[1], Barbara Hutka[1,2], András S. Tóth[1], Klára Gyires[1], Zoltán S. Zádori[1]
[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary[2]

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) can cause significant damage to the distal parts of the small intestine. This enteropathy is characterized by changes in the intestinal microbiota, which contributes to the development of tissue inflammation, but little is known about the mechanisms underlying NSAID-induced dysbiosis. Toll-like receptors (TLRs) are important part of the innate immunity and their activation by potentially pathogenic bacteria triggers a defensive immune response. A part of it is the stimulation of the production of antimicrobial peptides (AMPs), such as defensins and cathelicidin antimicrobial peptide (CAMP). Although interactions between TLRs, AMPs and gut microbiota are intensively studied in the context of inflammatory bowel diseases, they are poorly understood in NSAID enteropathy. In fact, there is only limited data on the effect of NSAIDs on intestinal expression of TLRs and AMPs.

Aim: Analyzing the changes in PRR and AMP expressions in NSAID-treated rats.

Methods: In the first experiment, male Wistar rats were treated once with a single large dose of 20 mg/kg indomethacin by gavage and were euthanized 6, 12, 24, 48 and 72 h later. A sixth group was treated with vehicle (1% hydroxyethylcellulose) and euthanized at 72 h. In the second experiment, rats were treated chronically (twice daily for two weeks) with indomethacin (2 or 4 mg/kg), naproxen (10 or 20 mg/kg) or vehicle. Intestinal injury was assessed macroscopically and by measuring the tissue level of inflammatory proteins. The mRNA levels of CAMP, α-defensin 5, β-defensin 2 and TLR-1, -2, -4, -6 and -9 were assessed by qPCR.

Results: A single high dose of indomethacin caused severe enteropathy within 3 days, characterized by weight loss, intestinal ulcers, and shortening of the small intestine. Mucosal inflammation was confirmed by elevated tissue levels of myeloperoxidase, cyclooxygenase-2 and pentraxin 3. Inflammation was accompanied by increased mRNA level of TLR-1, -2 and CAMP, and also that of α-defensin 5 tended to rise, whereas the expressions of TLR-4, -6, -9 and β-defensin 2 did not change significantly. Chronic treatment with NSAIDs yielded similar results. Indomethacin treatment increased the jejunal expression of TLR-1 and -2, and both drugs increased the level of CAMP and α-defensin 5. However, chronic treatment with naproxen also resulted in higher levels of TLR-4 and β-defensin 2. **Conclusion:** Here we characterize for the first time the time-dependent alterations of TLRs and AMPs in NSAID enteropathy. Whether these changes contribute to NSAID-induced intestinal dysbiosis is currently under investigation. Grants: NKFI FK 138842.

DRUG AND DOSE-DEPENDENT TISSUE INJURY IN THE RAT RENAL MEDULLA AFTER CHRONIC TREATMENT WITH NSAIDS

Samaneh Haghighi[1,2], Arezoo Haghighi[3], Zoltán S. Zádori[3], Gábor Kökény[1,2]
[1]Institute of Translational Medicine, [2]International Nephrology Research and Training Center, [3]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are used extensively in clinical medicine. Chronic NSAID use is known to reduce renal medullary blood flow thus might lead to severe and irreversible damage and papilla necrosis. Non-selective NSAIDs were reported to induce mitochondrial dysfunction and renal damage in rats. Since renal tubular epithelial cells are one of the most ATP demanding cell types of the body, alterations in mitochondria have been recognized as a hallmark of the initiation and progression of several kidney diseases. Moreover, chronic damage to the renal medulla increases the expression of pro-fibrotic EGR1 and TGF-beta1. Sirtuin 3 (SIRT3) plays pivotal role in maintaining mitochondrial homeostasis and mitochondrial dysfunction with altered SIRT3 expression has been described in experimental acute kidney injury. Yet, the possible renal effects of chronic NSAID treatment on SIRT3 and pro-fibrotic factors haven't been investigated.

Aim: Histological and gene expression analysis of renal cortex and medulla after chronic NSAID treatment.

Methods: Non-fasted male Wister rats were treated twice daily orally for two weeks with different NSAIDs as follows: indomethacin (2 mg/kg), naproxen (10 or 20 mg/kg), celecoxib (10 and 30 mg/kg) or vehicle (1% hydroxyethylcellulose). Kidneys were removed, the left kidneys were used for histology while right kidney cortex and medulla were separated and snap frozen. Histological damage scores were evaluated on PAS-stained slides according to El Nahas et al, assessing tubular dilatation, epithelial atrophy and mononuclear cell infiltration. Total mRNA was isolated from cortex and medulla, and gene expression of SIRT3, EGR1 and TGF-beta1 will be assessed by qPCR.

Results: Chronic treatment of NSAIDs caused tubular dilatation significantly in rat's kidney in all NSAIDs treated group except for indomethacin 2 mg/kg in histological analysis. Also, a significant increase in histological epithelial atrophy was confirmed for celecoxib 10, 30 and naproxen 10 mg/kg. This is while that mononuclear cell infiltration increased significantly just in celecoxib 10 mg/kg. Gene expression analysis is on progress. **Conclusion:** Here we characterize the dose-dependent tissue injury in the rat renal medulla after chronic treatment with different NSAIDs for the first time. Whether these histological changes because of the NSAIDs chronic treatment could be effective in kidney dysfunction are remains to be elucidated.

ANTICONVULSANT EFFECT OF MECLOFENAMATE VIA TRPM4 INHIBITION

Erzsébet Kövesdi[1], Laura Mundrucz[1], Attila Gyéresi[1], Máté Deák[1] and Miklós Kecskés[1]
[1]Institute of Physiology, Medical School, University of Pécs, H-7624, Pécs, Hungary

TRPM4 is a Ca^{2+}-activated non-selective cation channel regulating diverse physiological function of excitable cells. In our previous work we showed that TRPM4 is present and functionally active in hilar mossy cells. Furthermore, it contributes to mossy cells death following status epilepticus and therefore modulates seizure susceptibility and epilepsy-related memory deficits in the chronic phase of TLE.

Recently the non-steroidal anti-inflammatory drug meclofenamate has been identified as a potent TRPM4 blocker. Here we demonstrate that *in vivo* application of meclofenamate before induction of status epilepticus reduces the frequency and duration of seizures. Furthermore, we showed that mossy cell loss is reduced especially in the ventral hippocampus in meclofenamate treated mice after status epilepticus.

This data indicates that meclofenamate might have a so far unrecognized role in seizure development.

THYMUS ACTIVATION MAY AFFECT THE CARDIOTOXICITY CAUSED BY ANTI-PD-1 THERAPY

Márton Kocsis
Semmelweis University, Department of Pharmacology and Pharmacotherapy, Budapest

Immune checkpoint inhibitors (ICIs) have revolutionized cancer treatment and shown remarkable efficacy in a wide range of cancers, leading to improved patient outcomes and prolonged survival rates. However, along with their promising benefits, ICIs also have a distinct profile of adverse effects, including various forms of cardiotoxicity .As ICIs continue to gain excessive use in clinical practice, it is crucial to understand and manage their adverse effects effectively to ensure optimal patient care.

Azacitidine (AZA) is a nucleoside analogue that is commonly used for the treatment of myelodysplastic syndromes (MDS), a group of hematologic malignancies. It exerts its therapeutic effects by inhibiting DNA methylation, leading to hypomethylation and subsequent reactivation of tumor suppressor genes. Despite its effectiveness in treating MDS, azacitidine is associated with several adverse effects, also including cardiotoxicity.

In this study our aim was to investigate the combined therapy and effect of azacitidine and ICI which are both potential cardiotoxic agent on cardiac functions. Our hypothesis was that combined AZA and ICI treatment would result in a significant reduction in cardiac function compared to single AZA or ICI therapy.

We randomized C57Bl/6J mice based on their baseline echocardiography and weight to the following groups: Control + Vehicle (CON), ICI + Vehicle (ICI), AZA + Vehicle (AZA), and ICI+AZA combination treatment. The mice were injected intraperitoneally with 2.5mg/kg Azacitidine every day, and 200µg PD-1 inhibitor 3 times/week. Before termination we have measured the echocardiographic parameters of the animals.

The results were partly different from what we have expected. The EF was significantly decreased in ICI+VEH treated group (mean: 45.74 ± 1.76) vs CON+VEH group (54.75 ± 1.15) using two-way ANOVA, Tukey's multiple comparisons ($p=0.0012$). Also when terminal (TRM) data was compared to baseline (BL), significant decrease was seen only in ICI+VEH group (mean: 45.74 ± 1.76 TRM , 54.75 ± 1.37 BL) meaning the combined AZA + ICI treatment reverted the EF-decreasing effects of ICI (55.54 ± 1.34 TRM, 55.57 ± 1.37 BL) using two-way ANOVA, repeated measures, Sidak's multiple comparisons ($p>0.999$). In addition, strong atrophy was observed in the thymus of AZA+ICI treated animals , while no lesions were observed in animals treated with ICI+VEH.

This raises the question of whether the inflammatory millieu of the thymus potentially influences the cardiotoxic adverse effect of anti PD-1 therapy. To test this theory further, we will administer aged mice (16 month old – when thymic involution is present) with the same dosage and frequency PD-1 inhibitor to assess potential changes in cardiac functions.

THE COMBINED USE OF IMMUNE CHECKPOINT INHIBITORS AND OLAPARIB TO REDUCE CARDIOTOXIC SIDE EFFECTS

Tamás Kovács[1,2,3], Tamás G Gergely[1,2,3], Nabil V Sayour[1,2,3], Dániel Kucsera[1,2,3], Viktória E Tóth[1,2,3], Varga V Zoltán[1,2,3]
[1]Deparment of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, [2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest, [3]HAS-SU Momentum Cardiooncology and Cardioimmunology Research Group, Budapest

Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of many types of cancer. However, the use of ICIs can result in immune-related adverse events including cardiotoxicity. To treat life-threatening ICI-induced side effects, current therapeutic options are limited, often resulting in discontinuation of ICI therapy. The combination of ICIs with another class of anticancer drug that increases treatment efficacy and at the same time mitigating side effects would be extremely valuable in the clinical practice.

PARP inhibitors are indicated in primary peritoneal, fallopian tube and pancreatic cancers, as well as in advanced ovarian carcinomas, where use of ICI is limited. Several clinical trials are currently underway to investigate the use of PARP inhibitors in combination with ICIs in different cancer types, including metastatic melanoma.

PARP inhibitors have been shown to have beneficial effects in heart failure in preclinical models, in addition to their anti-cancer activity. However, the combined effect of PARP and immune checkpoint inhibition on the heart has not been previously investigated.

We aimed to test our hypothesis in a preclinical mouse model, to see if combination of a clinically used PARP inhibitor (Olaparib, OLA) and an ICI (anti-PD1) would reduce ICI-induced cardiotoxicity. We randomized 8 weeks old C57Bl/6J mice based on their baseline (BL) echocardiography and weight to the following groups: Control (CON), ICI-treated, OLA-treated with corresponding vehicle controls, and ICI+OLA combination treatment. The mice were injected intraperitoneally with 10 mg/kg Olaparib every day and with 200 µg anti-PD1 monoclonal antibody 6 times during the two weeks of the study. Before termination (TRM) we measured the echocardiographic parameters of the animals.

The results show that the anti-PD1 treatment led to reduced cardiac function. We compared treatment groups and time points with repeated measures two-way ANOVA, post-hoc Sidak's test. Ejection fraction (EF) significantly decreased in ICI group (45,74±1,78%) compared to CON (54,75±1,15%) and compared to ICI-BL (54,63±1,37%). No significant unfavourable change in echo parameters was observed with OLA treatment, in fact the combination therapy (ICI+OLA) resulted in significantly (p_{adj}=0,0029) better EF than ICI suggesting the cardioprotective effect of OLA.

Following the successful confirmation of the cardioprotective effect of Olaparib, we aim to further investigate the mechanisms by which the PARP inhibitor mitigates and protects against ICI cardiotoxicity.

PROTECTIVE ROLE OF DMT PRETREATMENT IN MYOCARDIAL ISCHEMIC REPERFUSION INJURY

Alexandra Gyöngyösi[1], Judit Zsuga[2], István Bak[1], Kitti Szőke[2], Ede Frecska[2], István Lekli[1]
[1]University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, Debrecen
[2]University of Debrecen, Faculty of Medicine, Department of Psychiatry, Debrecen

N'N-dimethyltryptamine (DMT) became a promising candidate for preventing or treating several pathological conditions due to its potent 5-HT_{1A} receptor agonist properties, which give anxiolytic effects. Its psychedelic effect is due to its 5-HT_{2A} receptor-activating capacity. In addition, DMT shows Sigma-1 (Sig-1R) receptor agonist activity, which helps neuronal survival against oxidative stress and it also regulating immune responses. The Sig-1R ER is for the survival against cellular, particularly ER stress. Due to the Sig-1R effects from DMT are investigated, and it could be helpful for the treatment of different forms of ischemia/reperfusion (I/R) injury. Including, to investigate the micro-rheological and metabolic parameters, and the effects of DMT in renal I/R in rats. Moreover, it reduced infarct size and improved functional recovery following transient focal brain ischemia in rats, as well as it attenuated spreading depolarization and restrains neurodegeneration. The aim of our study was to investigate whether, DMT-pretreatment has influance on myocardial I/R injury.

The experiments were accomplished using adult male SD rats. The rats were anesthetized and heparin was given. Chest cavities were opened, and hearts were excised. Aorta and vena pulmonalis were cannulated, and hearts were perfused with modified KHB buffer. After 10 minutes of working perfusion hearts were perfused with DMT solution for 10 minutes then, 30 min of global ischemia (ISA) was initiated. At the end of the ISA period, 120 min of reperfusion (REP) was started. Baseline parameters for each heart were registered following the 10 min of working perfusion before the DMT perfusion. To examine the recovery of the left ventricle, cardiac function was assessed after 30, 60, 90 and 120 min of REP. During the entire experimental procedure heart rate (HR), coronary flow (CF), aortic flow (AF) and aortic pressure (AoP) were measured. Cardiac output (CO) and stroke volume (SV) was calculated. Estimations of infarcted area were conducted using the TTC staining method. Western blot analysis was carried out on heart tissue in order to evaluate the expression changes of target proteins. Cardiac malondialdehyde (MDA) were also measured.

The results show that DMT pretreatment significantly decreased the prevalence of ventricular fibrillation after the ISA period compared to control group. There was no significant differences before and after ISA in HR, AoP and CF in comparison with control group. Whilst, the applied pretreatment during REP period significantly ameliorated post-ISA cardiac functions (AF, CO, SV). It was further noted that I/R-induced infarct size was remarkably diminished in the DMT treated hearts in contrast with untreated group.

Taken together, we may assume that DMT pretreatment could have a protective effects against myocardial I/R injury. However, additional experiments are needed to determine the underlying mechanisms.

Acknowledgments: NKFI-143360, 2020-4.1.1-TKP2020

AT1R LIGAND BINDING MECHANISM CHARACTERIZATION WITH WELL-TEMPERED METADYNAMICS

Ádám Misák[1], András Dávid Tóth[1,2,4], Miklós Cserző[1,3], László Hunyady[1,2,3], Gábor Turu[1,2]
[1]Department of Physiology, Semmelweis University, 1094 Budapest, Hungary, [2]ELKH-SE Laboratory of Molecular Physiology, Eötvös Loránd Research Network and Semmelweis University, 1085 Budapest, Hungary, [3]Research Center for Natural Sciences, Institute of Enzymology, 1117 Budapest, Hungary, [4]Department of Internal Medicine and Haematology, Semmelweis University, 1088 Budapest, Hungary.

Introduction: The angiotensin II type 1 receptor (AT1R) has a major role in the renin-angiotensin system, and is known to exhibit biased signaling. However, its ligand binding mechanism is not fully understood. The existing metadynamics binding protocols for seven-transmembrane receptors proved to be ineffective in the case of AT1R.

Aims: Therefore we set out to develop a metadynamics protocol to model the binding of ligands from the solvent to the orthosteric binding pocket of AT1R.

Methods: We used well-tempered metadynamics with two collective variables (CVs): The distance between the alpha carbon atom of the conserved Trp6.48 and the center of mass of the ligand. A coordination CV that measures the strength of the contact of the AT1R N-terminal to the second extracellular loop (ECL2).

Results: Our results indicate that the N-terminal can unbind from the groove of ECL2, this allows access to the orthosteric binding pocket from the extracellular side. After the binding of Angiotensin II (Ang II) the N-terminal "closes down" the binding pocket, and the N-terminal - ECL2 interaction is stabilized by Ang II. In the case of angiotensin receptor blockers binding this interaction is not stabilized by the ligand.

Conclusion: The N-terminal of the AT1R acts as a "lid" for the orthosteric binding pocket and can sterically block the binding/unbinding of the ligands. Our results can aid the development of new AT1R ligands.

Funding: FK 138862, K 139231. The scientific work/research and/or results publicised in this article was reached with the sponsorship of Gedeon Richter Talentum Foundation in framework of Gedeon Richter Excellence PhD Scholarship of Gedeon Richter.

HYPERCHOLESTEROLEMIA ABOLISHED THE CARDIOCYTOPROTECTIVE EFFECT OF ROFECOXIB IN CARDIAC MYOCYTE CELL CULTURE MODELS OF ISCHEMIA/REPERFUSION INJURY

Regina N. Nagy[1], Bernadett Kravcsenko-Kiss[1], Gábor B. Brenner[1], András Makkos[1], Dóra Reé[2], Ágota Apáti[2], Paola Poggi[3], Alexandros Chatgilialoglu[3], Zoltán V Varga[1], Zoltán Giricz[1], Péter Ferdinandy[1,4], Anikó Görbe[1]
[1]MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary, [2]ELKH-Research Centre for Natural Sciences, Institute of Enzymology, Budapest, Hungary, [3]Remembrane S.r.L., Imola, Italy, [4]Pharmahungary Group, H-6722 Szeged, Hungary

The hidden cardiotoxicity of the selective COX-2 inhibitor rofecoxib can be shown in early preclinical models of ischemia/reperfusion (I/R) injury. Paradoxically, rofecoxib also has cytoprotective effect. We hypothesize that cytoprotective effect of rofecoxib can be abolished in presence of metabolic comorbidities, unmasking its hidden cardiotoxicity. Therefore, we aimed to investigate the effect of rofecoxib in cellular models of I/R in the presence of hypercholesterolemia (HC).

Adult rat cardiac myocytes (ARCMs) and human induced pluripotent stem cell-derived cardiac myocytes (huIPS-CMs) were incubated in normocholesterolemic (NC) or HC conditions for 24 or 48 hours, respectively. ARCMs and huIPS-CMs were the pretreated with 1 µM rofecoxib for 1 hour, then subjected to simulated ischemia or normoxia for 3 or 24 hours, respectively, followed by 2 hours of reperfusion (normoxia or sI/R groups) with the supplemention by rofecoxib throughout the protocol. The cell survival was measured by fluorescence based viability assay.

In normoxic conditions, HC or rofecoxib treatment did not affect cell survival. In NC conditions, rofecoxib significantly improved cell survival of both ARCMs and huIPS-CMs following sI/R injury. In contrast, the viability-increasing effect of rofecoxib was abolished in presence of HC conditions after sI/R injury.

This is the first demonstration that the cardioprotective effect of rofecoxib on cardiac myocyte survival after sI/R injury in NC is abolished in HC conditions, which highlights the need for the application of complex co-morbidity assays in preclinical safety test systems in drug development programs.

INVESTIGATION OF FATTY ACID AMIDE HYDROLASE 1 (FAAH1) IN HUMAN SEBOCYTES

Tamara Nyitrai[1], Dorottya Ádám[1], József Arany[1], Mátyás Magyari[1], Christos C. Zouboulis[2], Attila Oláh[1]
[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary, [2]Departments of Dermatology, Venereology, Allergology and Immunology, Staedtisches Klinikum Dessau, Brandenburg Medical School Theodor Fontane, Dessau, Germany

One of the most common human skin diseases, acne, is characterized by elevated sebum production and pathological inflammatory processes. We have previously shown that one of the most important endocannabinoid-degrading enzymes, fatty acid amide hydrolase-1 (FAAH1), is a key positive regulator of the production and release of pro-inflammatory cytokines in human epidermal keratinocytes, and operates under the control of Toll-like receptor (TLR)-2 (Oláh et al., *Exp. Dermatol.*, 2016). We could also demonstrate that, similar to several TLRs, FAAH1 was also expressed on human sebocytes both *in vitro* and *in situ* (Zákány et al., *J. Invest. Dermatol.*, 2018), and, while its pharmacological inhibition with URB597 did not affect basal sebaceous lipogenesis, it significantly reduced the lipogenic effect of the endocannabinoid anandamide (unpublished observations), suggesting that pharmacological inhibition of FAAH1 could be beneficial in acne. Thus, within the confines of the current study, we aimed to further investigate the role of FAAH1 and the biological effects of URB597 by using human, immortalized SZ95 sebocytes.

First, we investigated whether different, “acne-relevant”, non-cytotoxic (MTT-assay) pro-inflammatory stimuli (the inflammatory lipid mediator arachidonic acid [AA; 50 µM], the Toll-like receptor [TLR]-2 activator lipoteichoic acid [LTA; 10 µg/ml], and the TLR4 activator lipopolysaccharide [LPS; 5 µg/ml]) influenced expression of FAAH1. We found that, although they induced characteristic inflammatory response (as monitored by the expression [Q-PCR] and release [ELISA] of well-known pro-inflammatory cytokines) over the course of 3- and 24-hr treatments, none of the said stimuli increased FAAH1 mRNA (Q-PCR) or protein level (western blot) expression. Interestingly, however, when applied at non-cytotoxic concentrations (≤10 µM; MTT-assay), URB597 significantly reduced AA-induced, pathologically elevated sebaceous lipogenesis without affecting the cell count (Nile Red staining – determination of the level of neutral and polar lipids, respectively; 48-hr treatments). In addition, when applied alone, URB597 (0.1-10 µM) did not increase the release of interleukin (IL)-6 and IL-8, but it suppressed the LPS-induced release of said cytokines (ELISA).

Collectively, we found that non-cytotoxic concentrations of the FAAH1 inhibitor URB597 could normalize the AA-induced, “acne-mimicking” sebaceous lipogenesis, and exhibited anti-inflammatory effects on human sebocytes. Thus, FAAH1-inhibition may become a promising, novel therapeutic strategy in the management of acne.

Supported by: NRDIO 134235, EFOP-3.6.3-VEKOP-16-2017-00009, EFOP-3.6.1-16-2016-00022, János Bolyai Research Scholarship (BO/00660/21/5), New National Excellence Program of the Ministry for Culture and Innovation (ÚNKP-22-5-DE-427).

ACCURACY AND PRECISION OF THE RECEPTORIAL RESPONSIVENESS METHOD (RRM) PERFORMED USING LOCAL (INDIVIDUAL), FURTHERMORE ONE- AND TWO-MODEL GLOBAL REGRESSION

Óvári Ignác[1], Viczján Gábor[1], Erdei Tamás[1], Tele-Héri Brigitta[2], Zsuga Judit[2], Juhász Béla[1], Szilvássy Zoltán[1], Gesztelyi Rudolf[1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, [2]Department of Psychiatry, Faculty of Medicine, University of Debrecen

The receptorial responsiveness method (RRM) is a procedure based on regression of concentration-effect (E/c) curves. RRM enables the estimation of a change in concentration of a degradable agonist near the receptor, from data of two E/c curves of a stable agonist of the given receptor, if the first curve has been generated before (or in the absence of), while the second one has been constructed after the change in concentration, in the same (or in an identical) biological system. RRM results in a surrogate parameter, which is the concentration of the stable agonist being equieffective with the change in concentration of the degradable agonist. The curve fitting can be implemented in several ways that, however, can affect accuracy, precision (reliability) and manageability (easy or difficult nature) of the determination.

In this investigation, known concentrations of stable agonists were estimated with RRM by performing local (individual) or global fitting (the latter one with one model or two models), combined with ordinary or robust manner of regression. Since all E/c curves were constructed with the agonist to be determined, herein estimates of the desired concentrations were directly obtained (instead of getting substitute parameters), enabling the comparison to the real values.

We found that accuracy and precision of the global regression were greatly increased when the appropriate E/c curves were fitted in pairs (so only two at once). The local fitting, the most difficult to use otherwise, proved to be the most accurate, closely followed by the moderately difficult two-model global fitting and the easy-to-perform one-model one. The most precise procedure was the two-model global fitting followed by the local fitting (closely) and the one-model global fitting (from afar). The ordinary or robust way of fitting did not significantly affect accuracy. Summarizing, the two-model global fitting, performed pairwise, is recommended for RRM, but the local fitting is also suitable.

Funding: GINOP-2.3.4-15-2020-00008, TKP2020-NKA-04 (financed under the 2020-4.1.1-TKP2020 funding scheme), Project no. TKP2021-EGA-18 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme.

EFFECTS OF BLUNTING ANNEXIN A1 EXPRESION IN SMALL CELL LUNG CANCER CELL LINES

Ágnes Paál[1], Ákos Takács[1], Zoltán V. Varga[1], István Peták[1], Anikó Görbe[1], Péter Ferdinandy[1], Dávid Dóra[2]
[1]MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, [2]Department of Anatomy, Histology and Embryology, Faculty of Medicine, Semmelweis University

The first advancement in small cell lung cancer (SCLC) therapy since the 1990s was the recent addition of immune checkpoint inhibitors to chemotherapy as first-line treatment of extensive-stage disease. However, response and resistance to Immunotherapy are not characterized in SCLC. Previous studies identified novel molecular subtypes of SCLC with distinct immune infiltration patterns. Still, tumors with strong immune infiltration are often resistant to Immunotherapy. A possible mechanism behind this phenomenon is the overexpression of immunosuppressive molecules, such as annexin A1 (ANXA1). We hypothesized that silencing ANXA1 expression in cell culture reduces growth rate, which would highlight the role of ANXA1 in SCLC therapy outcome. We aimed to investigate the effect of silencing ANXA1 expression in an SCLC cell line.

An SCLC cell line with high ANXA1 expression (SW1271) was transfected with control or ANXA1 silencing lentiviral short hairpin RNA (shRNA) vector. As a selection agent, 0,1% puromycin was continuously present in culture medium. Effectivity of ANXA1 silencing was tested with western blot. Growth rate of native, non-transfected cells and control shRNA transfected and ANXA1 silenced cells was compared with trypan blue exclusion assay and PrestoBlue™ Viability Assay. Statistical analysis was done with Kruskal-Wallis test followed by Dunn's test for the western blots and PrestoBlue™ viability assays, and mixed-effects analysis followed by Tukey's post hoc test for the trypan blue exclusion assays. The n number of the groups was 6 (n=6) in each experiment. In the growth rate assays, technical repeats were also performed.

Annexin A1 expression was stably silenced with lentiviral shRNA vector, shown by western blots. Growth rate of ANXA1 silenced cells was lower than the native and control shRNA transfected SCLC cells, and the difference increased with time of incubation, as seen in 120-hour-long trypan blue exclusion assays and Presto Blue™ Viability Assays.

Silencing annexin A1 expression has therapeutic relevance in small cell lung cancer. Reduction of growth rate by annexin A1 silencing is a feature that can help understand Immunotherapy resistance of this aggressive malignancy.

INVESTIGATION OF SPATIAL WORKING MEMORY PERFORMANCE IN THE SELF-ORDERED SPATIAL SEARCH TASK FOLLOWING PHARMACOLOGICALLY INDUCED COGNITIVE IMPAIRMENT IN NON-HUMAN PRIMATES

Rafaella Mínea Riszt[1,3], B. Knakker[1], A. Vitális-Kovács[1], J. Inkeller[1], A. Padányi[1,3], Sz. L. Khalil[1], E. Kiefer[1], I. Hernádi[1,2,3]
[1]Grastyán E. Translational Research Centre, University of Pécs, [2]Faculty of Sciences, University of Pécs, [3]Medical School, University of Pécs

The Self-ordered Spatial Search (SOSS) task is a non-verbal test paradigm probing visual spatial working memory (SWM) that is suitable for testing the performance of nonhuman primates in a translational experimental setting. As a model of cognitive decline associated with ageing, transient cognitive impairment was induced in rhesus macaques by the muscarinic receptor antagonist amnestic agent scopolamine. In addition, we used the acetylcholinesterase inhibitor donepezil to test the reversibility of the amnestic condition caused by scopolamine.

First, we trained 14 adult male subjects to perform the task and measured their performance in 1-hour sessions with 300-500 trials in each session. In every trial the animals were shown 4-8 identical blue squares on a touchscreen, each of which they had to touch exactly once, in an arbitrary order. Individual stimulus set sizes (n=4-7) were defined based on the animals' performance levels. In half of the trials a larger (n+1) set size was used. Each response was followed by a delay period (0.5-2 s). Besides the proportion of correct trials, we analyzed the frequencies of two error types: continuous perseverative errors (CPE: a stimulus is touched two times in a row) and recurrent perseverative errors (RPE: a formerly touched stimulus is touched again, not directly after the previous touch).

Increasing set sizes resulted in the selectively increased proportion of RPEs, that also typically occurred in the later phase of choice sequence. However, varying the length of the delay period did not have any further effects on the performance of the animals. These results suggest that within the SOSS task, set size effects on RPEs provide a viable candidate endpoint to measure SWM in non-human primates. Even though scopolamine treatment dosedependently deteriorated performance, it did not show similar selectivity to any of the error types, which shows a certain limitation of the cognitive impairment model. We observed a trend of donepezil reversing the effects of the evoked amnestic condition, but this effect was found to be significant only in the case of CPEs.

Based on our results, the SOSS task can be modified to provide a sensitive assessment of SWM. The specificity of the effects of scopolamine treatment on performance endpoints that were shown to be memory-related were weak but may be improved with further task development. Specifically linking the observed pharmacological effects to memory processes will optimize the SOSS task for preclinical determination of the efficacy of potential cognitive enhancer drug candidates.

DEVELOPMENTAL CHANGES OF GANGLIOSIDE GM1 METABOLISM OF NOCICEPTIVE PRIMARY SENSORY NEURONS

Péter Sántha[1], Gábor Jancsó[1]
[1]Department of Physiology, University of Szeged, Szeged

Primary sensory neurons (PSNs) show profound phenotypic changes during early postnatal development regulated by trophic factors, such as nerve growth factor (NGF). Ganglioside GM1 is implicated in the mechanism of NGF action, but developmental changes in ganglioside metabolism of PSNs are unclear. In the present study, the histochemical localization of choleratoxin B subunit (CTB), a specific biochemical marker of GM1 was examined in PSNs in the course of the early postnatal development.

Experiments were performed on Wistar rats ageing 1-30 days. Rats were injected with either capsaicin (50 mg/kg) or its vehicle at birth. Animals were given intraplantar injections of CTB-horse radish peroxidase (HRP) conjugate (1 µl, 1%) at postnatal days 2-30. Rats were sacrificed 2 days after the injection of the tracer and processed for HRP histochemistry using standard procedures.

In newborn rats, peripherally injected CTB-HRP was transported transganglionically to the spinal dorsal horn, including the substantia gelatinosa (Rexed's lamina II). Transganglionic labeling of the substantia gelatinosa gradually decreased towards later postnatal periods; at around postnatal day 30, the labeling pattern of the dorsal horn resembled that observed in adult rats, i.e. the substantia gelatinosa was practically devoid of labeling. In rats treated neonatally with capsaicin, CTB-HRP labeling of the substantia gelatinosa was dramatically reduced already on the 5th postnatal day following an injection of the tracer on day 3. In addition, unlike in capsaicin-treated rats, labeling of the substantia gelatinosa was still conspicuous on days 15-20 following an injection of CTB-HRP.

The present experiments corroborate earlier findings demonstrating a gradual decrease of CTB-HRP labeling of the substantia gelatinosa in the course of the early postnatal life. Since neonatal capsaicin treatment is known to rapidly and substantially eliminate C-fiber primary afferents terminating in the substantia gelatinosa, our observations suggest that the labeling of the substantia gelatinosa by CTB-HRP in the early postnatal days results from the uptake and transganglionic transport of the tracer by C-fiber primary afferents. The findings also indicate that CTB-HRP is not a specific tracer for myelinated primary afferents in the newborn rat, and the developmental loss of labeling of the substantia gelatinosa may be accounted for by a change in the molecular phenotype of C-fiber afferents, rather than withdrawal of myelinated spinal afferents from that area.

Supported by the NRDIO grant K-138568

THE IMPACT OF ASTROCYTIC PIEZO-1 CHANNELS ON NEOCORTICAL PYRAMIDAL NEURONS, EVALUATED BY CHANGES IN SLOW INWARD CURRENTS (SICS)

Andrea Csemer[1], Cintia Sokvári[1], Baneen Maamrah[1], Krisztina Pocsai[1], Balázs Pál[1]
[1]University of Debrecen

Astrocytes can critically affect neuronal excitability via eliciting tonic and phasic NMDA receptor dependent excitatory currents on them. Phasic NMDA receptor dependent neuronal currents - the consequences of astrocytic activity - are known as 'slow inward currents' (SICs). Although astrocytes are known to express Piezo-1 mechanosensitive channels, its significance on astrocyte-neuron communication and neuronal excitability has not been investigated yet. We aimed to investigate the impact of the Piezo-1 mechanosensitive channel on the astrocyte-dependent NMDA receptor dependent neuronal currents, and the consequential changes of neuronal activity.

We used 2-3 weeks old juvenile mice to prepare coronal slices of the temporal cortex and performed patch clamp recordings on layer III-IV. pyramidal cells.

The Piezo-1 opener Yoda (10 µm) significantly increased SIC activity on neurons. This action could be prevented by the NMDA receptor antagonist D-AP5 (10 µm). The Piezo-1 channel inhibitor Dooku (10 µm) exerted a mild inhibition on basal SIC activity and a stronger but not full inhibition on Yoda-evoked increase.

One can conclude that activation or inhibition of Piezo-1 channels have a significant impact on NMDA receptor mediated astrocyte-neuron communication and neuronal excitability in the neocortex.

MICRORNA (MIRNA) PROFILE AFFECTED BY H_2S-RELEASING VITAMIN C DERIVATIVE (BM-164) IN RAT HEARTS AFTER ISCHEMIA - REPERFUSION INJURY

Erzsébet Szabó[1,4], Virág Vass[1,3,4], Bence Tánczos[1,4], Miklós Lovas[2], Ilona Bereczki[2,4], Zsuzsanna Szabó[5], Kálmán Szenthe[6], Anikó Borbás[2], Pál Herczegh[2,4], Árpád Tósaki[1,4]
[1]Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, 4032, Debrecen, Hungary, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Debrecen, 4032, Debrecen, Hungary, [3]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy, University of Debrecen, 4032, Debrecen, Hungary, [4]ELKH-DE Pharmamodul Research Team, University of Debrecen, 4032, Debrecen, Hungary, [5]Department of Biopharmacy, Faculty of Pharmacy, University of Debrecen, 4032, Debrecen, Hungary, [6]RT-Europe Research Organization Kft, 9200, Mosonmagyaróvár, Hungary

The mechanism of arrhythmogenesis is a complex process, however, only a few data are available to explain the role of miRNA in ischemia-reperfusion induced injury. Studies have suggested that by altering key signaling elements miRNAs may also contribute to ischemia-reperfusion injury, including ventricular fibrillation (VF). We aimed to identify miRNAs that might play vital role in signalling mechanisms of ischemia-reperfusion induced VF. We synthesized a new water-soluble hydrogen sulfide (H_2S)-donor vitamin C derivative, BM-164. Studies have proved the cardioprotective effect of H_2S, but the relationship between H_2S and miRNAs has only recently become in the focus of cardiovascular research. Thus, we investigated the effect of BM-164 on the expression of miRNAs in rat hearts subjected to ischemia-reperfusion.

Langendorff isolated hearts were used for our studies. Hearts pretreated with BM-164 (30 µM) or vitamin C (30 µM) were subjected to ischemia (30 min) – reperfusion (120 min). At the end of the reperfusion, the hearts were collected in RNA shield reagent (Zymo Research) before RNA extraction. After extraction qualitative analysis of RNAs was performed by Agilent Bioanalyzer. For miRNA (miR) profiling the samples were processed using Rat miRNA panel v1.5 (with 425 types of miRNA) on NanoString SPRINT Profiler instrument (RT-Europe Research Center Ltd), and then the expression of miRNAs was validated with quantitative PCR (qPCR) by using specific TaqMan miRNA assays (LifeTechnology).

A total of 12 hearts (control non-ischemic, hearts developed VF and hearts did not develop VF upon reperfusion) were used for miRNA screening with Nanostring technology. Only a few miRNAs out of several hundreds (425) showed significant differences among the groups studied. Thus, comparing the control (non-ischemic) and the fibrillated groups, *rno-mir-99a* showed a significant difference, while the ischemic-non-fibrillated group showed a significant change in the expression of *rno-mir-191* compared to the control (non-ischemic) group. Validation by qPCR was extended to more samples, including hearts treated with vitamin C or BM-164, respectively. The following microRNAs were measured: *rno-miR-30d, rno-miR-99a, rno-miR-130a, rno-133a, rno-mir-181a and rno-miR-191*. By qPCR a significant decrease in the expression of *rno-miR-99a* in ischemic-VF group compared to non-ischemic control was detected, and even a more remarkable decrease was found in the BM-164 treated group. *rno-miR-191* proved a significantly lower expression in hearts showed VF than in the control non-ischemic hearts. *rno-miR-30d and rno-mir-181* were significantly upregulated in hearts pretreated with BM-164 in comparison with the non-ischemic control group. In the expression of other miRNAs, there was no significant difference between the treated groups.

Our results show that the expression of miRNAs is affected by ischemia-reperfusion, and BM-164 may have an impact on microRNAs.

Acknowledgement: The project was supported by the ELKH-DE Pharmamodul Research Team.

SIMULATED MICROGRAVITY LEADS TO ALTERED INTRACELLULAR CALCIUM HOMEOSTASIS AND PROTEIN EXPRESSION IN C2C12 CULTURES

Mónika Gönczi[1,2], Zsolt Ráduly[1,2], László Szabó[1,2], Péter Szentesi[1,2], Beatrix Dienes[1,2], László Csernoch[1,2]
[1]Department of Physiology, University of Debrecen, Debrecen, [2]ELKH-DE Cell Physiology Research Group, Debrecen

Skeletal muscle has the role to maintain body posture against a constant gravitational load. During aging, in low physical activity or even in spaceflight, the muscle mass is decreasing, resulting in impaired myogenesis and regeneration. The cytoskeleton, mechanosensitive ion channels, and calcium homeostasis play crucial role in maintaining normal myogenic processes. Simulated microgravity (SM) with 3D clinorotation is one of the accepted techniques to model gravitational unloading in vitro. C2C12 mouse myoblast cell line is a verified in vitro model system of myogenesis.

Using RPM 2.0, a Random Positioning Machine as a partial g simulator, by randomly rotating the accommodated experiment package around the Earth's gravity vector, we are able to investigate alteration of physiological processes caused by SM. In our experiments, territorial gravity is compared to 0 g, respectively. However, fusion of myoblasts was preserved with SM, the formation of myotubes was different as compared to the control environment. Expression of Myosin Heavy Chain 2 (MYH2) protein, which is an important structural protein and a marker molecule of myotube differentiation program, was significantly reduced in samples kept in RPM. Immunocytochemistry of myogenic cultures revealed decreased size and average nuclei content in differentiated myotubes. The expression level of Piezo1 channels, which are mechanically activated cation channels are also decreased under SM. Altered calcium transients evoked by KCl have been observed in myotubes formed under simulated microgravity.

Our results are in coherence with the recently published data. Our future goal is to analyze the architecture of cytoskeletal Septin7 protein and investigate how intracellular calcium concentrations and mechanotransduction is regulated by Piezo1 channels under microgravity condition.

EFFICIENT TREATMENT OF A PRECLINICAL INFLAMMATORY BOWEL DISEASE MODEL WITH ENGINEERED BACTERIA

Szilamer Ferenczi[1, 2], Norbert Solymosi[3], Istvan Horvath[2], Natalia Szeőcs[2], Zsuzsanna Grozer[2], Daniel Kuti[1], Balazs Juhasz[1], Zsuzsanna Winkler[1], Tibor Pankotai[4], Farkas Sukosd[5], Aniko Stagel[6], Melinda Paholcsek[6], David Dora[7], Nandor Nagy[7], Krisztina J. Kovacs[1], Ivan Zanoni[8], Zoltan Szallasi [7,8,9]
[1]Institute of Experimental Medicine, Laboratory of Molecular Neuroendocrinology, Budapest, Hungary, [2]Central European Biosystems, Gödöllő, Hungary, [3]Centre for Bioinformatics, University of Veterinary Medicine Budapest, [4] University of Szeged, Faculty of Science and Informatics, Department of Biochemistry and Molecular Biology, [5]University of Szeged, Faculty of Medicine, Department of Pathology, Laboratory of Molecular Pathology, [6]University of Debrecen, Faculty of Medicine, Department of Human Genetics, Debrecen, Hungary, [7]Semmelweis University, Faculty of Medicine, Department of Anatomy, Histology and Embryology, Budapest, Hungary., [8]Harvard Medical School, Boston Children's Hospital, Division of Immunology, Division of Gastroenterology Boston, MA, USA, [9]Danish Cancer Society Research Center, Copenhagen, Denmark, [10]Computational Health Informatics Program, Boston Children's Hospital, USA, [11]2nd Department of Pathology, MTA-SE NAP, Brain Metastasis Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary

We developed an orally administered, engineered, bacterium-based, RNA interference-mediated therapeutic method to significantly reduce the symptoms in the most frequently used animal model of inflammatory bowel disease. This bacterium-mediated RNA interference strategy was based on the genomically stable, non-pathogenic E. coli MDS42 strain, which was engineered to constitutively produce invasin and the listeriolysin O cytolysin. These proteins enabled the bacteria first to invade the colon epithelium and then degrade in the phagosome. This allowed the delivery of a plasmid encoding small hairpin RNA (shRNA) targeting tumor necrosis factor (TNF) into the cytoplasm of the target cells. The expression levels of TNF and other cytokines significantly decreased upon this treatment in dextran sulfate sodium (DSS)-induced colitis, and the degree of inflammation was significantly reduced. With further safety modifications this method could serve as a safe and side effect-free alternative to biologicals targeting TNF or other inflammatory mediators.

ANTIOXIDANT PROPERTIES OF NEWLY SYNTHETHISED CBD DERIVATES

Kitti Szőke [1,2], Richárd Kajtár [1], Alexandra Gyöngyösi [1], Ilona Bakai-Bereczki [3], Anikó Borbás [3] István Lekli [1]
[1] University of Debrecen, Faculty of Pharmacy, Department of Pharmacology, [2] University of Debrecen, Institute of Healthcare Industry, [3] University of Debrecen, Faculty of Pharmacy, Department of Pharmaceutical Chemistry

Cannabidiol (CBD) is one of the main nonintoxicating, nonpsychoactive phytocannabinoid in the annual herbaceous plant, Cannabis sativa. It has numerous health beneficial effects, such as antioxidant and anti-inflammatory activities. CBD is effective in the treatment of a wide variety of diseases, such as cardiovascular, neurodegenerative, cancer, and metabolic diseases, among others. Nowadays there is a growing interest for the synthetise of new CBD derivates to enhance its efficacy and pharmacokinetic properties. In our study we aimed to examine the antioxidant activities of newly synthetised CBD derivatives, ILKA653, ILKA653F and ILKA655.

MTT assay was carried out to monitor the possible cytotoxic effect of the compounds and IC_{50} values were determined. Cell viability was studied after treatment with synthetic CBD derivates and CBD under H_2O_2 induced oxidative stress. Then, LDH assay and trypan blue exclusion test were carried out to determine the possible protective effect of our compounds after 4h hypoxia and 3h reperfusion on cell viability. Direct antioxidant activity was measured by TAC Levels of antioxidant proteins, SOD, catalase, and HO-1 were evaluated by Western blot.

Calculated pIC_{50} values are the following ILKA653F: 4,113, ILKA653F: 3,995, ILKA655: 4,190 and CBD: 4,671 M. No cytotoxic effect was observed at concentrations of 3 and 10µM. The synthetic CBD derivates and CBD increased cell viability during H_2O_2 induced oxidative stress. The results of LDH and trypan blue test revealed that the cell death due to simulated hypoxia/reperfusion was decreased by ILKA653A, ILKA65F and CBD, especially in case of ILKA653F. Direct antioxidant activity of ILKA653A and ILKA653F was comparable but lower, then CBD antioxidant activity. ILKA655 showed no direct antioxidant activity. Pretreatment with CBD derivates and CBD increased the level of antioxidant proteins. We have noticed that the increment was the highest at ILKA653F treated group.

The results indicate that the newly synthesized CBD derivatives especially ILKA 653F has similar or even better protective effect as CBD.

Acknowledgment: GINOP-2.3.4-15-2020-00008, TKP2021-EGA-18, TKP2021-EGA, NKFI-143360, ÚNKP-22-4-I-DE-184

COLOCALIZATION OF THE PAC1 RECEPTOR WITH CA2+-BINDING PROTEINS AND COCHLEA EFFERENT MARKERS IN THE AUDITORY PATHWAY OF PACAP - KNOCK OUT AND WILD TYPE MICE

Daniel Pham[1], Balazs Daniel Fulop[1], Gergo Gyurok[1], Balazs Gaszner[1], Dora Reglodi[1], Andrea Tamas[1]
[1]Department of Anatomy, ELKH-PTE PACAP Research Team, Medical School, University of Pécs, Hungary,

Background, aims: The auditory system is also affected by the neuroprotective pituitary adenylate cyclase-activating polypeptide (PACAP). We discovered elevated hearing thresholds, increased apoptosis, and increased synthesis of Ca2+-binding proteins in hair cells of Corti's organ in PACAP knock out (KO) mice. The aim of this study was to examine the role of PACAP in the auditory pathway of 1.5, 4, and 8-month-old PACAP KO and wild type (WT) mice.

Materials and methods: The co-localization of PACAP specific PAC1 receptor (PAC1R) was visualized by immunostaining with calretinin-parvalbumin Ca2+-binding proteins and with choline acetyltransferase(ChAT)-tyrosine hydroxylase (TH) in the auditory pathway.

Results: The number of parvalbumin-positive cells in the ventral cochear nucleus (VCN) increased significantly with age in both genotypes, but the number of parvalbumin-PAC1R positive cells increased less markedly. In both the WT and KO young genotype groups, PAC1R colocalized with parvalbumin rather than calretinin in the dorsal cochlear nucleus (DCN). PAC1R was found in the fifth of both ChAT and TH positive cells in the superior olivary complex (SOC).

Conclusion: The age-related increase of parvalbumin in the auditory pathway is well known, but its decrease in PAC1R positive cells of VCN indicates that PACAP influences this age-related process. Our findings in the DCN show that PACAP action is not equal on all cell types. The presence of PAC1R in the SOC indicates that PACAP is involved in the descending control of the cochlea. Overall, we found that, in addition to the cochlea, PACAP influences the function of the auditory pathway.

Keywords: auditory pathway, cochlear nucleus, PACAP

EFFECTS OF H_2S-DONOR VITAMIN C DERIVATIVE AFTER ISCHEMIA/REPERFUSION INDUCED INJURY IN ISOLATED RAT HEARTS

Bence Tánczos[1,4], Virág Vass[1,3,4], Erzsébet Szabó[1,4], Miklós Lovas[2], Ilona Bereczki[2,4], Pál Herczegh[2,4], Anikó Borbás[2], Árpád Tósaki[1,4]
[1]Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [3]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [4]ELKH-DE Pharmamodul Research Team, University of Debrecen, Debrecen, Hungary

Hydrogen sulfide (H_2S) as a gasotransmitter plays an important role in vasorelaxation, anti-inflammatory processes and in case of myocardial ischemia/reperfusion induced injury. It can also regulate various signaling processes. In light of the experimental and clinical data provided in recent years, the need for H_2S releasing drugs is increasing.

Because of these aforementioned reasons our group synthesized a new, water soluble H_2S-releasing vitamin C derivative. Vitamin C is an essential antioxidant with excellent water solubility therefore it was chosen as the parent molecule of our new H_2S donor derivative, BM-164.

In our experiments, we tested the potential positive effects of BM-164 (30 µM), in case of 30 min of myocardial ischemia followed by 120 min reperfusion. As a potential drug BM-164 was used *in vitro* in Langendorff-heart model vs. non-treated (control) and vitamin C treated (30 µM) rat hearts. The H_2S release was measured by an H_2S sensitive sensor. Increased hydrogen sulfide concentration was detected in the coronary perfusate of the BM-164 pretreated group, showing that this molecule can successfully release H_2S in the myocardium.

The incidence of ventricular fibrillation was significantly reduced in the BM-164 treated (31 %) hearts compared to the control group (79 %) as well the size of the ischemia damaged area (infarct size) (Control: 28.1 ± 4.2 % vs. BM-164: 13.8 ± 1.8 %). Coronary flow and heart rate did not show statistically significant difference between the groups, however the BM-164 group resulted in better outcomes compared to the other experimental groups. In conclusion, the synthesis of a water soluble, H_2S releasing vitamin C derivative (BM-164) showed a better protection compared to the non-treated and vitamin C treated rat hearts.

Acknowledgement: The project was supported by the ELKH-DE Pharmamodul Research Team.

INVESTIGATION OF THE ROLE OF FRACTALKINE RECEPTOR (CX3CR1) IN MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME

Valéria Tékus[1], Nikolett Szentes [1,2], Krisztina Pohóczky[1,3,], Ádám Dénes[4], Serena Sensi[5,6], Andreas Goebel[5,6], Zsuzsanna Helyes[1,2,7]
[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, H-7624, Pécs, Hungary; [2]ELKH-PTE Chronic Pain Research Group, University of Pécs, H-7624, Pécs, Hungary; [3]Faculty of Pharmacy, Department of Pharmacology, University of Pécs, H-7624, Pécs, Hungary; [4]Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, H-1083, Budapest, Hungary; [5]Department of Translational Medicine, University of Liverpool, Liverpool L9 7AL, United Kingdom; [6]Department of Pain Medicine, The Walton Centre National Health Service Foundation Trust, Liverpool L9 7LJ, United Kingdom; [7]PharmInVivo Ltd., H-7629, Pécs, Hungary;

Background and aims
Complex Regional Pain Syndrome (CRPS) is a severe chronic pain condition accompanied by edema and autonomic disorders, which develop after a small injury. The pathophysiological mechanisms are unknown, but immune response against sensory nerve-derived antigens, complex neuro-immune-vascular interactions and neuroinflammation are involved. Since the treatment is unsatisfactory, it is necessary to identify the key mediators and new therapeutic targets. The inflammatory chemokine (fractalkine) receptor 1 (CX3CR1) expressed on microglia cells and macrophages plays a crucial role in neuroinflammatory processes. Here we investigated its involvement in a passive transfer-trauma translational CRPS mouse model.
Methods
Female CX3CR1-gene deficient and wildtype mice were treated daily with plasma IgG purified from CRPS patients or healthy volunteers. Plantar skin-muscle incision was performed on day 0 to model the microinjury. The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, astrocyte and microglia in pain-related central nervous system regions by glial fibrillary acidic protein (GFAP) and Iba1 immunohistochemistry. The CX3CR1 antagonist AZD 8797 (80µg/kg i.p) was administered daily to wildtype mice.
Results
Daily i.p. injections of CRPS IgG significantly increased the mechanical hyperalgesia, as well as astrocyte and microglia markers in the spinal cord dorsal horn, periaqueductal gray and somatosensory cortex during the 7-day experimental period after plantar skin-muscle incision compared to healthy IgG treatment in wildtype animals. Both CX3CR1 deficiency and the antagonist treatment significantly diminished the CRPS IgG-induced increased pain behavior and glia cell activation.
Conclusions
CX3CR1 activation is likely to mediate CRPS-associated pain and neuroinflammatory mechanisms suggesting that CX3CR1 inhibition might provide novel analgesic perspectives.
Support
National Brain Research Program 3.0, OTKA K- 138046; Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, Hungary, TKP2021-EGA-16, FIKPII-17886-4/23018/FEKUTSTRAT; PTE ÁOK Dr. János Szolcsányi Research Fund: PTE ÁOK_KA-2020-18

INVESTIGATION OF THE ROLE AND DIAGNOSTIC, PROGNOSTIC VALUE OF PACAP-38 IN MULTIPLE MYELOMA

Tünde Tóth[1], Hussain Alizadeh[2], Beáta Polgár[3], Renáta Csalódi[4], Dóra Reglődi[1], Andrea Tamás[1]
[1]Department of Anatomy, ELKH-PTE PACAP Research Team, Centre for Neuroscience, Medical School, University of Pécs, 7624 Pécs, Hungary; [2]1st Department of Medicine, Division of Hematology, Clinical Centre, University of Pécs, 7624 Pécs, Hungary; [3]Department of Medical Microbiology and Immunology, Clinical Centre, University of Pécs, 7624 Pecs, Hungary; [4]Department of Hematology, Balassa János Hospital of Tolna County, 7100 Szekszárd, Hungary.

Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide with well known antiapoptotic, anti-inflammatory and antioxidant effects. The antitumor effect of PACAP in multiple myeloma (MM) has been demonstrated in numerous studies. PACAP inhibits the growth of myeloma cells, regulates osteolytic bone destruction, and protects proximal tubule cells in various models of MM. The peptide also has an immunomodulatory effect and may influence the complex cytokine network of the bone marrow microenvironment.

The aim of our study was to investigate the plasma PACAP-38 levels of patients with MM using ELISA method (n=66; control: n=10). We correlated the changes of PACAP levels with various clinical and laboratory parameters.

Lower PACAP levels were measured in treated MM patients compared with the healthy control group, but this difference disappeared if the patient achieved better response than partial response after therapy. Significantly higher PACAP-38 levels were seen in younger individuals with lower stage, lower plasma cell fraction in bone marrow, lower tumor markers and in patients after lenalidomide therapy. Higher PACAP-38 levels in newly diagnosed MM patients predicted longer survival and higher probability of response to treatment.

Based on our findings, we suggest that this peptide may play an important role in the pathophysiology of MM and PACAP could be used as a valuable noninvasive alternative biomarker. However, further studies are needed to describe the exact patomechanism of the protective effect in this disease.

TRANSCRIPTOMIC PROFILING OF THE ADRENAL GLAND IN A TRANSLATIONAL RAT MODEL OF POSTINFARCTION HEART FAILURE

Váradi Barnabás[1,2], Tóth Viktória E.[1,2,3], Brenner Gábor B.[1], Makkos András[1,3], Ónódi Zsófia[1,2,3], Kucsera Dániel[1,2,3], Vörös Imre[1,2,3], Novák Julianna[1,2], Sayour Nabil Viktor[1,2,3], Ferdinandy Péter[1,4], Varga Zoltán V.[1,2,3,4]

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Faculty of Medicine, Budapest. [2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest. [3]HAS-SU Momentum Cardio-Oncology and Cardioimmunology Research Group, Budapest. [4]Pharmahungary Group, Szeged

Heart failure is a serious health problem due its prevalence and high mortality rate. It has long been known that the adrenal gland plays a major role in the pathomechanism of heart failure through the secretion of catecholamines, glucocorticoids, and mineralocorticoids, which can be modulated pharmaceutically to reduce mortality. Nevertheless, the molecular mechanism of adrenal gland activation in heart failure is less known.

We aimed to better understand the role of the adrenal gland in chronic heart failure and test the effect of the currently used renin-angiotensin-aldosterone inhibitor therapy.

Male Wistar rats were randomized to chronic myocardial infarction (MI, n=6), sham operated (SHAM n=6) and enalapril treated chronic infarction (IN, n=6) groups. The chronic infarction was induced by permanent occlusion of the left anterior descending coronary artery for 6 weeks. After termination the transcriptomes of whole adrenal glands were investigated by next-generation sequencing. Gene expression profiling was perfomed by HISAT2-featureCounts-DESeq2 bioinformatics workflow. Differential expression analysis was carried out by Wald tests, p-values were corrected by the Benjamini-Hochberg procedure due the multiple comparisons. Gene expression profiles were analysed comprehensively by principal component analysis, expression profiles were functionally analysed using GeneOntology terms.

Systolic function reduction was significant in MI group in comparison to SHAM group, which was improved slightly by the enalapril treatment. Ischemic heart failure had robust effect on the adrenal transcriptome, whereas enalapril treatment had minor modifying effect. In addition to changes related to steroid hormones and catecholamines, heart failure also induced interleukine-1 and monocyte/macrophage-mediated inflammation in the adrenal gland.

We have shown that heart failure induced robust changes in the adrenal transcriptome, and a central role of adrenal inflammation in the disease is suggested, in addition to expected changes in catecholamine, glucocorticoid, and mineralocorticoid synthesis. The current “gold-standard” therapy has minor effect on all of these processes.

This project was supperted by OTKA-FK-134751, and by the European Union’s Horizon 2020 Research and Innovation Programme under grant agreement no. 739593 and by project no. RRF-2.3.1-21-2022-00003 with the support provided by the European Union and by the Thematic Excellence Programme (2020-4.1.1.-TKP2020) of the Ministry for Innovation and Technology in Hungary, within the framework of the Therapeutic Development and Bioimaging thematic programmes of the Semmelweis University and by a Momentum Research Grant from the Hungarian Academy of Sciences (LP-2021-14 to ZVV). BV was supported EFOP-3.6.3-VEKOP-16-2017-00009.

ROLE OF CB1 RECEPTORS AND ENDOTHELIAL FACTORS IN ESTROGEN DEPENDENT VASCULAR RESPONSE AND REMODELING

Zsolt Vass[1,2], Bálint Bányai[2,3], Stella Kiss[1], György Nádasy[3], Anikó Balogh[1], Dóra Brandhuber[1], Gabriella Dörnyei[1], Eszter Mária Horváth[3], László Hunyady[3,4], Mária Szekeres[1,3]
[1]Department of Morphology and Physiology, [2]Doctoral School, [3]Department of Physiology, Semmelweis University, Budapest; [4]Institute of Enzymology, Eötvös Loránd Research Network, Research Centre for Natural Sciences, Budapest

Introduction: There is an important interplay between the endocannabinoid system (ECS) and the female reproductive system. Cannabinoids downregulate hypothalamic-pituitary axis (HPA) and estrogen production. Also, estrogens have been shown to significantly modulate cardiovascular functions. Thus we aimed to study the impact of ECS and the role of endothelial factors in estrogen-induced vascular response.

Methods: Experiments were performed on CB1 receptor knockout (CB1R-KO) and wild-type (WT) female mice. Animals were anaesthetized (pentobarbital 50mg/kg ip.) and abdominal aortas were isolated for myography and tissue immunohistochemistry (IHC). Contractile responses to phenylephrine and relaxation to acetylcholine were obtained to test vasomotor functions. Vascular effects of estradiol (ED) were obtained in control conditions and repeated in the presence of inhibitors of cyclooxygenase (COX, indomethacin) and nitric oxide synthase (NOS, nitro-L-arginine). IHC was performed to detect eNOS and COX2 expression of aortas.

Results: ED increased relaxation of aorta isolated from CB1R-KO mice ($p<0.05$) compared to that of WT mice, which effect was attenuated by NOS-inhibition. COX-inhibition slightly increased ED-relaxation in WT mice. Intima-media ratio was decreased in CB1R-KO mice compared to WT mice. Expression of eNOS increased and COX2 depressed in the absence of CB1R, compared to WT.

Conclusion: CB1R-KO mice are characterized by increased ED-induced vasorelaxation and remodeling, which can be associated with an increased utilization of endothelial NO with depressed levels of constrictor prostanoids and altered vascular structure. Increased ED-dependent vasodilation in CB1R-KO mice may be the consequence of a depressed cannabinoid-dependent inhibition on HPA.

Support: Hungarian National Grants OTKA K116954, K139231; ÚNKP-22-3-II-SE-6

REPERFUSION-INDUCED INJURY AND THE EFFECTS OF THE DITHIOACETATE TYPE HYDROGEN SULFIDE DONOR IBUPROFEN DERIVATIVE, BM-88, IN ISOLATED RAT HEARTS

Virág Vass[1,2,4], Erzsébet Szabó[1,4], Ilona Bereczki[2,4], Alexandra Gyöngyösi [1,4], Bence Tánczos[1,4], Anikó Borbás[2], Pál Herczegh[2,4], Árpád Tósaki[1,4]
[1]Department of Pharmacology, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [3]Doctoral School of Pharmaceutical Sciences, Faculty of Pharmacy, University of Debrecen, Debrecen, Hungary, [4]ELKH-DE Pharmamodul Research Team, University of Debrecen, Debrecen, Hungary

Hydrogen-sulfide (H_2S) plays an important role in cardiac protection by regulating various redox signaling associated with myocardial ischemia/reperfusion (I/R) induced injury. H_2S donors are able to release H_2S and thus may reduce the known side effects of widely used ibuprofen. The goal of the present investigations was the synthesis of a newly designed H_2S-releasing ibuprofen derivative, BM-88, and its pharmacological characterization regarding the cardioprotective effects in isolated rat hearts.

H_2S-release was measured by an H_2S sensor from the coronary prefusate in "Langendorff" rat hearts. Increasing concentrations of BM-88 (1.0 to 20.0 µM) were tested in in vitro studies. MTT cell viability assay was carried out in H9c2 cardiomyocyte cells to evaluate the cytotoxicity of BM-88. Preadministration of 10 µM BM-88 significantly reduced the incidence of reperfusion-induced ventricular fibrillation (VF) from its drug-free control value of 92% to 12%. However, no clear dose dependent reduction in the incidence of reperfusion-induced VF was observed while different concentrations of BM-88 were used. It was also found that 10 µM BM-88 provided a substantial protection and significantly reduced the infarct size in the ischemic/reperfused myocardium. However, this cardiac protection was not reflected in any significant changes in coronary flow and heart rates.

The results support the fact that H_2S release plays an important role mitigating the reperfusion-induced cardiac damage.

Acknowledgement: The project was supported by the ELKH-DE Pharmamodul Research Team.

THE EFFECT OF A FOUR-WEEK CANNABIDIOL TREATMENT ON THE INTERSTITIAL ADENOSINE LEVEL IN THE ATRIUM OF OBESE TYPE ZUCKER DIABETIC FATTY (ZDF) RATS

Viczján Gábor [1], Szilágyi Anna [1], Takács Barbara [1], Óvári Ignác [1], Erdei Tamás [1], Zsuga Judit [2], Szilvássy Zoltán [1], Juhász Béla [1], Varga Balázs [1], Gesztelyi Rudolf [1]
[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, [2]Department of Psychiatry, Faculty of Medicine, University of Debrecen

Cannabidiol (CBD) is a non-intoxicating phytocannabinoid, which has been found to exert anti-inflammatory, anticonvulsant, anxiolytic, antidepressant and antidiabetic effects. Our goal was to explore how CBD, a known adenosine transport inhibitor and presumed A_1 adenosine receptor (A_1 receptor) agonist, affects the function of the A_1 receptor in the myocardium.

Lean (healthy) and obese (suffering from type 2 diabetes mellitus: T2DM) types of male Zucker Diabetic Fatty (ZDF) rats were randomized into three groups: "Lean" (fed conventionally), "Obese" (maintained on a diabetogenic diet), and “CBD-treated Obese” (receiving 60 mg/kg/day CBD orally for 4 weeks in addition to the diabetogenic diet). Left atria were isolated, mounted and paced in organ baths filled with Krebs solution. Concentration-effect (E/c) curves were constructed by measuring the inotropic response to adenosine, showing rapid elimination and intense transport into the cells, and then (after washing) to N^6-cyclopentyladenosine (CPA), a selective A_1 receptor agonist with slow elimination and transport. The averaged adenosine and CPA E/c curves were corrected for the biasing effect of the interstitially accumulated endogenous adenosine, when appropriate, by means of the receptorial responsiveness method (RRM).

CBD treatment significantly reduced the response of obese ZDF rat atria to CPA, while it significantly increased their response to adenosine, providing functional evidence for the adenosine transport inhibitory property of CBD, and against its A_1 receptor agonist action. The opposite changes in the response to CPA and adenosine, elicited by CBD, were even more pronounced on the corrected E/c curves. Adenosine, equieffective with about 3 nmol/L CPA, was estimated to be accumulated in the CBD-treated atria of obese ZDF rats. The corrected E/c curves of both CPA and adenosine started from an effect of 25-30%, which indicates a significant, continuous activation of A_1 receptors in the CBD-treated atria. Thus, CBD can be considered a cardioprotective agent, which is effective in the myocardium subjected to the deteriorative effects of T2DM.

Funding: Project no. TKP2021-EGA-18 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the TKP2021-EGA funding scheme.

GINOP-2.3.4-15-2016-00002, TKP2020-NKA-04 (financed under the 2020-4.1.1-TKP2020 funding scheme)

PHARMACOKINETIC CHARACTERISTICS OF A MICRORNA MIMIC AFTER INTRAVENOUS INJECTION IN MICE

Imre Vörös[1,3], András Makkos[1], Gábor G Brenner[1], Bence Ágg[1], Péter Bencsik[2], Zoltán V. Varga[3], Anikó Görbe[1,4], Péter Ferdinandy[1,4]
[1]MTA-SE System Pharmacology Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, H-1089 Budapest, Hungary, [2]Department of Pharmacology and Pharmacotherapy, University of Szeged, [3]HCEMM-SU Cardiometabolic Immunology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, H-1089 Budapest, Hungary, [4]Pharmahungary Group, Szeged, Hungary

Abstract

Background: Oligonucleotides, especially microRNAs are novel therapeutic approaches to induce cardioprotection. However, pharmacokinetic properties of the microRNAs and their delivery into the heart are rarely investigated. Even less is known specifically about the microRNA mimics, inducers of miRNA-mediated effects.

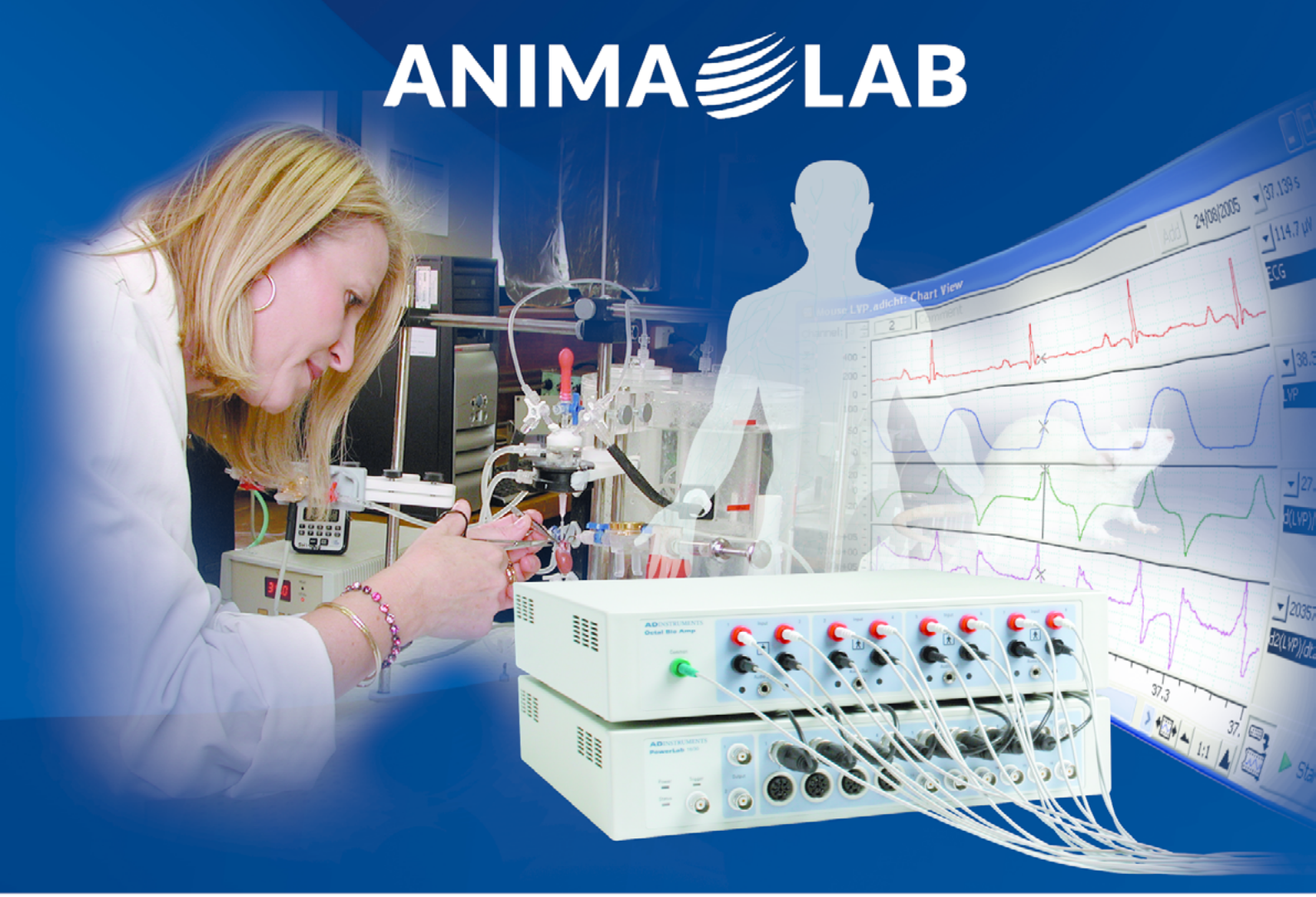
Aim: Here we aimed to characterize pharmacokinetic properties of a microRNA mimic, and its effect on selected cardiac target genes in mice.

Methods: A single injection of microRNA mimic in neutral lipid emulsion was administered intravenously to mice. Animals were sacrificed and plasma, heart, kidney and liver tissue samples were collected 1, 2, 4, 8 or 24 hours post-treatment. Total RNA was isolated for further analysis. MicroRNA expression was measured in plasma, heart, liver and kidney and expression of selected microRNA target genes was measured in the myocardium with qRT-PCR.

Results: we observed an 8-10-fold increase in the microRNA expression in the myocardium 1 hour following the administration, however, this elevation decreased rapidly. The expression of 2 out of 5 selected target mRNAs reduced significantly 8 hours after injection.

Conclusions: The applied intravenous microRNA mimic was able to reach the myocardium, and induce an elevation both in miRNA level and target gene expression. The observed difference in the time window of the induced miRNA and target mRNA changes reveals important information in terms of future dosing and application regimen.

ANIMALAB



Check Animalab offer for preclinical research and tests on laboratory animals

- Animal models with transport services
- Langendorff & Working Heart systems
- Cardiac DAQ systems
- Blood flow monitoring, laser speckle contrast
- Anesthesia, NIBP, IBP, surgical instruments
- Pain & inflammation systems for rodent tests
- Physiological and metabolic studies in group housed animals
- Imaging of neurons

charles river · ADINSTRUMENTS · ugo basile TRANSFORMING IDEAS INTO INSTRUMENTS · OXFORD OPTRONIX · TSE systems

The Jackson Laboratory · MappingLab · RWD · Kent Scientific CORPORATION · F·S·T FINE SCIENCE TOOLS

INNOVO

Amire a kísérletéhez szüksége lehet...egy helyen!

Még több termék, nagyobb választék! Partnereinkkel...

Laboratóriumi állatok forgalmazása

- *egér/patkány valamint aranyhörcsög modellek a JANVIER LABS partnereként*

JANVIER LABS Expertise In Rodent Research Models

ISOQUIMEN

Laborállat tenyésztés/bértartás

- *saját tengerimalac (DH) és nyúl (NZW) tenyészet*

Diagnosztikai szolgáltatások, genetikai monitorozás

- *élő állat, minták vizsgálata FELASA ajánlás szerint a GVG Diag.Serv.-nél*

GVG Diagnostics

Takarmány, alomanyag, környezetgazdagító termékek

- *minden, ami SAFE*

SAFE

Labor berendezések, egyéb eszközök

- *sebészeti eszközök, anesztézia berendezés az RWD-től és sok más*

RWD Stoelting

NB National Band & Tag Company INTERNATIONAL IDENTIFICATION INC. Family Operated Since 1902

Tisztatér technológia, berendezések

- *DYCEM tisztatéri szőnyeg, állattartó berendezések*

Dycem

Szállítás és kiegészítők

- *bérfuvar (export-import), szállítódobozok, zselék*

INNOVO Kereskedelmi és Szolgáltató Kft.
Tel: 06-28-582035 / E-mail: info@innovokft.hu
Weboldal: www.innovokft.hu

CLL-ben tételes finanszírozásban elérhető![4]

MÉLY VÁLASZ, TARTÓS REMISSZIÓ[1,2] és HOSSZABB TÚLÉLÉS[3]

VENCLYXTO®▼-val

VENCLYXTO® +obinutuzumab — 1L CLL

62,6%
5 ÉVES PFS ARÁNY
(VEN+O vs. 27% Clb+O) [2]

76%
uMRD ARÁNY
(VEN+O vs. 35% Clb+O; 3 hónappal a kezelés befejezését követően) [1]

1 ÉVES FIX IDEJŰ KEZELÉS[1]

VENCLYXTO® +rituximab — 2L+ CLL

54 HÓNAP
mPFS
(VEN+R vs. 17 hónap BR) [1]

64%
uMRD ARÁNY (EoT)[1]
(62% VEN+R vs. 13% BR; EoCT) [1]

2 ÉVES FIX IDEJŰ KEZELÉS[1]

VENCLYXTO® +AZA — 1L AML

66,4%
KOMPLETT REMISSZIÓ
(VEN+AZA vs. 28,3% PBO+AZA) [3]

14,7 HÓNAP
mOS
(VEN+AZA vs. 9,6 hónap PBO+AZA) [3]

JELENTŐSEN ALACSONYABB TRANSZFÚZIÓ IGÉNY[3]

AML: akut myeloid leukémia; **AZA:** azacitidin; **Clb+O:** chlorambucil+obinutuzumab; **CLL:** krónikus limfocitás leukémia; **mOS:** medián teljes túlélés; **PBO:** placebo; **mPFS:** medián progressziómentes túlélés; **VEN:** VENCLYXTO®; **VEN+O:** VENCLYXTO®+obinutuzumab; **VEN+R:** VENCLYXTO®+ rituximab; **EoT:** A 24 hónapos kezelés végén, **EoCT:** A kombinációs kezelés végén; **uMRD:** nem kimutatható minimális reziduális betegség; **BR:** bendamusztin+rituximab

Hivatkozások:
1. Venclyxto® (venetoklax) alkalmazási előírás, https://www.ema.europa.eu/en/documents/product-information/venclyxto-epar-product-information_hu.pdf
2.Othman Al-Sawaf et al. Venetoclax-obinutuzumab for previously untreated chronic lymphocytic leukemia: 5-year results of the randomized CLL14 study. Abstract S148, EHA 2022.
3. DiNardo CD et al. N Engl J Med 2020;383(7):617–629.
4. 9/1993. (IV. 2.) NM rendelet 2023. március 28-án hatályos változata

VENCLYXTO® 10 mg, 50 mg és 100 mg filmtabletta (venetoklax)
Bővebb információért olvassa el a gyógyszer alkalmazási előírását!
https://www.ema.europa.eu/en/documents/product-information/venclyxto-epar-product-information_hu.pdf

Forgalomba hozatali engedély jogosultjának helyi képviselete: AbbVie Kft., 1095 Budapest, Lechner Ödön fasor 7. Telefonszám: +36 1 455 8600. www.abbvie.hu
A VENCLYXTO® hipometiláló szerekkel kombinálva jelenleg közfinanszírozásban nem részesül.
A VENCLYXTO®/rituximab kombinációs kezelés, valamint a VENCLYXTO® monoterápia 2020. január 15-től, a VENCLYXTO®/obinutuzumab kombinációs terápia 2022. január 1-től a 9/1993 NM Rendelet 7/b13. indikációs pontja alapján tételes finanszírozás alá eső kezelés.

A közfinanszírozás alapjául elfogadott árak:
VENCLYXTO® 10 mg (14×): 23 139 Ft (térítési díj: 0 Ft); VENCLYXTO® 50 mg (7×): 56 288 Ft (térítési díj: 0 Ft); VENCLYXTO® 100 mg (7×): 111 536 Ft (térítési díj: 0 Ft); VENCLYXTO® 100 mg (14×): 222 033 Ft (térítési díj: 0 Ft); VENCLYXTO® 100 mg (112×): 1 771 412 Ft,(térítési díj: 0 Ft).

Forrás: www.neak.gov.hu. Az aktuális árak megtalálhatók a www.neak.gov.hu oldalon.
Letöltés dátuma: 2023. 03. 27.

▼ Ez a gyógyszer fokozott felügyelet alatt áll, mely lehetővé teszi az új gyógyszerbiztonsági információk gyors azonosítását.
Az egészségügyi szakembereket arra kérjük, hogy jelentsenek bármilyen feltételezett mellékhatást.

abbvie

HU-VNCLY-230005
Lezárás dátuma: 2023. 04. 24.

VENCLYXTO®

TÁMOGATÓK

abbvie

ACÉLLABOR

AKRONOM

ANIMA LAB
animal facility and laboratory equipment • animal research models

AURO-SCIENCE CONSULTING

BIOCENTER

Bio kasztel
ÉLVONAL SZEMÉLYRE SZABVA

BIOMEDICA

CEBIO
Central European Biosystems

noli turbare circulos meos

DIALAB

greiner
BIO-ONE

INNOVO

JK
Joó Katalin

nanosurf

MERCK

perform

RICHTER GEDEON

SARSTEDT

LNICAM
Magyarország Kft.

AmerisourceBergen
World Courier