PROGRAMME

ORAL & POSTER ABSTRACTS

Conference on Pharmacology, Pharmacokinetics & Innovation

HUPHAR 2024

MATRAHAZA **5-7, JUNE**

HUPHAR
Hungarian Society for Experimental and Clinical Pharmacology

HCEMM
Translational Medicine

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 HPV generátorok és rendszerek és fertőtlenítő kamra
3. ***Laborállat forgalmazás***
   - ENVIGO laborállatok forgalmazása
4. ***Labortechnológiai berendezések***
   - laborállat altatógépek (gáz vagy folyadék alapú), lélegeztető gépek, eutanázia berendezések
   - szövetbank, plazma és gyors fagyasztók -86ºC-ig, vérlemezke inkubátorok és keverők
   - laborhűtők +4ºC, kombinált labor hűtő-fagyasztók +4ºC/-20 ºC
   - plazma és őssejt kiolvasztók
   - laboratóriumi jégkészítők, hordozható hűtő-fagyasztó dobozok, inkubátorok széles választéka
   - klimatizált kamrák, szárító szekrények +130ºC-tól +300ºC-ig
   - 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***

WELCOME

Dear Colleagues,

On behalf of the organizing committee, we are pleased to inform you and all interested colleagues that the Hungarian Society of Experimental and Clinical Pharmacology (HUPHAR) will organize the HUPHAR2024 – Pharmacology, Pharmacokineics & Innovation conference in Mátraháza, Hungary, between 5-7 June 2024.

We will pay special attention to young researchers: provide great opportunities to present and discuss their results. Besides the outstanding scientific program, there will be recreational and social activities too.

The main topics of the conference are:

- Oncopharmacology
- Gastrointestinal pharmacology
- Analytical tools in drug development
- New therapies for inflammatory, cardiovascular and rheumatological diseases
- Novelties in drug innovations
- Neuropharmacology
- Extracellular vesicle-based pharmacology
- Key questions in translational pharmacology
- Personalized medicine
- In vitro – in vivo models, new tools

Information on Gyoftex/ Oftex accreditation scores for participant with registration numbers will be provided later.
We sincerely hope to see you again in 2024 for an outstanding, enjoyable and memorable meeting.

ORGANIZER OF THE CONFERENCE:

HUNGARIAN SOCIETY FOR EXPERIMENTAL AND CLINICAL PHARMACOLOGY

CO- ORGANIZER OF THE CONFERENCE:

HUNGARIAN CENTRE OF EXCELLENCE FOR MOLECULAR MEDICINE

CONFERENCE CHAIRS:

Katalin Monostory (HUN-REN Research Centre for Natural Sciences)
Zsuzsanna Helyes (Secretary General of HUPHAR, University of Pécs)
Péter Ferdinandy (President of HUPHAR, Semmelweis University)

ORGANIZING COMMITTEE

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Éva Borbély (University of Pécs)
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Dinnyés András (BioTalentum Ltd.)
Péter Hegyi (Semmelweis University)
Gábor Varga (Semmelweis University)

VENUE OF THE CONFERENCE

Mátraháza
HOTEL ÓZON & LUXURY VILLAS**** superior

TECHNICAL ORGANISER OF THE EVENT

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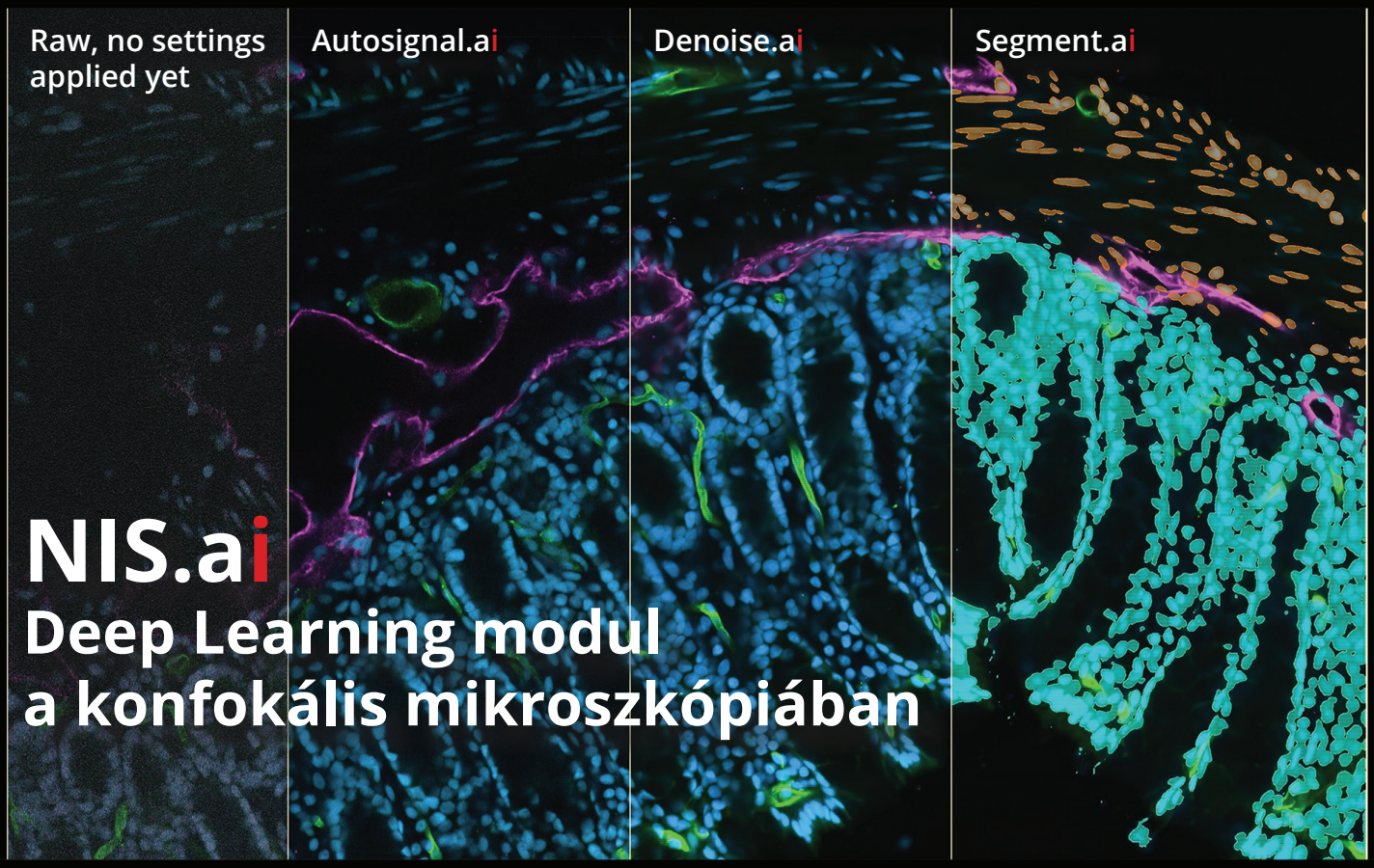
- Szuperrezolúciós 3D konfokális képalkotás



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GENERAL INFORMATION

Onsite Registration

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

5th June 2024, 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 is English. 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, Foyer on the 1st floor and Galya Room.*

Posters should be exhibited until 12.00 on Wednesday 5th June and kept outside until 5:00pm on Friday 7th 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 7th June 2024 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, Foyer of Kékes Room and Foyer on the 2nd floor*

Lunches and Dinners

*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 6th and 7th June at 7:00 am, meeting at the reception of HOTEL ÓZON & LUXURY VILLAS****.*
Thursday, 7th June 2024, 20:00 pm, Mátra Restaurant: **Gala dinner.**
Party after dinner will be in the Music Lounge.
Saturday, 9th June 2024, **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 lectures: **K***- Kékes Room* **KA***- Kékes Room A,* **KB***- Kékes Room B,* **KC***-Kékes Room C*
Numbers:
1st number: number of the session within a day
2nd number: serial number within a session

ALTAGRA 25

Az Altagrában idén jubileumot ünnepelünk! Büszkék vagyunk az elmúlt 25 év munkájára, a sikeres rendezvényeinkre és a sok kedves visszatérő partnerünkre!

Örömmel állunk megbízóink rendelkezésére a következő negyedszázadban is!

Altagra Szervező és Utazási Iroda Kft.

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GREINER – morning 5K run Mátraháza

Come on and run with us on 6 and 7 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!

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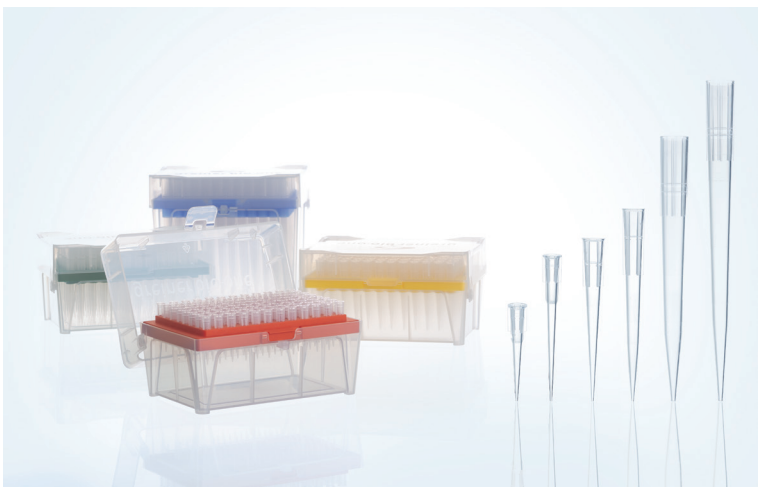

További információért keresse kollégáinkat a Greiner standon vagy az alábbi elérhetőségeken:

Kelet-Magyarország:
Merva László
+36 30 321 5190
laszlo.merva@gbo.com

Nyugat-Magyarország és Budapest:
Dr. Jancsó András
+36 30 498 9689
andras.jancso@gbo.com

Exhibitor game for the participants on HUPHAR 2024 – Pharmacology, Pharmacokinetics & Innovation Conference

Dear Participant,

Three high-value gift packages wait for their owners, which will be raffled at the Gala dinner on 6th June, among the participants who participate in the announced prize draw. The prize draw takes place as follows:

When visiting the exhibition, you need to collect stamps/signature from the representative of each company at each stand. Anyone who obtains the necessary stamp/signatura at all stands and, based on the information gathered during the stand visit, correctly answers the questions concerning the exhibitors will participate in the draw. Printed forms are available at the exhibition area and at the registration desk. Completed forms needs to be handle to the registration staff by 5:30 p.m. on Thursday at the Conference registration desk.

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

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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: Cellink, Cytena, Dispendix

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

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- Nitrogén, hidrogén és zéró levegő generátorok

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PROGRAMME

Conference on Pharmacology, Pharmacokinetics & Innovation

HUPHAR 2024

MATRAHAZA 5-7, JUNE

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June 5, 2024 Wednesday

10:00		**Registration – Hotel Lobby**
12:00-13:00		**Lunch - Mátra Restaurant**
13:00-13:45		**Opening of the Conference** **Issekutz Presentation and Award Ceremony** **Knoll-Szolcsányi Award Ceremony** **Award ceremony of the Young Pharmacologist Researcher competition**
13:45-14:15	**W-K1**	**KEYNOTE 1 - András Perczel - Sponsored by Auro-Science Consulting Ltd.** Laboratory of Structural Chemistry and Biology, Institute of Chemistry, Eötvös Loránd University, Budapest, Hungary *A drug-drug interaction (valproate and a carbapenem) explained by the first cryo-EM determined 3D structures of the mammalian acylaminoacyl peptidase*
14:15-15:45	**W-K2**	**Epigenetics and human disease** Chairs: Gábor Szabó, Lóránt Székvölgyi
	W-K2-1	[1]Viktória Tisza, [2]Laura Vízkeleti, [1]Csaba Kiss, [1]**Sándor Spisák 15'+3'** [1]HUN-REN TTK, Institute of Molecular Life Science, Epigenetics and Genome Editing Research Group, Budapest, Hungary [2]Semmelweis University, Department of Bioinformatics, Budapest, Hungary *Identification of Epigenetic Mechanisms Influencing Cell Differentiation Block in Colorectal Cancer Development*
	W-K2-2	[1]**Dalma Müller**, [1,2]Balázs Győrffy **15'+3'** [1]Semmelweis University, Department of Bioinformatics, Budapest, Hungary [2]TTK Oncology Biomarker Research Group, Budapest, Hungary *EpigenPlot: a tool for the gene-level methylation analysis of colorectal tumors*
	W-K2-3	[1]Márton Dániel Tóth, [1]Muhyiddeen Muazu, [2]Dóra Kővári, [2]Andrea Kadar, [3]Tamatey Virgil, [1]Mária Ashaber, [4]Klára Lévay, [5]András Budai, [4]András Fülöp, [2]Csaba Fekete, [3]Flora Szeri, [1,3]**Tamás Arányi 15'+3'** [1]Department of Molecular Biology, Semmelweis University, Budapest, Hungary [2]Institute of Experimental Medicine, Budapest, Hungary [3]Research Centre for Natural Sciences, Institute of Molecular Life Sciences, HUN_REN, Budapest, Hungary [4]Department of Surgery, Transplantation and Interventional Gastroenterology, Semmelweis University, Budapest, Hungary [5]2nd Department of Pathology, Semmelweis University, Budapest, Hungary

Hepatocyte-specific Dnmt3a and DNMT3b mice develop pre-cancerous phenotype

W-K2-4 **[1]Gábor Szabó**, [1]Péter Nánási, [1]László Imre **15'+3'**

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

Nucleosomes: old and new pharmacological targets

W-K2-5 **[1]Lóránt Székvölgyi 15'+3'**

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

The role of one-carbon metabolism in R-loop-associated transcriptional changes and mutagenesis

15:45-16:15 **Coffee break**

16:15-16:30 **W-K3** **KEYNOTE 2- Igor Pongrac - Sponsored by Merck Life Science Ltd. 15'**

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16:30-17:15 **W-K4** **PLENARY LECTURE - Sir Mark Caulfield 45'**

Faculty of Medicine and Dentistry, Queen Mary University of London, UK

Transforming Pharmacogenomics in Healthcare

17:15-17:25 **Break**

17:25-19:15 **W-K6** **Young Investigator Session**

Chairs: Éva Szőke, Zoltán Varga

W-K6-1 **Barbara Takács**, Wachal Zita, Szilágyi Anna, Szabó Adrienn Mónika, Priksz Dániel, Bombicz Mariann, Pelles-Taskó Beáta, Juhász Béla, Szilvássy Zoltán, Varga Balázs **10+5'**

Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecen

Key findings from bgp-15 treatment on retinal function improvement in zdf and sprague-dawley rat models

W-K6-2 **[1,2]Márton Kocsis,** [1,2] Sayour Viktor Nabil, [1,2]Tóth Viktória, [1,2]Gergely Tamás, [1,2]Kovács Tamás, [1,2]Szabó Lilla, [1,2]Varga Zoltán **10+5'**

[1]Semmelweis University, Budapest
[2]HCEMM, Szeged

Thymic modulation of immune checkpoint inhibitor-induced cardiotoxicity: new perspectives in immunotherapy

W-K6-3 **Angelika Bodó**[1,2,3] Bali ZK[1,3], Bruszt N[1,2,3,4], Reisinger Cs[1,3,4], Hernadi I[1,2,3,4] **10+5'**

[1]Translational Neuroscience Research Group, Grastyán Translational Research Centre, [2]Medical School [3]Szentágothai Research Centre, [4]Institute of Biology, Faculty of Sciences, University of Pécs, Pécs, Hungary

A pilot study using dreadd technology to develop a novel model of cognitive impairment

W-K6-4 [1,2]**Zita Képes,** [1,2]Csaba Csikos, [1]Fekete Anikó, [3]Vágner Adrienn, [3]Nagy Gábor, [1,4]Gyuricza Barbara, [1,5]Arató Viktória, [6]Kárpáti Levente, [7]Mándity István, [8]Bruchertseifer Frank, [9]Halmos Gábor, [1]Szikra Dezső, [1,2]Trencsényi György **10+5'**

University of Debrecen

[1]Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, Faculty of Medicine, University of Debrecen, Nagyerdei St. 98, H-4032 Debrecen, Hungary.
[2]Gyula Petrányi Doctoral School of Clinical Immunology and Allergology, Faculty of Medicine, University of Debrecen, Nagyerdei St. 98, H-4032 Debrecen, Hungary.
[3]Scanomed Ltd., Debrecen, Nagyerdei St. 98, H-4032 Debrecen, Hungary.
[4]Doctoral School of Chemistry, Faculty of Science and Technology, University of Debrecen, Egyetem square 1, H-4032 Debrecen, Hungary.
[5]Doctoral School of Pharmaceutical Sciences, University of Debrecen, Nagyerdei St. 98, H-4032 Debrecen, Hungary.
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[8]European Commission, Joint Research Centre (JRC), Karlsruhe, Germany.
[9]Department of Biopharmacy, Faculty of Pharmacy, University of Debrecen, Nagyerdei St. 98, H-4032 Debrecen, Hungary.

Evaluation of the therapeutic efficacy of ^{213}bi-labelled dota-conjugated alpha-melanocyte stimulating hormone peptide analogues in melanocortin-1 receptor positive preclinical melanoma model

W-K6-5 [1,2]**Szonja Anna Kovács,** [3]Kovács Tamás, [3]Hegedűs Zsombor, [3]Paál Ágnes, [1,2]Fekete János Tibor, [3]Varga Zoltán, [1,2]Győrffy Balázs **10+5'**

[1]Semmelweis Egyetem, Bioinformatika Tanszék, Budapest
[2]HUN-REN Természettudományi Kutatóközpont, Enzimológiai Intézet, Budapest
[3]Semmelweis Egyetem, Farmakológiai és Farmakoterápiás Intézet, Budapest

Yap1 inhibitor verteporfin potentiates the effects of anti-pd-1 immunotherapy in melanoma

W-K6-6 **Emese Ritter**[1], Kata Csekő[1,2], Péter Mátyus[5,6], Ágnes Kemény[1], András Garami[7], Eszter Pákai[7], Zsuzsanna Helyes[1,2,3,4] **10+5'**

University of Pécs

[1]Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School
[2]National Laboratory for Drug Research and Development, Budapest; [3]HUNREN-PTE Chronic Pain Research Group; [4]PharmInVivo Hungary Ltd, Pécs; [5]Veterinary University, Budapest; [6]E-Group Ltd, Budapest; [7]Department of Translational Medicine, University of Pécs, Medical School

The novel multi-target drug candidate szv-1287 inhibits inflammatory lung function alterations in the optimized endotoxin-induced acute pneumonitis mouse model

W-K6-7 **Brigitta Bernát,** Vécsei Vencel, Garami Gréta, Bombicz Mariann, Tarjányi Vera, Óvári Ignác, Szilvássy Zoltán, Juhász Béla, Priksz Dániel **10+5'**

Department of Pharmacology and Pharmacotherapy, Debrecen

Investigation of the cardiac effects of ertugliflozin utilizing high-resolution echocardiography in SHR rat model

19:30-21:00 **Dinner - Mátra Restaurant**

June 6, 2024 Thursday

8:00		**Registration- Hotel Lobby**
9:00-10:00	**T-KA**	**PLENARY LECTURE Thomas Wieland 60'** Medical Faculty, Mannheim Heidelberg University, Germany *New Avenues to Increase Cardiac Contractility in Heart Failure*
10:00-10:30		**Coffee break**
10:45-12:30	**T-KA1**	**Fusion Pharmacology: cardio-inflammation in focus** Chairs: Clive Page, Soraia Costa
	T-KA1-1	**Gilberto De Nucci 50'** Faculty of Medical Sciences Unicamp (Campinas-SP, Brazil) *Pharmacological Actions of Novel Endogenous Catecholamines*
	T-KA1-2	**Aisah A Aubdool,**[1] Kristen J Bubb, PhD,[1,2] Amie J Moyes, PhD,[1] Sarah Lewis, MD,[3] Jonathan P Drayton, MD,[1]Owen Tang, PhD,[2] Vedanta Mehta, PhD,[4] Ian C Zachary, PhD,[4] David J Abraham, PhD,[3] Janice Tsui, MD,[3] and Adrian J Hobbs, PhD[1,] **30'** [1]William Harvey Research Institute, Barts & The London School of Medicine & Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK [2]University of Sydney, Kolling Institute of Medical Research, St Leonards, 2065, Australia [3]Centre for Rheumatology and Connective Tissue Diseases, University College London Medical School, Royal Free Campus, London, NW3 2PF, UK [4]Centre for Cardiovascular Biology and Medicine, Division of Medicine, The Rayne Building, University College London, London WC1E 6JJ, UK *Endothelium-derived C-type natriuretic peptide is a critical regulator of angiogenesis and vascular remodelling*
	T-KA1-3	[1,2]**Elizabeth S. Fernandes**, [2]Liziane C. M. da Silva, [3]Catielen P. Pavi, [3]Beatriz P. Savi, [4]Seigo Nagashima, [5]Samara Damasceno, [5]Ayda H. Schneider, [3]Izabella T. Silva, [3]Gislaine Fongaro, [6]Maria R. Q. Bomfim, [7]Adara Aurea, [8]Sérgio J. Macedo Júnior, [9]João Valente, [5]Thiago M. Cunha, [4]Lucia de Noronha, [7]Joao B. Calixto, [9]Susan D. Brain **30'**

[1]Faculdades Pequeno Príncipe, Curitiba, Brazil
[2] Instituto de Pesquisa Pelé Pequeno Príncipe, Curitiba, Brazil
[3]Universidade Federal de Santa Catarina, Florianópolis, Brazil
[4]Pontifícia Universidade Católica Paraná, Curitiba, Brazil
[5]Universidade de São Paulo, Ribeirão Preto, Brazi[l]
[6]Universidade CEUMA, São Luis, Brazil
[7]Centro de Inovação e Ensaios Pré-clínicos, Florianópolis, Brazil
[8]Universidade Federal do Paraná, Curitiba, Brazil
[9]King´s College London, London, UK

Unveiling the mechanisms of Chikungunya-induced pain

10:30-12:30 **T-KB1** **Opioid research: past, present and future**

Chairs: Mahmoud Al-Khrasani, Pál Riba

T-KB1-1 **[1]Michael Schaefer,** [1]Mohammed Shaqura, [1]Shaaban Mousa **15'**

[1]Dep. of Anaesthesiology, Campus Benjamin Franklin, Charite University Berlin, Hindenburgdamm 30, 12203 Berlin, Germany

Distinct expression and functional profile of Mu-, Delta-, and Kappa-Opioid receptors in human dorsal root ganglia

T-KB1-2 **[1]Carmela Parenti**, [2]Simona Denaro, [2]Nunzio Vicario, [2]Rosalba Parenti, [1]Rita Turnaturi, [1,2]Annamaria Fidilio, [1]Margherita Grasso, [1]Lorella Pasquinucci **15'**

[1]Department of Drug and Health Sciences, University of Catania, Italy
[2]Department of Biomedical and Biotechnological Sciences, University of Catania, Italy

Dual-target ligands and pain: our experience

T-KB1-3 **[1]Shaaban Mousa**, [1]Mohammed Shaqura, [1]Michael Schaefer **15'**

[1]Dep. of Anaesthesiology, Campus Benjamin Franklin, Charite University Berlin, Hindenburgdamm 30, 12203 Berlin, Germany

Peripheral analgesic effects of opioids for painful diabetic neuropathy

T-KB1-4 **[1]Sanzio Candeletti**, [1]Laura Rullo, [1]Camilla Morosini, [1]Loredana M. Losapio, [1]Antonio Lacorte, [1]Marco Cristani, [1]Patrizia Romualdi **15'**

[1]Dept. of Pharmacy and Biotechnology, Alma Mater Studiorum – University of Bologna, Bologna, Italy

The affective component of chronic pain and the opioid system

T-KB1-5 **[1]Patrizia Romualdi**, [1]Laura Rullo, [1]Camilla Morosini, [1]Loredana M. Losapio, [1]Antonio Lacorte, [1]Marco Cristani, [1]Sanzio Candeletti **15'**

[1]Dept. of Pharmacy and Biotechnology, Alma Mater Studiorum-University of Bologna, Bologna, Italy

Opioids in chronic pain treatment and risk of OUD

T-KB1-6 **Tibor Soós** **15'**

HUN-REN Research Centre for Natural Sciences, Institute of Organic Chemistry, Budapest

Dawn of a novel pain treatment: Synthesis and structural plasticity of the most potent atypical opioid kratom alkaloid

T-KB1-7 [1]**Al-Khrasani Mahmoud**, [1]Galambos Anna Rita, [1]Karádi Dávid Á., [1]Nariman Essmat, [1]Sarah K. Abbood, [1]Király Kornél, [2]Lakatos Péter P., [1,4]Zádor Ferenc, [3]Köles László, [2]Tábi Tamás, [1]Riba Pál, [1]Ifj. Hársing G. László, [1]Fürst Susanna **15'**

[1] Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, Semmelweis University, Nagyvárad tér 4, H-1445 Budapest, Hungary
[2] Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Nagyvárad tér 4, Budapest, Hungary
[3] Department of Oral Biology, Semmelweis University, H-1089 Budapest, Hungary
[4] Pharmacological and Drug Safety Research, Gedeon Richter Plc, H-1475 Budapest, Hungary

Glycine transporter 1 and AT1 receptor inhibitors: novel strategies to decrease morphine analgesic tolerance

10:30-12:30 **T-KC1** **Beyond technicality: analytical science as an attitude**

Chairs: Csaba Szántay, Pál Szabó

Chairmen's introductory remarks 10'

T-KC1-1 **Zoltán Béni 20'**

Spectroscopic Research Department, Gedeon Richter Plc.

NMR at the frontier: structure elucidation of mysterious trace components

T-KC1-2 **Pál Szabó 10'**

HUN-REN Research Centre for Natural Sciences, Budapest

The role of high resolution mass spectrometry in the identification of 5-F-cumylpegaclon metabolites

T-KC1-3 **László Valkai 20'**

In vitro Metabolism Laboratory, Gedeon Richter Plc, Budapest

Behind the scenes: light absorption-based detection on in vitro ADME test samples

T-KC1-4 [1]**Tibor Renkecz**, [1,2,3]Aliz Széles, [1]Károly Schöll, [1]Ilona Pasics, [4]Scopchanova Sirma, [1]Gábor Hirka, [2]Katalin Monostory **20'**

[1]Toxi-Coop Toxicological Research Center, Budapest,
[2]HUN-REN Research Center for Natural Sciences, Budapest,
[3]Semmelweis University, Budapest,
[4]SCC Scientific Consulting Company, Bad Kreuznach

Different derivatization approaches to enable toxicokinetic analysis of curious analytes

Panel discussion 30'

12:30-13:30 **Lunch - Mátra Restaurant**

13:30-14:00 **T-KA2** **KEYNOTE 4 - Sönke Behrends 30'**

[1]Pharmacology, University of Braunschweig, Germany
[2]Semmelweiss University Budapest, Asklepios Campus Hamburg, Germany

Precision pharmacology: targeting enzyme isoforms for tailored therapeutics

13:30-14:00 **T-KB2** **KEYNOTE 5 - Soraia Costa 30'**

[1]Jorge L Dallazen, PhD, [1]Larissa G Santos, MSc, [1]Simone A Teixeira, PhD, [2]John Wallace, PhD, [1]Marcelo N Muscará, PhD, **[1]Soraia K P Costa, PhD.**

[1]Departamento de Farmacologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof Lineu Prestes, 1524 São Paulo/SP, 05508-000, Brazil
[2]Department of Physiology and Pharmacology, University of Calgary, Calgary, AB, T2N 1N4, Canada.

Exploring opportunities and challenges of hydrogen sulfide-releasing non-steroidal anti-inflammatory drugs for effective pain control and gastric integrity

14:00-15:30 **T-KA3** **Novel approaches to treat gastrointestinal and pancreatic diseases**

Chairs: Zoltán Zádori, József Maléth

T-KA3-1 **[1,2,9]Viktória Venglovecz**, [2,3]Anna Grassalkovich, [2,3,4]Emese Tóth, [1]Attila Ébert, [1]Eleonóra Gál, [1]Marietta Margaréta Korsós, [3,5,6]József Maléth, [7]Zoltán Rakonczay Jr., [8]Zsolt Galla, [8]Péter Monostori, [2,9,10,11]Péter Hegyi **15+3'**

[1]Department of Pharmacology and Pharmacotherapy, University of Szeged, Hungary,
[2]Translational Pancreatology Research Group, Interdisciplinary Center of Excellence for Research Development and Innovation, University of Szeged, Hungary,
[3]Department of Medicine, University of Szeged, Hungary,
[4]Department of Health Sciences, Department of Theoretical and Integrative Health Sciences, University of Debrecen, Hungary,
[5]HCEMM–SZTE Molecular Gastroenterology Research Group, University of Szeged, Hungary,
[6]ELKH–USZ Momentum Epithelial Cell Signaling and Secretion Research Group, University of Szeged, Hungary,

[7]Department of Pathophysiology, University of Szeged, Hungary,
[8]Metabolic and Newborn Screening Laboratory, Department of Paediatrics, University of Szeged, Hungary,
[9]Institute for Translational Medicine, Medical School, University of Pécs, Hungary,
[10]Centre for Translational Medicine, Semmelweis University, Hungary,
[11]Institute for Pancreatic Disorders, Semmelweis University, Hungary

Orkambi is a potential therapeutic option for acute pancreatitis

T-KA3-2 [1]**Eszter M. Horváth**, [1]Máté Bencsics, [1]Ke Haoran, [1]Bálint Bányai, [1]Roland Csépányi-Kömi, [1]Péter Sasvári, [2]Dantzer Françoise, [2]Hanini Najat, Rita Benkő **15+3'**

[1]Department of Physiology, Semmelweis University, Budapest, Hungary, [2]UMR7242, Biotechnology and Cell Signaling, CNRS/Université de Strasbourg, Strasbourg, France

T-cell specific PARP-2 downregulation in LPS induced inflammation of the large intestine

T-KA3-3 [1]**Zoltán S. Zádori**, [1,2]Barbara Hutka, [1]Arezoo Haghighi, [1]András S. Tóth, [1]Szilvia B. László, [1]Zsuzsanna Demeter, [1]Gerda Wachtl, [1]Klára Gyires **15+3'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary,
[2] Pharmacological and Drug Safety Research, Gedeon Richter Plc, Budapest, Hungary

Searching for new targets for treatment of NSAID enteropathy

T-KA3-4 [1]**Edyta Korbut**, [1]Głowacka Urszula, [1]Krukowska Kinga, [1,2]Wierdak Mateusz, [3]Vignane Thibaut, [1]Magierowska Katarzyna, [1,4]Bakalarz Dominik, [3]Filipovic Milos R, [1]Magierowski Marcin **15+3'**

[1]Department of Physiology, Jagiellonian University Medical College, Cracow, Poland,
[2]2nd Department of Surgery, Jagiellonian University Medical College, Cracow, Poland
[3]Leibniz-Institut für Analytische Wissenschaften-ISAS e.V. Dortmund, Germany
[4]Department of Forensic Toxicology, Institute of Forensic Research, Cracow, Poland

Controllable gaseous mediators delivery, pathway-specific proteins persulfidation and translational insights into Barrett's esophagus pathogenesis

T-KA3-5 [1,2]**József Maléth** **15+3'**

[1]Department of Medicine, University of Szeged, Szeged, Hungary; ELKH-USZ Momentum Epithelial Cell Signaling and Secretion Research Group, University of Szeged, Szeged, Hungary;
[2]HCEMM-USZ Molecular Gastroenterology Research Group, University of Szeged, Szeged, Hungary

Novel therapeutic targets in chronic pancreatitis: preclinical findings and translational possibilities

14:00-15:30 **T-KB3** **Advances in the pharmacotherapy of pain and inflammation**

Chairs: Valéria Tékus, Zsuzsanna Helyes, Peter Bai

T-KB3-1 Henrietta Papp[1,2,*], Judit Bóvári-Biri[3,*], Krisztina Bánfai[3,*], Tóth Emese[8,*], Péter Juhász[4], Mohamed Mahdi[5], Lilian Cristina Russo[6], Dávid Bajusz[7], Adrienn Sipos[8,9], László Petri[7], Tibor Viktor Szalai[7], Ágnes Kemény[2,10,11], Gyula Batta[12], Orsolya Mózner[13], Dorottya Vaskó[14], Edit Hirsch[14], Péter Bohus[15], Gábor Méhes[4], József Tőzsér[5], Nicola J. Curtin[16], Zsuzsanna Helyes[2,10], Attila Tóth[17], Nicolas C. Hoch[6], Ferenc Jakab[1,2], György M. Keserű[7], Judit E. Pongrácz[2], **Péter Bai[8,9,18,19]**
15+5'

[1]National Laboratory of Virology, University of Pécs, 7624, Pécs, Hungary and Institute of Biology, Faculty of Sciences, University of Pécs, 7624, Pécs, Hungary
[2]Szentagothai Research Centre, University of Pécs, 7624, Pécs, Hungary
[3]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, University of Pécs, 7624, Pécs, Hungary
[4]Department of Pathology, Faculty of Medicine, University of Debrecen, 4032, Debrecen, Hungary;
[5]Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen, 4032, Hungary;
[6]Department of Biochemistry, Institute of Chemistry, University of São Paulo, São Paulo, Brazil;
[7]Medicinal Chemistry Research Group, Research Centre for Natural Sciences, 1117, Budapest, Hungary
[8]Department of Medical Chemistry, Faculty of Medicine, University of Debrecen, 4032, Debrecen, Hungary;
[9]MTA-DE Cell Biology and Signaling Research Group ELKH, Debrecen, 4032, Hungary;
[10]Department of Pharmacology and Pharmacotherapy, Medical School; Centre for Neuroscience, 7624, Pécs, Hungary
[11]Department of Medical Biology, Medical School, Pécs, 7624, Hungary
[12]Department of Organic Chemistry, Faculty of Science and Technology, University of Debrecen, 4032, Debrecen, Hungary
[13]Doctoral School of Molecular Medicine, Semmelweis University, 1094, Budapest, Hungary and Institute of Enzymology, Research Centre for Natural Sciences, 1117, Budapest, Hungary
[14]Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, 1111, Budapest, Hungary
[15]Erzsébet Hospital, Sátoraljaújhely, 3980, Hungary
[16]Translational and Clinical Research Institute, Newcastle University Centre for Cancer, Faculty of Medical Sciences, Newcastle University, NE2 4HH, Newcastle upon Tyne, UK
[17]Section of Clinical Physiology, Department of Cardiology, University of Debrecen, Debrecen, 4032, Hungary;

Repurposing PARP inhibitors for treating COVID-19-related inflammation

T-KB3-2 **[1]Ivica Matak 15+5'**

[1]University of Zagreb School of Medicine, Zagreb

Antinociceptive action of botulinum toxin A and recombinant botulinum toxin-based molecules

T-KB3-3 **[1,2,3]Valéria Tékus**, [1,3,4]Nikolett Szentes, [1,3,4]Barbara Fülöp, [1]Jennet Pirkulyeva, [1,3]Éva Borbély, [5]Ádám Dénes, [6,7]Andreas Goebel, [1,3,4,8]Zsuzsanna Helyes **15+5'**

[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs, Hungary,
[2]Faculty of Health Sciences, Department of Laboratory Diagnostics, University of Pécs, H-7624, Pécs, Hungary;
[3]Hungarian Research Network, University of Pécs, Pécs, Hungary,
[4]National Laboratory for Drug Research and Development, Budapest, Hungary,
[5]Momentum Laboratory of Neuroimmunology, Institute of Experimental Medicine, Budapest, Hungary,
[6]Pain Research Institute, University of Liverpool, Liverpool, United Kingdom,
[7]Department of Pain Medicine, The Walton Centre National Health Service Foundation Trust, Liverpool, United Kingdom,
[8]PharmInVivo Ltd., Pécs, Hungary

Fractalkine (CX3CR1) and Interleukin-1 (IL-1) receptors mediate neuroinflammation and related hypersensitivity in mouse models of chronic primary pain

T-KB3-4 [1]Göntér Kitti, [2]László Szabolcs, [2]Wagner Ödön, [1]Pozsgai Gábor, [1]Pintér Erika, **[1]Zsófia Hajna 10+5'**

[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Pécs
[2]Department of Inorganic and Analytical Chemistry, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, Budapest

In vivo investigation of combined capsaicin-diclofenac containing transdermal patch in rat models of acute pain

T-KB3-5 **[1,3]Patrik Szekér**, [1]Anna Hajdara, [1]Gábor Rácz, [1]József Murányi, [1]Ágota Csóti, [1]Nikoletta Ngo Hahn, [1]Márton Megyeri, [1]Tamás Kitka, [1]Attila Brunyánszki, [2]Ágnes Kemény, [2]Erika Pintér, [2]Zsuzsanna Helyes, [4]György Panyi, [1]Sándor Farkas, [1]Zalán Péterfi, [1]Péter Hornyák, [3]Norbert Gyöngyösi **10+5'**

[1]VRG Therapeutics Ltd., Budapes,
[2]PharmInVivo Ltd., Pécs,
[3]Semmelweis University, Institute of Biochemistry and Molecular Biology Department of Molecular Biology, Budapest,
[4]Pharmion LP.,Debrecen

Developing selective KV1.3 inhibitors for the treatment of chronic inflammatory diseases

14:00-15:30 **T-KC3** **Heart failure - remodeling - seeking answers to open questions - From bench to bedside**

Chair: Przemysław Leszek

T-KC3-1 **Przemysław Leszek 5'**

Heart Failure and Transplantology Department; Mechanical Circulatory Support and Transplant Department, National Institute of Cardiology, Warsaw, Poland

Clinical need for rhythm optimization

T-KC3-2 **Michał Mączewski 10'**

Department of Clinical Physiology, Medical Centre of Postgraduate Education, Kiełpin, Poland

Experimental studies - what they suggest to clinicians

T-KC3-3 [1,2,3]**Zoltán V. Varga**, [1,2,3]Márk E. Jakab, [1,2,3]Al-Haddad R. Ayham, [1,2,3]Zsófia Onódi, [1,4]Péter Ferdinandy **7,5'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest,
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest,
[3]MTA-SE Momentum Cardio-oncology and Cardio-immunology Research Group, Budapest,
[4]Pharmahungary Group, Szeged, Hungary

Antidiabetic drugs repurposed for heart failure

T-KC3-4 [1,2,3]**Zsófia Onódi**, [1,4]Péter Ferdinandy, [1,2,3]Zoltán V. Varga **7,5'**

[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, Semmelweis University, Budapest, Hungary,
[4]Pharmahungary Group, Szeged, Hungary

Anti-gout medications repurposed for heart failure

T-KC3-5 **Przemysław Leszek 5'**

Heart Failure and Transplantology Department; Mechanical Circulatory Support and Transplant Department, National Institute of Cardiology, Warsaw, Poland

Iron deficiency - a clinician's perspective

T-KC3-6 **Aleksandra Paterek 10'**

Centre of Postgraduate Medical Education, Warsaw, Poland

What basic research teaches us about iron deficiency?

T-KC3-7 **Michał Mączewski 7,5'**

Department of Clinical Physiology, Medical Centre of Postgraduate Education, Kiełpin, Poland

Epicardial fat - how it affects the myocardium

T-KC3-8 **Aleksandra Paterek 7,5'**

Centre of Postgraduate Medical Education, Warsaw, Poland

Intramyocardial fat - a novel proarrhythmic factor

T-KC3-9 [1,2,3]**Tamás Kovács**, [1,2,3]Ágnes Paál, [1,2,3]Zsombor Hegedűs, [1,2,3]Lilla Szabó, [1,2,3]Zoltán Varga **7,5'**

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

Melanoma subtype-dependent cardiotoxicity to immune checkpoint inhibitor therapy

T-KC3-10 [1,2]**Zsombor I. Hegedűs**, [1,2,3]Gergely G. Tamás, [1,2,3]Tamás Kovács, [1,2,3]Zsófia Onódi, [4]Bálint Barta, [4]Sayour Alex Ali, [4]Tamás Radovits, [4]Béla Merkely, [1,5]Péter Ferdinandy, [1,2,3]Zoltán V. Varga **7,5'**

[1]Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest,
[2]HCEMM-SU Cardiometabolic Immunology Research Group, Budapest,
[3]MTA-SE Momentum Cardio-oncology and Cardio-immunology Research Group, Budapest,
[4]Heart and Vascular Center, Semmelweis University, Budapest, Hungary,
[5]Pharmahungary Group, Szeged, Hungary

Expression of key immune checkpoints in end-stage heart failure

15:30-15:50 **Coffee break**

15:50-17:50 **T-KA4** **Innovative models in pharmacological research**

Chair: Dóra Zelena

T-KA4-1 [1]Dávid Czimer, [1]Panna Kaluzsa, [2]Krisztina Fülöp, [2]Viola Pomozi, [2]András Váradi, **[1]Máté Varga 15+5'**

[1]Department of Genetics, ELTE Eötvös Loránd University, Budapest,
[2]Institute of Molecular Life Sciences, HUN-REN Research Centre for Natural Sciences, Budapest

From tank to bed in PXE? Using zebrafish to search for pseudoxanthoma elasticum treatments

T-K4-2 **[1]Zoltán Veréb 15+5'**

[1] Regenerative Medicine and Cellular Pharmacology Laboratory, Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary;

3D tissue printing in toxicology research

T-K4-3 István Fodor, Réka Svigruha, Éva Molnár, Tibor Kiss, and **Zsolt Pirger 15+5'**

[1]Ecophysiological and Environmental Toxicological Research Group, HUN-REN Balaton Limnological Research Institute, Tihany, 8237, Hungary

„Top-down" effects of psychoactive compounds on a defined simpler nervous system encoding associative memory

T-K4-4 **[1]Judit Hargitai 15+5'**

[1]Charles River Laboratories Hungary Kft, Veszprém, Hungary

The use of in vitro models in toxicology studies

T-K4-5 **[1]Dávid Szép**, [1]Ferenc Budán, [2]Kristóf Csepregi, [1]Kinga Dávid, [1]Bianka Pál-Dittrich, [1]Attila Sik **15+5'**

[1]University of Pécs, Medical School, Institute of Physiology, Pécs,
[2]University of Pécs, Faculty of Natural Sciences, Institute of Biology, Pécs

The power of zebrafish: swimming to success to find plant-based antiepileptic drug candidates

T-K4-6 **[1]Kornélia Szebényi 15+5'**

HUN-REN Research Centre for Natural Sciences, Budapest, Hungary

Human induced pluripotent stem cell-derived organoids for disease modeling

15:50-17:50 **T-KB4** **Novel findings of the TRP channel research by the Hungarian scientists**

Chair: Erika Pintér

T-KB4-1 **István Nagy 30'**

Imperial College London

TRPV1: back on the list of targets for analgesia

T-KB4-2 **András Garami 25'**

Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pecs, Pecs, Hungary

Mechanisms of the thermoregulatory effects of TRPV1 antagonists

T-KB4-3 *[1]Márk Racskó, [1,2]Árpád Kunka, [1,2] Judit Bohács, [1]Erika Lisztes, [2]Rita Marincsák,* **[1] Balázs István Tóth 20'**

[1]Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen,
[2]Faculty of Dentistry, University of Debrecen, Debrecen

Sensory TRP channels in the human dental pulp and their role in pulpitis

T-KB4-4 *[1]Erzsébet Kövesdi, [1]Laura Mundrucz, [7]Angela Kecskes, [1]Attila Gyéresi, [1]Máté Deák, [2]Balázs Gaszner, [4]Cecília Szekeres-Paraczky, [4]Zsófia Maglóczky, [3]Rudi Vennekens, [7]Viktória Kormos,* **[1]Miklós Kecskés 20'**

[1] *Institute of Physiology, Medical School, University of Pécs, H-7624, Pécs, Hungary,*
[2] *Department of Anatomy, Medical School and Research Group for Mood Disorders, Centre for Neuroscience, Szentágothai Research Centre, University of Pécs, H-7624, Pécs, Hungary ,*
[3] *Laboratory of Ion Channel Research, Biomedical Sciences Group, Department of Cellular and Molecular Medicine, VIB-KU Leuven Center for Brain & Disease Research, KU Leuven, 3000, Leuven, Belgium,*
[4] *Human Brain Research Laboratory, HUN-REN Institute of Experimental Medicine, H-1083 Budapest, Hungary,*
[7] *Department of Pharmacology and Pharmacotherapy, Centre for Neuroscience, Medical School, University of Pécs, H-7624, Pécs, Hungary*

TRPM4 in hilar mossy cells, a role in epilepsy

T-KB4-5 [1]**Péter Sántha,** [2]Ivett Kozma-Szeredi, [2]Orsolya Oszlács, Anett Somogyi and [1,2]Gábor Jancsó

[1]University of Szeged, Department of Anatomy, Histology and Embryology, Szeged
[2]University of Szeged and Department of Physiology

Different contributions of primary sensory neuron subpopulations to the initiation of nerve injury induced spinal microglia activation

15:50-17:50 **T-KC4** **Translational medicine leading to pharmacology applications**

Chair: Zoltán Varga

T-KC4-1 [1,2]**Eszter Farkas**, [1,2]Réka Tóth, [1,2]Anna Törteli, [3]Noémi Kovács, [4] Ildikó Horváth, [3,4]Domokos Máthé, [1,2]Ákos Menyhárt **15+5'**

[1]HCEMM-USZ Cerebral Blood Flow and Metabolism Research Group, HCEMM Nonprofit Ltd., Szeged,
[2]Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged,
[3]HCEMM-SU In Vivo Imaging Advanced Core Facility, Budapest,
[4]Department of Biophysics and Radiation Biology, Semmelweis University-Faculty of Medicine, Budapest

Modulation of Aquaporin-4 Expression by Trifluoperazine Augments Functional Recovery after Experimental Ischemic Stroke

T-KC4-2 [1]Kolos Nemes, [1]Alexandra Á. Benő, [1]Gabriella Mihalekné Fűr, [1,2]Éva Magó, [1]Petronella Topolcsányi, **[1]Lőrinc S. Pongor 15+5'**

[1]Cancer Genomics and Epigenetics Core Group, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary,
[2]Genome Integrity and DNA Repair Core Group, Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Szeged, Hungary

Predicting Drug Response Using Gene Expression Signatures in Cell Line Models

T-KC4-3 [1,2]**Tibor Pankotai 15+5'**

[1] Hungarian Centre of Excellence for Molecular Medicine (HCEMM), Genome Integrity and DNA Repair Core Group, University of Szeged, Szeged, Hungary,
[2] Department of Pathology, University of Szeged, Szeged, Hungary

The clinical significance of epigenetic, RNAPII and transcriptional variabilities occurring in clear cell renal cell carcinoma as a potential prognostic marker

T-KC4-4 **[1]Karri Lamsa 15+5'**

[1]Hungarian Centre of Excellence for Molecular Medicine Research Group for Human neuron physiology and therapy, Szeged, Hungary

How neurons in human brain are different from animal model cells, and why this is important?

T-KC4-5 **[1,2,3] Gábor M. Mórotz**, [1,2,3]Nabil V. Sayour, [1,2,3]Tamás G. Gergely, [1,2,3]Viktória É. Tóth, [1,2,3]Tamás Kovács, [1,2]Barnabás Váradi, [1,4,5]Bence Ágg, [1,4,5]Péter Ferdinandy, [1,2,3]Zoltán V. Varga **10+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]MTA-SE System Pharmacology Research Group, Budapest, [5]Pharmahungary Group, Szeged

Adrenal Inflammation in heart failure

T-KC4-6 [1,2]Viktor Szegedi, **[1]Ádám Tiszlavicz**, [1]Szabina Furdan, [1]Abdennour Douida, [1,2] Emoke Bakos, [3]Pal Barzo, [4]Gabor Tamas, [5] Attila Szucs, [1,2]Karri Lamsa **10+3'**

[1]Hungarian Centre of Excellence for Molecular Medicine Research Group for Human Neuron Physiology and Therapy, Szeged, Hungary,
[2]Department of Physiology, Anatomy and Neuroscience, University of Szeged, Hungary,
[3]Department of Neurosurgery, University of Szeged, Hungary,
[4]MTA-SZTE Research Group for Cortical Microcircuits, Department of Physiology, Anatomy and Neuroscience, University of Szeged, Hungary,
[5]Neuronal Cell Biology Research Group, Eötvös Loránd University, Budapest, Hungary

Aging-associated weakening of the action potential in fast-spiking interneurons in the human neocortex

17:50-18:20 **T-KA5** **KEYNOTE 6- BioTech Hungary Ltd. and GeneTiCa Ltd. 30'**

T-KA5-1 **Ágnes Angyal 10'**

GeneTiCA Ltd.

The power of multiomics

T-KA5-2 **Josef Uskoba 10'**

BioTech a.s.

		BioTech – A Key Partner in Structural Biology Solutions for a Pharmaceutical Industry
	T-KA5-3	**Péter Keresztúri 10'** BioTech Hungary Ltd. *Deeper insights into cell models with Agilent Cell Analysis instruments*
18:20-19:20		**POSTER SESSION- Galya Room on the 1st floor and Foyer on the 1st floor**
20:00		**Gala Dinner - Mátra Restaurant**
22:00		**Dance- Music Lounge**

June 7, 2024 Friday

8:00 **Registration- Hotel Lobby**

9:00-10:00 **F-KA** **PLENARY LECTURE -Wan-Wan-Lin 60'**

Department of Pharmacology, College of Medicine, National Taiwan University

Oxidative stress and cell death: Roles of PARP1 and AMPK

10:00-10:30 **Coffee break**

10:30-12:30 **F-KA1** **Novel innovative potentials of cyclodextrins in drug formulation and targeted pharmacotherapy**

Chairs: Éva Szőke, Éva Fenyvesi

F-KA1-1 [1]**Lajos Szente**, [1]Éva Fenyvesi **20+5'**

[1]CycloLab Ltd.

Antiviral therapies: Cyclodextrins in dual function

F-KA1-2 [1]**Rita Ambrus**, [1]Anett Motzwickler-Németh, [1]Patrícia Varga, [1]Csilla Balla-Bartos, [1]Ildikó Csóka **20+5'**

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

Application of cyclodextrin in traditional and alternative drug formulation; case studies

F-KA1-3 [1]Ágnes Rusznyák, [1]Csenge Urgyán, [1] Katalin Réti-Nagy, [2]István Hajdu, [2]György Trencsényi, [1]**Ferenc Fenyvesi 20+5'**

[1]Department of Molecular and Nanopharmaceutics, University of Debrecen, Debrecen,
[2]Division of Nuclear Medicine and Translational Imaging, Department of Medical Imaging, University of Debrecen, Debrecen

Targeting cancer cells by cyclodextrins via endocytosis

F-KA1-4 [1]**Levente Szőcs**, [1]Éva Fenyvesi **20+5'**

[1]CycloLab Cyclodextrin Research & Development Laboratory, Ltd. Budapest

Methylated cyclodextrins: understanding quality – bioactivity relationships

F-KA1-5 [1,2,3]**Andrea Nehr-Majoros**, [1,2,3]Maja Payrits, [1,2,3]Noémi Bencze, [1,4]Ágnes Kemény, [1,2,3,5]Zsuzsanna Helyes, [1,2,3,5]Éva Szőke **15+5'**

[1]Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Pécs, Pécs, [2]National Laboratory for Drug Research and Development, Budapest, [3]Centre for Neuroscience, University of Pécs, Pécs, [4]Department of Medical Biology, Faculty of Medicine, University of Pécs, Pécs, [5]HUN-REN PTE Chronic Pain Research Group, Pécs

Analgesia via lipid raft disruption by cyclodextrins

10:30-12:30 **F-KB1** **Searching for new therapies for neurodegenerative diseases**

Chairs: Erika Pintér and Anikó Borbás

F-KB1-1 **Sabina Podlewska**, Bugno Ryszard, Satała Grzegorz, Bojarski Andrzej J., Przewłocki Ryszard **15+5'**

Maj Institute of Pharmacology Polish Academy of Sciences, Smętna 12, 31-343 Kraków, Poland

Machine learning methods in the serve of new drugs development - case study of biased agonists of mu opioid receptor

F-KB1-2 **Jadwiga Handzlik 20+5'**

Department of Technology and Biotechnology of Drugs, Faculty of Pharmacy, Jagiellonian University, Medical College, Krakow, Poland

Chalcogen-containing 1,3,5-triazine compounds in search of breakthrough therapy for neurodegenerative diseases

F-KB1-3 [1]**Erika Pintér**, [1]Viktória Kormos, [1]Petra Prókay, [1]János Konkoly,[1]Maja Payrits, [1]Éva Borbély, [2]Balázs Gaszner, [3]Dóra Zelena **20+5'**

[1]Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, Hungary,
[2]Department of Anatomy, Medical School, University of Pécs, Hungary,
[3]Department of Physiology, Medical School, University of Pécs, Hungary

Could the TRPA1 be a promising target in the treatment of CNS diseases?

F-KB1-4 [1]**Dániel Priksz**, [2]Balázs Harangi, [3]Mária Lódi, [4]Zoltán Ujhelyi, [5]Dóra Ujvárosy, [1]Rita Erdélyi, [1]Brigitta Bernát, [1]Mariann Bombicz, [1]Vera Tarjányi, [1]Zoltán Szilvássy, [1]Béla Juhász **15+5'**

[1]Department of Pharmacology and Pharmacotherapy, University of Debrecen, Debrecen, Hungary, [2]Department of Data Science and Visualization, University of Debrecen, Debrecen, Hungary, [3]Department of Neuroanatomy and Molecular Brain Research, Ruhr University Bochum, Medical Faculty, Bochum, Germany, [4]Department of Pharmaceutical Technology, University of Debrecen, Debrecen, Hungary, [5]Department of Emergency Medicine, University of Debrecen Clinical Centre, Debrecen, Hungary

Assessment of the Effects of a Hydroxamic Acid Derivative Drug Candidate on Cognitive Function of Aged Rats

F-KB1-5 [1,2]Anna Anoir Abbas**,** [2]Jimoh Idris J., [3]Anikó Göblös, [4]Barker A. Roger, [3]Zoltán L. Veréb, [5]Johan Jakobsson, [1,3]Lajos Kemény, [2]Mária Judit Molnár, [1,2,5]**Karolina Pircs 10+5'**

[1] HCEMM, Szeged, [2] Semmelweis University, Budapest, [3] University of Szeged, Szeged, [4] University of Cambridge, Cambridge, [5] Lund University, Lund

Studying the effect of cariprazine in induced neurons directly reprogrammed from Huntington's disease patient's fibroblasts

F-KB1-6 [1]**Kinga Vörös**, [2]Dimitris Apostolopoulos, [1]Anna A. Abbas, [1]Danics Les, [2]Fazal Shaline, [2]Barker A. Roger, [1,3]Karolina Pircs **10+5'**

[1]HCEMM-Semmelweis University, Budapest, Hungary, [2]University of Cambridge, Cambridge, UK, [3]Lund University, Lund, Sweden.

Felodipine efficiency analysis on induced neurons derived from Huntington's disease FELL-HD clinical trial patients

10:30-12:30 **F-KC1** **Pharmaceutical Medicine Session**

Chairs: Kata Mazalin and Sandor Kerpel-Fronius

F-KC1-1 Anna Katalin Baráné Gilicze[1], **Viola Bardóczy[1] 24'**

National Center for Public Health and Pharmacy Department of Centralised Procedures and Biologicals

Quality requirements for biologicals in clinical trials

F-KC1-2 [1]**Sandor Kerpel-Fronius**, [2]Alexander L Becker **24'**

[1]Semmelweis University Department of Pharmacology and Pharmacotherapy
[2]Consultants in Pharmaceutical Medicine, Dover Heights, Australia

The value and importance of a professional ethical code for medicines development

F-KC1-3 **Krisztina Szabone Nemesy 24'**

National Center for Public Health and Pharmacy

Clinical trials in Hungary from the perspective of the competent authority

F-KC1-4 **Lilla Szabó 24'**

AstraZeneca Kft., Budapest

The role of medical affairs in pharma - focus on real world evidence generation

F-KC1-5 **Kata Mazalin 24'**

Boehringer Ingelheim RCV, Vienna

Modalities of early and supported patient access to medicines

12:30-13:30 **LUNCH - Mátra Restaurant**

13:30-14:00 **F-KA2** **KEYNOTE 8- Gábor Zacher 30'**

Albert Schweitzer Hospital, Hatvan

14:00-15:30 **F-KA3** **New concepts in cardiovascular pharmacology**

Chair: István Baczkó

F-KA3-1 [1]Attila Kiss **20'**

[1]Center for Biomedical Research and Translational Surgery, Medical University of Vienna, Vienna, Austria

Cardiovascular benefits of SGLT2i

F-KA3-2 **[1,2]Péter Bencsik**, [1,2]Tamara Szabados, [2,3]András Makkos, [2,3]Bettina Benczik, [2,3]Barnabás Váradi, [2,3]Bence Ágg, [3]Zoltán V. Varga, [1,2,3]Anikó Görbe, [2,3]Péter Ferdinandy **20'**

[1]Cardiovascular Research Group, Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Dóm tér 12, H-6720, Szeged, Hungary,
[2]Pharmahungary Group, Hajnóczy 6, H-6722, Szeged, Hungary,
[3]Cardiometabolic and MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Nagyvárad tér 4, 1089, Budapest, Hungary

Cardioprotection by exogenous microRNA-125b mimic in a mouse model of acute myocardial infarction*

F-KA3-3 **[1]Tibor Hornyik**, [2]Ilona Bodi, [2]Lea Mettke, [2]Konstantin Michaelides, [4]Stefan Meier, [3]Saranda Nimani, [2]Stefanie Perez-Feliz, [5]Ibrahim el-Battrawy, [6]Heiko Bugger, [2]Manfred Zehender, [7]Michael Brunner, [4,8]Jordi Heijman, [2,3]Katja E. Odening **20'**

[1]Department of Pharmacology and Pharmacotherapy, University of Szeged Albert Szent-Györgyi Medical School,
[2]Institute of Experimental Cardiovascular Medicine, Heart Center University of Freiburg, Medical Faculty, Freiburg, Germany;
[3]Translational Cardiology, Department of Cardiology, Inselspital, Bern University Hospital, and Department of Physiology, University of Bern, Bern, Switzerland,
[4] Department of Cardiology, Cardiovascular Research Institute Maastricht, Maastricht University and Maastricht University Medical Center, Maastricht, NL,
[5]First Department of Medicine, Faculty of Medicine, University Medical Centre Mannheim, University of Heidelberg;
[6]Department of Cardiology, University Heart Center Graz, Medical University of Graz, Graz, Austria;
[7]Department of Cardiology and Medical Intensive Care, St. Josefskrankenhaus, Freiburg, Germany.
[8]Gottfried Schatz Research Center, Division of Medical Physics and Biophysics, Medical University of Graz, Graz, Austria

Beneficial APD/QT normalizing effects of L-Carnitine in transgenic SQT1 rabbit model

F-KA3-4 **[1]Anikó Görbe**, [1]Zoltán Giricz, [1,2]Péter Ferdinandy **20'**

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

Hidden cardiotoxicity and cardioprotection: development of preclinical test platforms from in vitro to in vivo models

14:00-15:30 **F-KB3** **Pharmacological aspects of the neurovascular unit**

Chairs: Mária Deli, István Krizbai

F-KB3-1 **Ádám Dénes 20'**
HUN-REN Institute of Experimental Medicine
Role of microglia in modulation of cerebral circulation and neurovascular coupling

F-KB3-2 **Imola Wilhelm**, Kinga Molnár, Ádám Mészáros, Csilla Fazakas, István Krizbai **20'**
HUN-REN Biological Research Centre, Szeged
Targeting the brain metastatic environment

F-KB3-3 [1]**Szilvia Veszelka**, [1]Mária Mészáros, [1,2]Anikó Szecskó, [1,2]Gergő Porkoláb, [1]Koppány Párdi, [1]Janet Adegbite, [1] Mária Deli **20'**
[1]HUN-REN Biological Research Centre, Szeged,
[2]Doctoral School of Biology, University of Szeged, Szeged
Protection of brain endothelial cells as a therapeutic target in central nervous system diseases

F-KB3-4 [1]**Mária Mészáros**, [2]Thi Ha My Phan, [1,3]Judit P. Vigh, [1,3]Gergő Porkoláb, [1]Anna Kocsis,[1] Emese K. Páli, [1,4]Tamás F. Polgár, [1]Fruzsina R. Walter, [2]Jeng-Shiung Jan, [5]Tamás Janáky, [1]Szilvia Veszelka and [1]Mária A. Deli **15'**
[1]Institute of Biophysics, HUN-REN Biological Research Centre, Temesvári krt. 62, H-6726 Szeged, Hungary;
[2]Department of Chemical Engineering, National Cheng Kung University, Tainan 70101, Taiwan;
[3]Doctoral School of Biology, University of Szeged, Dugonics tér 13, H-6720 Szeged, Hungary;
[4]Theoretical Medicine Doctoral School, University of Szeged, Tisza Lajos krt. 97, H-6722 Szeged, Hungary;
[5]Department of Medical Chemistry, Albert Szent-Györgyi Medical School, University of Szeged, Dóm tér 8, H-6720 Szeged, Hungary
Alanine and glutathione targeting of dopamine- or ibuprofen-coupled polypeptide nanocarriers elevates crossing across the blood-brain barrier and protective effects

F-KB3-5 [1,2]**Szilvia Kecskés**, [1,2]Akos Menyhart, [1,2]Eszter Farkas **15'**
[1]HCEMM-USZ Cerebral Blood Flow and Metabolism Research Group, HCEMM Nonprofit Ltd., Szeged, Hungary,
[2]Department of Cell Biology and Molecular Medicine, University of Szeged, Szeged, Hungary
Dasatinib and Quercetin are protective in focal cerebral ischemia in aged rats

14:00-15:30 F-KC3 **The power of systematic reviews and meta-analyses in the translation of available clinical evidence and to initiate further research**
Chair: Gábor Varga

F-KC3-1 [1,2,3]**Péter Hegyi 40'**

[1]Centre for Translational Medicine, Semmelweis University, Budapest,
[2]Institute of Pancreatic Diseases, Semmelweis University, Budapest, Hungary,
[3]Institute for Translational Medicine, Medical School, University of Pécs, Pecs, Hungary

Translational Medicine – From bedside to bench and to bedside again

F-KC3-2 **[1,2]Dezső Csupor 25'**

[1]Institute of Clinical Pharmacy, University of Szeged, Szeged,
[2]Institute for Translational Medicine, University of Pécs, Pécs

TRANSLATIONAL MEDICINE – Clinical investigations of natural products initiated by meta-analyses

F-KC3-3 **[1,2]Gábor Varga 25'**

[1]Centre for Translational Medicine, Semmelweis University, Budapest,
[2]Department of Oral Biology, Semmelweis University, Budapest

Translational Medicine – From molecular physiology to meta-analyses to molecular pharmacology of epithelial ion transport and transport products

15:30-15:50 **Coffee break**

15:50-17:20 **F-KA4** **Cell and gene therapy – the way to clinical application**

Chairs: András Dinnyés, Péter Ferdinandy

F-KA4-1 **András Dinnyés[1,2,3]**, Anita Fehér[1], Suchitra Muenthaisong[1], Laura Colar Zanjko[1,3], Andrea Balogh[1], Kornél Kistamás[1], Krisztina Bánfai[1] **15+3'**

[1]BioTalentum Ltd., Gödöllő, [2]Department of Cell Biology and Molecular Medicine, USZ, [3]Department of Physiology and Animal Health, Institute of Physiology and Animal Nutrition, MATE, Gödöllő

Progress report on developments towards human cell and gene therapy and xenoorgan transplantation

F-KA4-2 **László Cervenak**, Kajdácsi Erika, Bihari György, Vadicsku Dorina, Kocsis Boglárka, Debreczeni Márta Lídia, Demeter Flóra **15+3'**

Cell Biology and Cell Therapy Group, Department of Internal Medicine and Haematology, Semmelweis University, Budapest

A multipurpose anti-inflammatory therapeutic agent: mesenchymal stem cells

F-KA4-3 **[1,2]Bence Ágg**, [1,2]Benczik Bettina, [1]Balogh Olivér, [1]Váczy-Földi Máté, [1]Bereczki Zoltán, [1]Pétervári Mátyás, [1,2]Ferdinandy Péter **15+3'**

[1]Cardiometabolic and HUN-REN-SU System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest,
[2]Pharmahungary Group, Szeged

Network theoretical and machine-learning-based analysis of the interactome for the development of oligonucleotide therapies in cardiovascular diseases

F-KA4-4 **Zoltán Veréb[1,2]**, Diána Szűcs[1,2], Tamás Monostori[1,2], Lajos Kemény[1,2,3] **15+3'**

[1] Regenerative Medicine and Cellular Pharmacology Laboratory, Department of Dermatology and Allergology, University of Szeged, Szeged, Hungary;
[2] Centre of Excellence for Interdisciplinary Research, Development and Innovation, University of Szeged, Szeged, Hungary
[3] Hungarian Centre of Excellence for Molecular Medicine-USz Skin Research Group, University of Szeged, Szeged, Hungary.

Pre-clinical development of a cell therapy product

F-KA4-5 **Péter Ferdinandy 15+3'**

MTA-SE System Pharmacology Research Group, Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; and Pharmahungary Group, Szeged, Hungary

Development of small non-coding RNA therapeutics: the example of protectomiR miR-450a mimic

15:50-17:20 **F-KB4** **Research relationships between industry and academia**

Chair: Viktor Román

F-KB4-1 **[1,2]Balazs Lendvai 20'**

[1]Gedeon Richter Plc, Pharmacological and Drug Safety Department, Budapest
[2]Department of Richter, Semmelweis University, Budapest

Ecosystem network around Gedeon Richter Plc.

F-KB4-2 **Zsolt Némethy 20'**

Gedeon Richter Plc., Laboratory of Systems Biology, Budapest

Optimization of novel α7 nicotinic acetylcholine receptor positive allosteric modulators and the discovery of a preclinical development candidate molecule

F-KB4-3 **Szilvia Benkő 20'**

Department of Physiology, Faculty of Medicine, University of Debrecen, Debrecen

Intracellular pattern recognition nod-like receptors (NLRs) in different macrophage subpopulations

F-KB4-4 **[1,2,3]István Hernádi**, [1,2]Anna Padányi, [1]Evelin Kiefer, [1]Antonietta Vitális-Kovács, [1]Rafaella M. Riszt, [1]Balázs Knakker **20'**

[1]Grastyán Translational Research Centre, University of Pécs – Gedeon Richter Plc., Hungary,
[2]Medical School, University of Pécs, Hungary,
[3]Institute of Biology, Faculty of Sciences, University of Pécs, Hungary

Development of a complex translational test battery for the investigation of cortical excitability in non-human primates

17:20-17:40 **F-KA5** **AWARDS CEREMONY- Young Investigators- Oral and Poster**
Closing of the Conference

Odyssey F: The Fastest Point Scanner on the Market

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LUMIPROBE és QBIT ASSAY KITEKKEL KOMPATIBILIS

SPECIFIKÁCIÓ:

Specifikáció	Paraméterek
Mintatérfogat	1-20ul
Mintakapacitás	1db
Cső kompatibilitás	0.5mL PCR cső
Tesztidő	≤5s/minta
Linearitási tartomány	5 nagyságrend
Fényforrás	LED
Gerjesztési hullámhossz	Kék 460-480nm, piros 630-650nm
Emissziós hullámhossz	Zöld 500-535nm, Piros 670-710nm
Érzékenység	dsDNS 0.01ng/ul
Kalibráció	2 vagy 3 pont
Kijelző	5 hüvelykes érintőképernyő

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sejtanyagcsere elemző

KROMAT

A Seahorse XF sejtanyagcsere elemző készülékek lehetővé teszik a különböző sejtek mitokondriális funkciójának és glikolízisének a vizsgálatát.

Kifejezetten hatóanyag vizsgálatokhoz javasolt.

- A tradicionális respirométerekhez képest sokkal nagyobb minta befogadási képességgel rendelkezik, melyek mellett 4 különböző hatóanyag is injektálható mérés közben.
- A Clark elektródához képest 10-20-szor kevesebb biológiai minta és felhasznált reagens / fogyóanyag szükséglet
- Kinetikus mérések egy készüléken belül
- Publikációkban megjelent felhasználási területek: glikolitikus ráta, az energiaköltség (például sejtes aktiváció, proliferáció, differenciálódás), valós idejű ATP termelési ráta, szubsztrát oxidáció, mitokondriális funkció, sejthalál, általános sejtes homeosztázis, immunológia, rák, elhízás, immuno-onkológia, gyógyszerkutatás, cukorbetegség, anyagcsere-betegségek, őssejtek, kardiovaszkuláris funkció, neurodegeneráció, virológia és öregedés.

Főbb alkalmazási területek

- Fenotípusos szűrés
- Számos feltétel egyidejű vizsgálata
- Dózis-hatás vizsgálatok
- OMICS funkcionális megerősítés
- Szferoidok

Bővebb információ

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ORAL ABSTRACTS

Conference on Pharmacology, Pharmacokinetics & Innovation

HUPHAR 2024

MATRAHAZA **5-7, JUNE**

WEDNESDAY
5 JUNE 2024

W-K1 A DRUG-DRUG INTERACTION (VALPROATE AND A CARBAPENEM) EXPLAINED BY THE FIRST CRYO-EM DETERMINED 3D STRUCTURES OF THE MAMMALIAN ACYLAMINOACYL PEPTIDASE

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The first high-resolution 3D structures of a mammalian acylaminoacyl peptidase (AAP) in free form and covalently complexed with meropenem have been determined by cryo-EM, showing that the serine protease is inhibited by a carbapenem antibiotic. Since AAP is an integrated modulator of the ubiquitin-proteasome degradation system, the revealed drug-drug interaction between a widely used antipsychotic (valproate) and a carbapenem antibiotic is of key importance. We found that Ser587, the key element of the catalytic serine protease triad of AAP, alternates between its active and latent states, manifested by an inserted Pro residue in a β-strand of the central β-sheet, providing sufficient conformational freedom to the segment containing the catalytic Ser587. The flexibility of the active site suggests that the dual function of catalysis and substrate selection is fulfilled by a mechanism that is novel among oligopeptidases. Substrate entry is regulated by flexible loops forming a double-gated channel system. Displacement and protonation of His707 of the catalytic triad prevents AAP from fully processing the antibiotic and thus meropenem remains covalently bound to Ser587.

We show that while carbapenems have sufficiently small substituents on their β-lactam rings to fit into the shallow substrate specificity pocket of the enzyme, AAP is susceptible to self-association due to its unusually protected active sites and flexible catalytic triads.

Self-association of the monomers results in the toroidal quaternary structure of the AAP tetramer, a process guided by an amyloidogenic β-edge. Using cryo-EM data, we are in the process of revealing the first amyloid-like tubular structure of AAP.

The pAAP structure will enable structure-based modeling and drug design targeting of human AAP, an upstream regulator of the ubiquitin-proteasome system.

W-K2-1 IDENTIFICATION OF EPIGENETIC MECHANISMS INFLUENCING CELL DIFFERENTIATION BLOCK IN COLORECTAL CANCER DEVELOPMENT

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Normal colon development relies heavily on cellular differentiation.
This process is maintained by the homeostatic balance of the BMP/Wnt signaling pathway. Canonical mutations in colorectal cancer (CRC), including Adenomatous polyposis coli (APC, occurring in 80% of CRC cases), disrupt this balance by inducing hyperactive Wnt signaling, which results in the expansion of a stem cell-like population. We developed a dual endogenous reporter system to effectively monitor stem cell-like behavior and cellular differentiation processes, enabling medium-high throughput genetic perturbation and drug screens. In my presentation, I will demonstrate the application areas of this reporter system and discuss possible intervention options to effectively restore cellular differentiation and specifically treat CRC.

W-K2-2 EPIGENPLOT: A TOOL FOR THE GENE-LEVEL METHYLATION ANALYSIS OF COLORECTAL TUMORS

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Introduction. Aberrant DNA methylation patterns are frequent in colorectal adenocarcinoma (CRC) tissues. The investigation of this layer of epigenetic regulation holds the promise of a better understanding of tumour progression and the identification of novel biomarker candidates.

Purpose. Our goal was to collect and analyse publicly available DNA methylation data of colorectal samples and create a user-friendly tool for exploring and visualizing the CRC methylome.

Methods. We used Illumina Infinium Methylation Arrays, which is a popular platform for genome-wide methylation analysis of large cohorts. Gene Expression Omnibus database (GEO) and TCGA-COAD (The Cancer Genome Atlas Colon Adenocarcinoma) datasets including normal colorectal mucosa, adenoma, and adenocarcinoma samples were systematically collected and filtered. Differentially methylated regions were identified using the Kruskal-Wallis test. Genes with significant methylation changes were further analysed using ROC (Receiver Operator Characteristic) analysis. Finally, we established a web application using the shiny R package.

Results. We assembled a database including methylation data of 2,586 samples. Adenoma and adenocarcinoma samples were globally hypomethylated. Hypermethylation ($p < 0.05$, $\Delta\beta >= 0.2$) in adenocarcinoma samples was predominantly present in regions near proximal promoters. Out of the 74 previously proposed biomarkers examined, 50 had a cross-validated AUC of over 0.8. FDA-approved biomarkers BMP3, NDRG4, and SEPT9 have shown the highest performance in the TSS200 region (cvAUC = 0.8, 0.83, and 0.76, respectively). Genes with the highest performance were ITGA4 (5'UTR, first exon: cvAUC = 0.9), MDFI (5'UTR: cvAUC = 0.9), CNRIP1 (5'UTR, first exon: cvAUC = 0.89). The established web application can visualize methylation at CpG sites and gene regions, and KEGG pathway genes. The platform is available at https://epigenplot.com.

Conclusion. We collected and analysed a sizeable database of colorectal tissue data. Gene regions with the best performance include previously proposed and novel diagnostic biomarker candidates. The web platform provides an tool for the efficient exploration of the assembled data..

W-K2-3 HEPATOCYTE-SPECIFIC DNMT3A AND DNMT3B MICE DEVELOP PRE-CANCEROUS PHENOTYPE

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DNA methylation and hydroxymethylation play major role in differentiation and maturation in various tissues. Although DNMT3b has been reported to play a protective role against liver fibrosis and hepatocellular carcinoma, our knowledge about the role of *de novo* DNA methyltransferases in postnatal liver maturation and during adulthood is limited. Our aim in this study was to characterize the phenotype of mice with the liver-specific elimination of the *de novo* DNA methylation apparatus.

Dnmt3a$^{Flox/Flox}$/*3b*$^{Flox/Flox}$ mice carrying Albumin-Cre (*Alb-Cre*) transgene were generated to abolish the expression of both *de novo* DNMTs (DKO) specifically in hepatocytes. Mice were viable without apparent phenotype, and the *Alb-Cre* transgene distribution followed the Mendelian rule. Since *Alb-Cre*$^{+/+}$ showed a more pronounced knockout effect than *Alb-Cre*$^{+/-}$, we used *Alb-Cre*$^{+/+}$ DKO mice in our experiments and compared them to their wildtype (*Alb-Cre*$^{-/-}$, WT) littermates. We investigated DNA methylation, gene expression, histone modifications, metabolic activity, and liver histology.

We observed only minor differences, in 3 weeks old young mice between DKO and WT animals. To test the effect of stress on livers of DKO mice, we performed partial hepatectomy followed by liver mass regeneration on 8-week-old mice, but no major difference was observed between DKO and WT animals suggesting that *de novo* methyltransferases are not involved in the regeneration process. DNA methylation and gene expression patterns have significantly changed during maturation and became prominent between 40 weeks (10months old, adult) and 3weeks old young mice. Significant DNA methylation increase was observed in WT mice, which was absent in DKO animals suggesting the primary role of *de novo* methyltransferases in methylation changes during ageing. We also observed histone modification alterations at 40 weeks of age. RNA-seq analysis revealed expression alterations of transcription factors and metabolic genes in the DKO mice. Finally, histologic analysis demonstrated that the 40 weeks old DKO mice underwent severe pathologic processes (portal fibrosis, small cell change, and necrosis) corresponding to precancerous state. In conclusion, our results show the involvement of *Dnmt3a/b* in epigenetic aging and the DKO presents a unique mouse model of hepatocellular precancerous state.

W-K2-4 NUCLEOSOMES: OLD AND NEW PHARMACOLOGICAL TARGETS

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Enzymes functioning in the cell nucleus, those playing crucial role in DNA repair and others contributing to epigenetic regulation, have entered clinical practice in a wide range of diseases, cancer in particular. Nucleosomes, composed of the canonical and also of variant histones, both categories carrying special combinations of posttranslational modifications as epigenetic marks, are generally perceived as structural components of lesser importance from the pharmacological perspective. By way of amending this notion, three lines of experimental evidence will be discussed in support of the view that nucleosomes themselves are to be considered among the important targets of drugs in the nucleus. We will demonstrate (a) such an aspect of the mechanism of action of anthracyclins; demonstrate (b) that specific interference with intermolecular interactions involving certain nucleosomes by peptides mimicking particular histone tails can lead to epigenetic modulation; and show (3) that inhibitors of certain histone modifying enzymes can modulate an all-important feature of nucleosomes, i.e. their stability.

Regarding (a), we will discuss data obtained with anthracyclins that demonstrate their nucleosome destabilizing effect at pharmacologically relevant concentrations what may contribute to their effects as well as side-effects; concerning (b) we will show that a nanopeptide corresponding to the C-terminal, unstructured tail of the histone variant H2A.Z can be introduced into live cells with conspicuous consequences for epigenetic regulation; and in the context of (c), an example of epigenetic modulation via inhibiting the KDM4a,b,c histone demethylases will be demonstrated.

Funding: OTKA 138524

References:
1. Pang et al., Nat Commun. 2013;4:1908
Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin.
2. Imre et al., Sci Rep. 2017 Oct 6;7(1):12734
Nucleosome stability measured in situ by automated quantitative imaging.

W-K2-5 THE ROLE OF ONE-CARBON METABOLISM IN R-LOOP-ASSOCIATED TRANSCRIPTIONAL CHANGES AND MUTAGENESIS

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The discovery of chromosomal R-loop structures, which act as genomic instability focal points in the genome, has brought significant progress in understanding the pathomechanism of human diseases. Uncontrolled R-loop formation poses a risk to the integrity of chromosomes, so it is crucial to identify the molecules that sense, bind and regulate R-loop structures. In my presentation, I present experimental approaches that enable the identification and characterization of R-loop regulatory genes (so-called R-loop regulators) in various human disease models. Based on our results, R-loop regulators can be potential epigenetic markers or therapeutic targets in malignant tumors associated with one-carbon metabolism.

W-K3 EXPERIENCE THE NEXT GENERATION MULTIPLEXING WITH LUMINEX® XMAP® INTELLIFLEX SYSTEM AND MILLIPLEX® MULTIPLEX ASSAYS

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[1] Merck Life Science | Science and Lab Solutions, Croatia

Multiplex assays are a type of immunoassay capable of simultaneous measurement of multiple analytes. Luminex® xMAP® technology combines advanced fluidics, optics, and digital signal processing with proprietary microsphere technology to deliver both high-density and high-throughput multiplexed assay capabilities at the same time.

We will introduce xMAP® INTELLIFLEX system, the latest addition to the Luminex® portfolio of instruments. It is a flow-based, multiplex platform that simplifies workflows by combining the established performance of xMAP® technology with advanced features to elevate assay performance and empower innovation in assay development. By combining low and high-plex capabilities, the xMAP® INTELLIFLEX system gives fast, reliable results.

We will also introduce MILLIPLEX® multiplex assays based on Luminex® xMAP® technology that offer applications throughout the life sciences and capabilities across a variety of bioassays. With over 1,000 analytes available for measurement of protein biomarkers and intracellular proteins, MILLIPLEX® assays represents the largest and fastest growing portfolio of multiplex biomarker assays.

W-K4 TRANSFORMING PHARMACOGENOMICS IN HEALTHCARE

Sir Mark Caulfield
Faculty of Medicine and Dentistry, Queen Mary University of London, UK

Pharmacogenomics is focused on understanding the consequence when our genome affects our response to medicines, which may manifest as medication inefficacy or harm. In the 100,000 Genomes Project we undertook whole genome sequencing for rare disease, cancer and infection but these genome sequences could also be used to identify known pharmacogenomic gene-drug pairs. Across 76,000 whole genomes we discovered that 99.5% harbour at least one recognised Clinical Pharmacogenetics International Consortium gene-drug pair. Importantly, 25% of the genes contained 4 gene drug pair haplotypes where harm or inefficacy may result. This was the case across all ancestries. Focusing on underserved communities in research we evaluated the frequency of known gene-drug pairs in 44,000 people from Genes&Health of British Bangladeshis and Pakistani ancestry. This revealed that variants in CYP2C19 that make it impossible to activate clopidogrel, a commonly prescribed antiplatelet therapy, in 57% of the Bangladeshi and Pakistani population compared with 30% frequency in white Europeans. This was associated with higher risk of recurrent myocardial infarction in those with the variant that doesn't activate clopidogrel. This underscores the need to understand the genetic architecture of pharmacogenomic variants in different ancestries to enable future healthcare to avoid harm from genome-drug interactions.

W-K6-1 KEY FINDINGS FROM BGP-15 TREATMENT ON RETINAL FUNCTION IMPROVEMENT IN ZDF AND SPRAGUE-DAWLEY RAT MODELS

Takács Barbara, Wachal Zita, Szilágyi Anna, Szabó Adrienn Mónika, Priksz Dániel, Bombicz Mariann, Pelles-Taskó Beáta, Juhász Béla, Szilvássy Zoltán, Varga Balázs

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Retinal vascular disorders and the ensuing metabolic disruptions in the eye are great concerns for healthcare systems. Many of the retinal disorders are characterized by ischemia-reperfusion injury in their early stages and these retinal changes can be diagnosed by electroretinographical testing (ERG). Test results can be utilized for early diagnosis and to monitor disease progression. We have tested a small molecule, BGP-15 [O-(3-piperidino-2-hydroxy-1-propyl) nicotinic amidoxime dihydrochloride] that appeared to be an ideal candidate to improve I/R injury based on its previously reported attributes. To address the question of whether systemic or topical administration is better, we administered the drug candidate orally and in the form of an eyedrop.

In our first study, the long-term tolerability and retinoprotective effect of BGP-15 were tested on male Zucker-Diabetic Fatty rats (fa/fa) for 52 weeks starting from 16 weeks of age. The treated group received 10 mg/kg BGP-15 in methyl-cellulose mucilage, while animals in the Lean and control ZDF groups were gavaged with vehicle only ($n = 10$ in each group). In the second study, we used male, 10-week-old Sprague-Dawley rats, and triggered I/R injury with the ligation of blood vessels: for 60 minutes, ocular ischemia was maintained, followed by a 7-day reperfusion period. During the reperfusion phase, BGP-15 was administered in an eyedrop formulation containing a solubility enhancer, sulfobutylether-β-cyclodextrin (SBECD) as well. BGP-15 in this case was administered twice a day at a dose of 100 mg/ml, eyedrops were dripped into the eye through a pipette tip using a manually adjustable pipette. The control group received vehicle only ($n = 10$ in each group).

The results of the first study imply that BGP-15 was a well-tolerated molecule and improved survival during long-term treatment in ZDF rats. The endpoint ERG revealed that scotopic a- and b-wave amplitudes were significantly higher in BGP-treated ZDF rats compared to the control ZDF animals ($p < 0.0001$). The Western blot analysis showed increased expression of HSP70 and decreased expression of NFkB in BGP-treated ZDF animals compared to control ZDF rats ($p < 0.05$). In the second experiment, we found that the topical application of BGP-15 treatment improved scotopic a- and b-waves and oscillatory potential amplitudes in the I/R group compared to the Control I/R group. The histological analysis showed that treatment significantly increased thickness in different retinal layers ($p< 0.0001$).

In summary, we demonstrated through animal models of retinal ischemia-reperfusion (I/R) injury that BGP-15 can be a promising asset to the treatment of acquired retinal vascular disorders in the future.

W-K6-2 THYMIC MODULATION OF IMMUNE CHECKPOINT INHIBITOR-INDUCED CARDIOTOXICITY: NEW PERSPECTIVES IN IMMUNOTHERAPY

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Immune checkpoint inhibitors (ICI) are monoclonal antibodies that enhance the anti-tumor activity of the immune system. However, this therapy could lead to immune overactivation, resulting in severe side effects associated with high mortality rates. Previously, we demonstrated that ICI increases the expression of inflammatory genes in the thymus, and furthermore in clinical studies, ICI therapy for thymoma treatment caused severe cardiotoxicity. Based on these findings, we hypothesized that thymus activity could play an important role in ICI-induced autoimmune side effects.

The main aim of our study was to investigate the correlation between thymus activity and the pathophysiological processes of immune-mediated cardiotoxic and systemic side effects during ICI therapy using a preclinical mouse model. Since the thymus gradually atrophies and thymic activity decreases with age, we examined the side effects of ICIs in two differently aged groups.

In our epxeriment, we treated young and aged mice with a PD-1 inhibitor administered intraperitoneally three times a week along with vehicle control. Furthermore, we repeated the experiment in a new group of young mice with the addition of azacitidine treatment, which causes artificial involution and inactivation of the thymus based on literature data. We analyzed cardiac functions using echocardiography, systemic morphological changes using histological examination, examined thymic gene expression changes using qrtPCR and RNA sequencing and analyzed the thymic tissue using flow cytometry.

Myocardial dysfunction was detected by echocardiography after 2 weeks of treatment in young animals, while ejection fraction remained preserved in aged and combined-azacitidine treated animals with fatty degenerated and atrophied thymus compared to the control groups. These results were validated by immunhistology, assessing oxidative stress markers in heart sections. Furthermore, we evaluated the most commonly affected organs histologically, such as the colon, lung, and kidney and detected significant changes in gene expression in the thymic tissue.

Based on our results, thymus immune response plays a central role in ICI-induced cardiotoxicity in mice. Thymus involution induced by treatment with ICIs in aged animals and combined immunotherapy with azacitidine significantly reduced unwanted immune-mediated side effects, indicating that the immune response following ICI treatment varies depending on thymus activity. Thus, modulation of thymus activity may serve as a therapeutic target in reducing immune-mediated side effects and also monitoring the morphology and activity may be an important factor in predicting the severity of side effects and identifying high-risk patients.

W-K6-3 A PILOT STUDY USING DREADD TECHNOLOGY TO DEVELOP A NOVEL MODEL OF COGNITIVE IMPAIRMENT

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Introduction: Designer Receptors Exclusively Activated by Designer Drugs (DREADD) is a novel chemogenetic technology where genes of modified human receptors without any endogenous ligands are expressed. These receptors can be reversibly activated by specific actuators, small molecules selectively binding to their DREADDs and not to any naturally occurring receptors. Thus, DREADD is a powerful tool that can be widely applied in basic research and preclinical drug development.

Aims: The primary aim of our experiments was to investigate the changes in performance of rats during multiple behavioral experiments as a result of silencing the targeted brain areas using DREADDs as part of a long-term goal to develop a novel translational model representing the pathophysiology of cognitive decline in humans.

Methods: We stereotaxically injected 500 nl of adeno-associated virus vector serotype 5 (AAV5) carrying the gene of the modified human M4D(Gi) cholinergic receptor into either the hippocampus (HC), the anterior cingulate cortex (ACC), the infralimbic cortex (IL), the ventral tegmental area (VTA) or the nucleus basalis (NBM) in rats. After recovery, 1) general alertness (arousal) and sustained attention were measured in the psychomotor vigilance task (PVT) 30 min after the administration of 3 different doses of DCZ or VEH. 2) Explicit memory was assessed in the novel object recognition (NOR) test after DCZ administration and compared exploration time of the novel and the old object. 3) Spatial learning skills were tested using the Morris water maze (MWM) task after injecting high doses (1.0 mg/bwkg) of DCZ, measuring escape latency on 3 training days.

Results: In the IL group, increased reaction time was detected in the PVT, whereas HC operated animals showed significantly prolonged motor response, and decreased number of missed trials. The NBM operated animals showed definite increase of missed trials and reaction time, whereas we observed an increasing number of premature trials in the VTA group, possibly indicating impulsive behavior. In the NOR test, the HC targeted animals could not recognize the novel object if they received DCZ treatment. The MWM test showed significantly increased escape latency in the ACC group compared to the control group, indicating impaired spatial learning.

Discussion: We have shown the feasibility of inhibitory DREADDs for modeling certain conditions resembling different aspects of cognitive decline. Our further plans include proving the therapeutic utility of this model by testing multiple cognitive enhancers on the previously described experimental setup.

W-K6-4 EVALUATION OF THE THERAPEUTIC EFFICACY OF 213BI-LABELLED DOTA-CONJUGATED ALPHA-MELANOCYTE STIMULATING HORMONE PEPTIDE ANALOGUES IN MELANOCORTIN-1 RECEPTOR POSITIVE PRECLINICAL MELANOMA MODEL

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Melanocortin-1 receptor (MC1-R) targeting alpha-melanocyte stimulating hormone-analogue (α-MSH) biomolecules labelled with α-emitting radiometal seem to be valuable in the targeted radionuclide therapy of MC1-R positive melanoma malignum (MM). Herein is reported the anti-tumor *in vivo* therapeutic evaluation of MC1-R-affine [^{213}Bi]Bi-DOTA-NAPamide and HOLDamide treatment in MC1-R positive B16-F10 melanoma tumor-bearing C57BL/6J mice. On the 6th, 8th and 10th days post tumor cell inoculation; the treated groups of mice were intravenously injected with approximately 5 MBq of both amide derivatives. Beyond body weight and tumor volume assessment, [^{68}Ga]Ga-DOTA-HOLDamide and NAPamide-based PET/MRI scans, and *ex vivo* biodistribution studies were executed 30,- and 90 min postinjection. In the PET/MRI imaging studies the B16-F10 tumors were clearly visualized with both ^{68}Ga-labelled tracers, however, significantly lower tumor-to-muscle (T/M) ratios were observed by using [^{68}Ga]Ga-DOTA-HOLDamide. After alpha-radiotherapy treatment the tumor size of the control group was larger relative to both treated cohorts, while the smallest tumor volumes were observed in the NAPamide-treated subclass on the 10th day.

Relatively higher [^{213}Bi]Bi-DOTA-NAPamide accumulation in the B16-F10 tumors (%ID/g: 2.71 ± 0.15) with discrete background activity led to excellent T/M ratios, particularly 90 min postinjection. Overall, the therapeutic application of receptor selective [^{213}Bi]Bi-DOTA-NAPamide seems to be feasible in MC1-R positive MM management.

Keywords: Alpha-melanocyte stimulating hormone-analogue (α-MSH analogue); Bismuth-213; Melanocortin-1 receptor (MC1-R); Melanoma malignum (MM); Radionuclide therapy; [(213)Bi]Bi-DOTA-HOLDamide; [(213)Bi]Bi-DOTA-NAPamide.

W-K6-5 YAP1 INHIBITOR VERTEPORFIN POTENTIATES THE EFFECTS OF ANTI-PD-1 IMMUNOTHERAPY IN MELANOMA

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Although immune checkpoint inhibitors such as anti-PD1 have revolutionized cancer treatment, their efficacy remains challenging. To maximize the treatment benefits of immunotherapy, we need more robust biomarkers.

Our goal was to identify biomarkers that can predict resistance to immune checkpoint inhibitors and can be targeted by FDA-approved drugs.

We previously established a web platform (https://rocplot.com/immune) with 1434 samples from different tumors. In the anti-PD-1 melanoma cohort (*n=501*), yes-associated protein 1 (YAP1) was the most promising targetable biomarker, which was overexpressed in non-responders (*p=3.22E05, FC=2.21, AUC=0.725*). For the investigation of the synergistic effect of anti-PD1 therapy and YAP1 inhibition by verteporfin (VP), B16-F10 and YUMM1.7 melanoma cell lines were injected into C57BL/6J mice. The impact of verteporfin monotherapy on these cells was assessed by viability assay.

In the YUMM1.7 group, tumor volume was reduced by the combination of VP + anti-PD1 compared to the control (ANOVA Dunnett's t, *p=0.014*) or anti-PD1 monotherapy groups (ANOVA Dunnett's t, *p=0.009*). Verteporfin monotherapy did not affect tumor size compared to the control group (*p=0.261*). No effect was observed with either treatment in the highly aggressive B16-F10 injected animals. However, *in vitro* experiments showed that verteporfin treatments at 5,- and 10 μM concentrations significantly reduced the growth of both B16-F10 (*p=0.01, p=0.04* respectively) and YUMM1.7 (*p=0.0005 , p=0.002* respectively) cell lines compared to non-treated cells or vehicle control (*p=0.002* for 5 μM and *p=0.04* for 10 μM in B16-F10, or *p=0.0001* for 5 μM and *p=0.001* for 10 μM in YUMM1.7).

In conclusion, we have established a database for discovering and validating immunotherapy biomarkers. A druggable, predictive biomarker (YAP1) was selected for *in vivo* studies. The combination of the YAP1 inhibitor verteporfin and anti-PD1 showed an increased efficacy in melanoma growth inhibition. Verteporfin alone was also effective under *in vitro* conditions (Animal Experiment Permit: ÁTET PE/EA/01017-6/2022).

W-K6-6 THE NOVEL MULTI-TARGET DRUG CANDIDATE SZV-1287 INHIBITS INFLAMMATORY LUNG FUNCTION ALTERATIONS IN THE OPTIMIZED ENDOTOXIN-INDUCED ACUTE PNEUMONITIS MOUSE MODEL

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SzV-1287, our novel, multi-target drug candidate patented for the treatment of neuropathic pain, has successfully completed Phase Ia clinical trials. It irreversibly inhibits copper-containing amine oxidase 3 (AOC3, formerly called semicarbazide-sensitive amine oxidase: SSAO) expressed in the lung, which produces tissue irritants activating the Transient Receptor Potential Ankyrin1 (TRPA1) involved in airway inflammation. SzV-1287 also directly antagonizes TRPA1 and TRP Vanilloid 1 (TRPV1), and its main metabolite, oxaprozin, is a cyclooxygenase inhibitor. Here we investigated the effect of SzV-1287 in comparison with the selective AOC3 inhibitor LJP-1207 in the endotoxin-induced acute airway inflammation mouse model optimized and validated with the reference compound glucocorticoid dexamethasone. Interstitial pneumonitis was induced by intratracheal administration of 100, 50, 20 and 5 μg endotoxin (lipopolysaccharide: LPS; E. coli O111:B4; 60 μl in phosphate buffer: PBS) in female C57BL/6J mice to determine the optimal dose for compound testing, control mice received PBS. Dexamethasone (5 mg/kg i.p.) was used as a positive control. Respiratory functions were measured by restrained whole body plethysmography 24 h after LPS, core body temperature was monitored. Lungs were then excised, weighed and myeloperoxidase activity was measured. Based on the obtained results 5 μg LPS-treated animals were divided into: i) LPS+vehicle (Kolliphor ip.), ii) LPS+SzV-1287 (20 mg/kg ip.), iii) LPS+LJP-1207 (20 mg/kg ip.).

LPS significantly increased breathing frequency, decreased tidal volume, inspiratory and expiratory time, increased lung weight and myeloperoxidase activity and 10% body weight loss 24 h after its administration and hypothermia after 2 h without dose-dependency, but only the 5 μg LPS effect was decreased by the reference compound dexamethasone. Body weight loss was blocked by both SzV-1287 and LJP-1207. However, inflammatory functional alterations and lung edema were decreased only by SzV-1287, but aggravated by LJP-1207.

Our multi-target candidate SzV-1287, but not the selective AOC-3 inhibitor improves airway inflammation, which suggests that besides AOC-3 blockade TRPA1/V1 ion channel and COX inhibition through its metabolite are likely to be involved in its effectivity. SzV-1287 represents a promising novel tool for the therapy of lung inflammation.

Funding: TKP2021-EGA-13, Hungarian Research Network (HUNREN, Chronic Pain Research Group), RRF-2.3.1-21-2022-00015

W-K6-7 INVESTIGATION OF THE CARDIAC EFFECTS OF ERTUGLIFLOZIN UTILIZING HIGH-RESOLUTION ECHOCARDIOGRAPHY IN SHR RAT MODEL

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Introduction: Recent literature suggests that several sodium-glucose co-transporter 2 (SGLT-2) inhibitors, used in the treatment of diabetes, exhibit cardioprotective properties. However, there is limited data available on the cardiac effects of ertugliflozin (ERTU). Preclinical studies indicate a potential reduction in myocardial hypertrophy with ERTU administration in mouse models.

Objective: This study aims to explore the myocardial alterations induced by hypertension and assess the impact of ertugliflozin in a spontaneous hypertensive rat (SHR) model, employing high-resolution small animal echocardiography.

Methods: Hypertensive and normotensive rats were left untreated until reaching 18 months of age. Subsequently, one group of SHR rats received 5 mg/kg bw ertugliflozin (SHR+ERTU) daily via oral gavage, while the other two groups received vehicle alone (SHR, CONTROL). After a treatment duration of 2 months, high-resolution echocardiography was conducted under ketamine/xylazine anesthesia with concurrent ECG monitoring. Blood glucose levels and blood pressure were also measured non-invasively.

Results: Although there were no significant differences in blood pressure levels between the ERTU-treated group and the untreated SHR group, notable variations in cardiac functional parameters were observed. Ventricular wall thickness was significantly increased in SHR animals compared to aged controls, accompanied by a reduction in left ventricular cavity size. These parameters exhibited significant improvement in ERTU-treated animals. While the ejection fraction was higher in the SHR group, stroke volume and cardiac output were diminished, whereas in SHR+ERTU animals, they approximated those of healthy controls. Additionally, normalization of left ventricular hypercontractility was evidenced by strain echocardiography. ERTU treatment also led to an approximation of the myocardial performance index to control levels. Noteworthy differences in aortic velocity and pressure gradient values were not observed. Diastolic function was compromised in the SHR group compared to controls, as indicated by reduced tissue e' wave, E wave deceleration time, and isovolumetric relaxation time (IVRT) stretched to its multiple, which all significantly ameliorated in the SHR+ERTU group.

Conclusion: Ertugliflozin treatment demonstrates cardioprotective effects in SHR rats. Although the effects on diastolic function were less pronounced, attenuation of ventricular hypertrophy and significant improvements in systolic parameters and performance index were evident following treatment.

Funding: This research was supported by the National Fund for Research Development and Innovation under the TKP2021-EGA grant program (project number TKP2021-EGA-18), GINOP-2.3.4-15-2016-00002, and ÚNKP-23-3-I-DE-46.

THURSDAY 6 JUNE 2024

T-KA NEW AVENUES TO INCREASE CARDIAC CONTRACTILITY IN HEART FAILURE

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Heart failure remains a leading cause of mortality in the Western world. An important hallmark of heart failure in many patients is reduced myocardial contractility. Therefore, positive inotropic agents were initially used as a means of enhancing cardiac pump function aimed at alleviating congestive symptomology, but most attempts failed due to adverse effects. In our research we attempt to find new avenues directed a novel moleular targets. For example regulators of G protein signaling (RGS) proteins have emerged as potential therapeutic targets due to their function in cardiac signaling. We could show that a mutant of the long isoform of the RGS3, RGS3LN460A, functions as a switch in the signaling of muscarinic, M_2 receptors cardiomyocytes by redirecting the Gi-mediated Rac1 activation into a Gi-mediated RhoA/ROCK activation. Mechanistically, the switch is mediated by a complex formation of RGS3LN460A with and nitration of the GTPase-activating protein p190ARhoGAP. Functionally, this switch resulted in a reduced production of reactive oxygen species in cardiomyocytes and an increased inotropic response. AAV-mediated overexpression of RGS3LN460A in rats in vivo, increased the contractility of ventricular strips ex vivo.

Another target are nucleoside diphosphate kinases (NDPK), which can for example supply GTP for G protein activation. Thereby they contribute to cAMP formation and cardiac muscle contraction. One isoform, NDPK C is a pivotal mediator in the interaction with G proteins and likely contributes to a the detrimental chronic activation of Gi in heart failure. Consequently, we screened for thus far unknown small molecule inhibitors of the enzymatic activity of NDPK C and identified SanWie3 as as prototypic allosteric inhibitor. In cardiomyocytes), SanWie3 had similar effects as the depletion of NDPK C. Especially in the SERCA/PLB subdomain in cardiomyocytes, isoprenaline-induced cAMP levels were significantly increased by 1.5-fold in the presence of SanWie3. In accordance, we observed an increase in isoprenaline-induced inotropy and lusitropy in rat left ventricular muscle strips by applying SanWie3 to the organ bath. As an underlying mechanism, we could verify that the interaction of NDPK C with Gi is suppressing cAMP levels in the SERCA/PLB subdomain chronically. The inhibition of NDPK C by SanWie3 enhanced the phosphorylation of phospholamban (PLB) accompanied with an enhanced Ca^{2+} load in sarcoplasmic reticulum further causing an elevated systolic Ca^{2+} release. Repeated in vivo applications of SanWie3 for 7 days in mice did not reveal detrimental effects, but significantly increased the ejection fraction, fractional shortening and steady state phosphorylation of PLB.

We conclude that both strategies, the AAV-mediated overexpression of RGS3LN460A in cardiomyocytes as well as the inhibition of NDPK C by small molecule inhibitors are alternative strategies to potentially increase cardiac contractility in heart failure patients. A validation of these strategies in relevant animal models of heart failure is ongoing.

T-KA-1 PHARMACOLOGICAL ACTIONS OF NOVEL ENDOGENOUS CATECHOLAMINES

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Several vascular tissues, including human umbilical cord vessels, human arterial and popliteal vein strips, aortic rings from *Chelonoidis carbonarius* and *Panterophis guttatus*, as well as thoracic aorta and pulmonary artery rings from Callithrix spp present basal release of 6-nitrodopamine (6-ND), as identified by liquid chromatography coupled to tandem mass spectrometry. In these vascular tissues, 6-ND is a potent vasorelaxant agent, acting as a dopamine D2-like receptor antagonist. Rat isolated atria and ventricles also present basal release of 6-ND, in the heart 6-ND is the most potent endogenous positive chronotropic and positive inotropic agent and has remarkable ability to synergize with the classical catecholamines dopamine, noradrenaline and adrenaline. The presentation will cover some other actions of 6-ND in vascular (rabbit corpus cavernosum) and non-vascular (human and rat vas deferens) tissues and will discuss both 6-ND mechanisms of action and biosynthetic pathways. In addition, identification of other novel endogenous catecholamines, such as 6-cyanodopamine and 6-bromodopamine will be presented.

Keywords: Langendorff, nitrocatecholamines, erectile function, premature ejaculation.

T-KA1-2 ENDOTHELIUM-DERIVED C-TYPE NATRIURETIC PEPTIDE IS A CRITICAL REGULATOR OF ANGIOGENESIS AND VASCULAR REMODELLING'

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Angiogenesis and vascular remodelling are complementary, innate responses to ischemic cardiovascular events, including peripheral artery disease and myocardial infarction, which restore tissue blood supply and oxygenation; the endothelium plays a critical function in these intrinsic protective processes. C-type natriuretic peptide (CNP) is a fundamental endothelial signalling species that coordinates vascular homeostasis. Endothelium derived C-type natriuretic peptide (CNP) plays an important role in regulating vascular tone, blood pressure, leukocyte flux and the integrity of the vessel wall. We sought to delineate a central role for CNP in angiogenesis and vascular remodelling in response to ischemia. Clinical vascular ischemia is associated with reduced levels of CNP. Endothelial CNP play a critical role in the vascular response to ischemia and injury, promoting angiogenesis and remodelling. The pharmacological activation of the CNP signalling pathway may be a potential therapeutic target to improve angiogenesis in ischemic patients.

T-KA1-3 UNVEILING THE MECHANISMS OF CHIKUNGUNYA-INDUCED PAIN

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Chikungunya virus (CHIKV) is a single stranded RNA virus that causes an illness characterized by debilitating joint pain that may become chronic in more than 50% of the infected patients. In the last decade in Brazil, more than 1 million cases of CHIK infection were reported, meaning that an important section of the population is chronically affected by joint pain. The mechanisms of CHIKV-induced pain are not yet fully understood but have been associated with a strong immune response to viral debris and antigens found in synovial fluid and tissues. Herein, the role of transient receptor potential channels were investigated in a translational animal model of CHIKV-induced joint pain. Following approval of the Ethics Committee, male and female (n = 6) C57BL/6 wild type, TRPV1 and TRPA1 knockout mice received a single injection of CHIKV in the ipsilateral joint; the contralateral joint received a single intra-articular injection of saline. Animals that received a single intra-articular injection of saline in the ipsilateral joint were used as controls. Secondary mechanical (up and down method) was evaluated at different time-points over 56 days and compared to baseline measurements. For analysis of joint and dorsal root ganglia (DRG) alterations, animals were culled at 7- and 21-days post-injection. DRG inflammatory mediator levels and joint cartilage damage were assessed. The involvement of TRPV1, TRPA1 and TRPC5 were investigated by using selective antagonists and knockout mice. Results are expressed as mean + SEM (behavioural and inflammatory mediator analysis) or median (IQR) for histology scores. Statistical significance was determined by repeated measures ANOVA or one-way ANOVA followed by Bonferroni's post hoc test, or unpaired t test. CHIKV intra-articular injection induced pronounced time-dependent bilateral secondary mechanical and thermal nociceptive responses in comparison to saline-injected mice. Increased DRG levels of IL-6 and IL-10, as well as cartilage damage in both joints were observed in CHIKV-injected mice. Mechanical hyperalgesia was absent The TRPV1 and TRPA1 antagonists (SB366791 and HC-030031) and knockout mice. Interestingly, CHIKV-injected mice exhibited increased expression of TRPA1 and TRPV1 in their joints. Overall the results indicate that both TRPV1 and TRPA1 substantially contribute to the joint articular pain triggered by CHIKV.

Funding: Institutlo de Pesquisa Pelé Pequeno Príncipe, CAPES, CNPq and INCT-INOVAMED

T-KB1-1 Distinct expression and functional profile of Mu-, Delta-, and Kappa-Opioid receptors in human dorsal root ganglia

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Introduction: Peripheral analgesic effects of opioids has been shown for animals and humans. Corresponding opioid receptor mRNA, protein and signalling have been shown in dorsal root ganglia of animals. Here we show for the first time the distinct mRNA and protein expression of all components of the opioid system (receptors and their endogenous ligands) and their respective location within certain subtypes of dorsal root ganglion neurons.

Methods: All human tissue samples were supplied by AnaBios Corporation which received IRB approval for research. Real-Time PCR, western Blot, and immunohistochemistry were performed according to previously published protocols.

Results: Mu-, delta-, and kappa-opioid receptors were abundantly expressed (mRNA and protein) in lumbar human dorsal root ganglion neurons. Expression level for mu-opioid receptors were highest compared to delta-, and kappa-opioid receptors. In addition, the opioid precursor peptides proopiomelanocortin, proenkephalin, and prodynorphin were all expressed in lumbar human dorsal root ganglion neurons with proenkephalin revealing the highest level of expression compared to proopiomelanocortin and prodynorphin. Opioid receptors were mainly located in nerve growth factor-dependent subpopulations of lumbar human dorsal root ganglion neurons co-localizing with well-known pain signalling molecules.

Conclusion: This is the first time that peripheral opioid receptors and their endogenous ligands are shown to be expressed in human dorsal root ganglion neurons. The relative expression levels might give some indication for the clinical use of locally applied opioids.

T-KB1-2 DUAL-TARGET LIGANDS AND PAIN: OUR EXPERIENCE

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The development of new analgesics endowed with mu/delta opioid receptors (MOR/DOR) activity represents a promising alternative to MOR selective compounds as their better therapeutic and tolerability profile. Recently, we synthetized the dual-target MOR/DOR agonist LP2 that showed a long lasting antinociceptive activity in the tail flick test. Notably LP2 also resulted effective in the mouse formalin test, a model of inflammatory pain: LP2 (0.25-1.00 mg/kg, i.p.) exhibited dose-dependently efficacy in counteracting both first and, above all, the second phase of formalin test where spinal cord neuronal sensitization occurs. This prompted us to investigate the pharmacological profile of LP2 in a neuropathic pain model the chronic constriction injury (CCI). Results showed that LP2 significantly ameliorated mechanical allodynia from the early phase of treatment up to 21 days post-ligatures. We additionally showed that LP2 prevented CCI-induced upregulation of connexin 43 (Cx43), the core glial gap junction-mediated cell coupling, which plays a role in the pathogenesis of neuropathy. The pathogenic role of neuroinflammation in neuropathic pain development has recently gained more attention. Pro-inflammatory cytokines, such as TNF and IL-6, are considered key modulators in the crosstalk among immune cells, neurons, and glia. We showed that LP2 was able to significantly downregulate the mRNA expression level of both CCI-induced TNF and IL-6. Notably, our data showed that neither NLX nor NTD were able to reverse LP2-mediated TNF reduction in CCI rats, indicating that concomitant MOR and DOR agonism was important to reduce the cytokine increase.

To investigate the pivotal role of the stereocenter at the *N*-substituent of the 6,7-benzomorphan scaffold, 2*R*- and 2*S*-diastereoisomers of LP2 were synthesized and their pharmacological profile was evaluated. 2*S*-LP2 showed an improved pharmacological profile in comparison to LP2 and 2*R*-LP2. *In vivo* effect of 2*S*-LP2 was consistent with the improved MOR/DOR efficacy profile assessed by radioligand binding assay and BRET assay, to evaluate the capability to promote receptor/G-protein and receptor/β-arrestin 2 interaction. 2*S*-LP2 was able to activate G-protein pathway over β-arrestin 2-mediated signaling, with a biased agonist profile at MOR and mainly at DOR. We examined also the correlation between anti-allodynic effect of 2*S*-LP2, and TGF-β1 signaling in neuropathic CCI model. We detected a significant decrease of active TGF-β1 and its type II receptor TGFβ-R2 levels in the spinal cord from CCI rats and a selective deficit of TGF-β1 in microglia cells both at days 11 and 21 post-ligature, as assessed by immunofluorescence analysis. 2*S*-LP2, administered from day 11 to 21post-ligature, was able to reduce CCI-induced mechanical allodynia by rescue of TGF-β1 and TGFβ-R2 levels. Thus, the dual-target approach could represent a novel pharmacological approach to increase the analgesic efficacy of MOR agonists.

T-KB1-3 PERIPHERAL ANALGESIC EFFECTS OF OPIOIDS FOR PAINFUL DIABETIC NEUROPATHY

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Introduction: Painful diabetic neuropathy is poorly controlled by analgesics and requires high doses of opioids that triggers side effects and reduces patients' quality of life. Since down regulation of G-protein coupled receptors limits the physiological response to external signals and contributes to a loss in agonist efficacy, we investigated the molecular mechanisms governing peripheral sensory neuron mu-opioid receptor (MOR) down regulation and the loss in opioid responsiveness under diabetic conditions.

Methods: In rats with streptozotocin (STZ)-induced painful diabetic neuropathy, we assessed changes in opioid antinociception, MOR number, G-protein coupling and inhibition of TRPV1 activity of peripheral sensory neurons. To examine MOR lysosomal targeting, we used subcellular fractionation combined with Western blot as well as electron microscopy. Finally, we applied intrathecal Rab7-siRNA to silence the endogenous Rab7 and intrathecal NGF to reverse NGF deprivation in peripheral sensory neurons.

Results: Impaired opioid analgesia is associated with a loss in MOR number, G-protein coupling and inhibition of TRPV1 activity in peripheral sensory neurons of diabetic rats. MOR colocalizing with upregulated Rab7 are enclosed in perinuclear LampI-positive lysosomes. Diminishing Rab7 by siRNA or by reversing NGF deprivation not only prevents MOR targeting to lysosomes and restores MOR plasma membrane density but also rescues opioid responsiveness towards better pain relief.

Conclusion: Our findings demonstrate in diabetic neuropathic pain that enhanced Rab7 dependent lysosomal targeting of MOR leads to a loss in opioid antinociception. This finding is different from peripheral sensory neuron MOR up-regulation and antinociception in inflammatory pain suggesting that regulation of opioid responsiveness is dependent on pain pathogenesis.

T-KB1-4 THE AFFECTIVE COMPONENT OF CHRONIC PAIN AND THE OPIOID SYSTEM

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Chronic neuropathic pain is a complex experience characterized by a sensory component that allows for discrimination of a painful stimulus location and intensity, as well as by an emotional component that modulates the negative affective and aversive response to a painful stimulus.

The dynorphin/kappa opioid receptor (Dyn/KOR) system is involved in pain transmission at spinal level, where dynorphin exerts either antinociceptive or pronociceptive effects, probably related to its opioid and non opioid actions. Interestingly, persistent pain of inflammatory or neuropathic origin induces alterations of the Dyn/KOR system in supraspinal CNS areas.

In this frame, we carried out studies to investigate the alterations of this opioid system in the mesocorticolimbic system of neuropathic pain-suffering mice (chronic constriction injury of the sciatic nerve). In this model, a marked increase in prodynorphin mRNA levels in the anterior cingulate cortex (ACC) and prefrontal cortex (PFC) was observed. In addition, KOR gene expression was increased in the PFC, and was decreased in the ACC and nucleus accumbens (NAc). Since the activation of the dynorphinergic system produces dysphoric, aversive and depressive effects either in humans and in rodents, this dysregulation of the Dyn/KOR system detected in the reward mesocorticolimbic system can be related to its role in the modulation of the tonic-aversive component of pain.

These Dyn/KOR system dysfunctions in the mesocorticolimbic reward circuitry, observed during experimental chronic pain conditions, correlates with the high comorbidity of persistent pain conditions and mood disorders, including the risk of substance abuse disorders. In this context, we observed that a persistent inflammatory pain condition induces negative affective states together with gene and protein expression dynamic alterations of the Dyn/KOR system in a sex- and time-dependent manner, in the rat. Furthermore, the pharmacological blockade of KORs in NAc of female rats inhibited the negative affective state induced by inflammatory pain.

Finally, a dysregulation of the Dyn/KOR system was also detected in the PFC and the amygdala of a murine model of Fabry disease, a genetic pathology causing multi-system disorders including neurological manifestations, such as painful neuropathy and mood disorders.

These observations, in agreement with other literature data, support the hypothesis of an important role played by the Dyn/KOR system in the modulation of the negative affective component of prolonged pain conditions.

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T-KB1-5 OPIOIDS IN CHRONIC PAIN TREATMENT AND RISK OF OUD

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Tolerance and physical dependence are two pharmacological phenomena that develop after chronic exposure to opiates: they are connected and distinct from the psychic dependence of OUD (Opioid Use Disorder). It is now believed that neuronal adaptation phenomena to chronic opiates occur, involving a complex series of molecular and cellular events, including receptor desensitization, down-regulation and internalization (Romualdi and Candeletti, 2016).

Opiates produce strong analgesia but their use is limited by OIH. Molecular mechanisms underlying these phenomena include genetic differences, variants of mu opioid receptor, LTP, neuronal sensitization, glial neuroinflammation and epigenetics (Roeckel et al, 2016). The psychic dependence is a chronic recurrent disorder characterized by a compulsive behaviour, that is, the loss of control over the search and intake of drugs of abuse. The reinforcing effects of substances of abuse are due to the mesocorticolimbic system, consisting of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the shell of the nucleus accumbens (NAc), the amygdala, and the prefrontal cortex (PFC). The vulnerability to develop addiction depends on factors related to the substance effects, genetics and environment. Recently, it has been proposed that the development of psychic dependence and the vulnerability to relapse are the result of CNS neuroadaptative processes.

To date, a therapeutically appropriate use of opiates for the treatment of chronic pain has been hindered by the incorrect belief that their use will inevitably lead to the psychic dependence. Recent data suggest that the therapeutic use of opiates does not associate conditioning environmental stimuli so important in determining the positive reinforcement leading to the compulsive use. The condition in which the drug is taken, and above all the underlying painful pathology, does not provide the substrate and the context in which the patient seeks for the drug and clinical findings confirm that the phenomenon of abuse is observed very rarely (Maremmani et al, 2015). It has been demonstrated that during chronic pain condition a large release of □-endorphin induce desensitization of μ opioid receptors located on dopaminergic neurons projecting to NAc, as well as microglial BDNF-induced effects. Both phenomena cause a strong reduction of DA release-related reward (Niikura et al, 2010; Taylor et al 2015). The opioid epidemic time happened in USA since 2000s can consist in the concomitance of abuse, misuse and diversion due to commercial and medical interests leading to 600.000 deaths: the majority of them were not chronic pain suffering people. Recent results suggest that the use of opioids in severe chronic pain does not activate the limbic brain areas responsible for gratification, thus reducing the risk of their abuse.

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T-KB1-6 DAWN OF A NOVEL PAIN TREATMENT: SYNTHESIS AND STRUCTURAL PLASTICITY OF THE MOST POTENT ATYPICAL OPIOID KRATOM ALKALOID

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Pain management is one of the oldest challenges for medicine, and opioids targeting μ-opioid receptors (MORs) play a fundamental role in the treatment of moderate-to-severe acute and chronic pain. Although opioid agonists are still the most effective analgesics, there is a growing concern about the safety of their long-term administration owing to various adverse effects, such as respiratory depression, constipation, and addiction. Additionally, the current upsurge in opioid abuse, coupled with the spread of illicit synthetic opioids, has reached an epidemic level in the United States, wreaking havoc on public health, society, and the economy. In addition to introducing new legislation and public education, the development of safer analgesics is a major goal in pain management.

In this context, alkaloids from Mitragyna speciosa (commonly known as kratom) have recently emerged as a promising analgesic alternative for pain management with considerably fewer side effects than those associated with opioids. Kratom is a tropical tree-like herb native to Southeast Asia that has been used for centuries for its distinctive psychotropic properties. Kratom has been noted to have dose-dependent stimulant and opioid-like effects, and its illicit use to treat chronic pain and drug dependence is on the rise globally (>15 million estimated kratom users in the US alone).

Systematic investigations have also shown that the opioid agonistic effect of Mitragyna speciosa cannot be fully explained by that of its main alkaloid, mitragynine (230 nM for MOR). Finally, more potent analgesics, which were the oxidative metabolites of mitragynine, were identified in *in vitro* assays. Accordingly, the main alkaloid metabolizes *in vivo* to 7-OH-mitragynine (37 nM for MOR), which is followed by a subsequent 1,2-semipinacol rearrangement in human plasma to the more potent mitragynine pseudoindoxyl (0.8 nM for MOR, G-protein-biased MOR partial agonist). Thus far, access to these mitragynine's oxidative metabolites has relied exclusively on low-yielding biomimetic semisynthesis from mitragynine, which generates the pseudoindoxyl ring in a 1,2-semipinacol rearrangement. Although these synthetic studies demonstrated the power of late-stage functionalization strategies the attainable chemical space did not allow extensive structure–activity relationship (SAR) studies or deep-seated modifications of the unique pseudoindoxyl scaffold, thus hampering further advances in drug development.

Triggered by this inherent potential, we embarked on and accomplished the first scalable total synthesis of mitragynine pseudoindoxyl, the most potent atypical opioid kratom metabolite. This synthetic study also allowed the discovery of its not-yet-know property: the structural plasticity.[1]

[1] P. Angyal, K. Hegedüs, B. B. Mészáros, J. Daru, Á. Dudás, A. R. Galambos, N. Essmat, M. Al-Khrasani, Sz. Varga, T. Soós, *Angew. Chem. Int. Ed.* **2023**, *62*, e202303700.

T-KB1-7 GLYCINE TRANSPORTER 1 AND AT1 RECEPTOR INHIBITORS: NOVEL STRATEGIES TO DECREASE MORPHINE ANALGESIC TOLERANCE

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Background: μ-Opioid receptor (MOR) agonists are a mainstay in the management of moderate to severe pain entities. However, in the long term, their use is associated with the development of analgesic tolerance. To maintain adequate analgesia, dose escalation is required but at the price of side effects. In this regard, several attempts have been made to delay the development of opioid tolerance either by developing opioids that are devoid of side effects or combining opioid analgesics with drugs affecting other systems. The former strategy encompasses developing β-arrestin-biased ligands for MORs. The components of the combination strategy are drugs targeting different systems contribute to the development of opioid tolerance such as ionotropic N-methyl-D-aspartate receptors (iNMDARs). In fact, drugs blocking GluN2B such as ketamine or MK801 have been proven to delay the development of opioid analgesic tolerance. The prerequisite for the activation of extrasynaptic GluN2B is simultaneous binding of glutamate and glycine to GluN2BR. Therefore, changes in either glutamate or glycine could influence the GluN2B receptor functions. The glial glycine transporter 1 (GlyT1) regulates extrasynatic glycine level and has bidirectional operation. Furthermore, astrocytes release D-serine that serves as a co-agonist for the glycine site on iNMDARs and is being considered as an important modulator in pain transmission in spinal cord. AT1R antagonists have also been shown to inhibit NMDA overactivity.

Experimental Approach: Male Wistar rats (170-260g) were treated for 10 consecutive days with morphine, morphine + NFPS (GlyT1 inhibitor) or telmisartan (AT1R inhibitor) or selective PPARγ antagonist (GW9662), test drugs alone or vehicles. To follow the development of morphine tolerance, the pain threshold of animals was determined prior to treatment and at the 10th day of treatment by tail-flick, a thermal pain assay. The motor function was measured by rotarod test. The cerebrospinal fluid levels of glycine and D-serine were measured by a capillary electrophoresis-laser induced fluorescence detection method.

Key Results: Simultaneous administration of NFPS or telmisartan or GW9662 and morphine delayed the development of morphine analgesic tolerance. NFPS or telmisartan alone in applied doses had no significant effect on pain threshold and were devoid from motor dysfunction. NFPS raises the glycine level, whereas telmisartan decreases D-serine content of CSF.

Conclusions: GlyT1 inhibitors delay the development of morphine tolerance by a mechanism which may be related to their ability to inhibit GlyT1-operated reverse mode that results in decrease the glycine level at the vicinity of GLuN2B. On the other hand, the effect of telmisartan on decreasing D-serine content might also contribute to decreasing GLuN2B overactivity as well as its antagonist effect on PPARγ.

Fund: FK_138389 and TKP 2021 EGA-25

T-KC1-1 NMR AT THE FRONTIER: STRUCTURE ELUCIDATION OF MYSTERIOUS TRACE COMPONENTS

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Working in drug discovery and development (DDD) as a nuclear magnetic resonance (NMR) expert is a privileged experience for many reasons. Among others, this role presents numerous opportunities to meet exceptional technical and scientific challenges in NMR spectroscopy and structural chemistry. Since structure elucidation problems can arise at all stages of the DDD process (discovery, development, mass production, quality control, regulatory affairs), it offers the chance to witness the entire lifecycle of a drug molecule (in fortunate cases from its inception to its application). In some cases, the exact structure elucidation in some of these cases, especially when dealing with trace components besides the active pharmaceutical ingredient, remains a highly challenging task, even with the availability of well established, highly efficient spectroscopic methodologies and spectrometers with ultra-high resolution and sensitivity.

Some examples of such structure determination problems and their complex analytical solutions will be presented here, aligning with the spirit of the title/motto of the section: *Beyond technicality: analytical science as an attitude* by highlighting the aha! moments and the bumpy road leading to retrospectively trivial solutions, while also placing some focus on the human aspects of the problems. Hopefully, these small molecule API development and production related structure elucidation stories may be interesting to experts working in a field other than spectroscopy or (analytical) chemistry.

T-KC1-2 THE ROLE OF HIGH RESOLUTION MASS SPECTROMETRY IN THE IDENTIFICATION OF 5-F-CUMYLPEGACLON METABOLITES

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Mass spectrometry coupled with HPLC plays a key role in metabolite identification and quantitation. The most frequently used mass spectrometric analyzer is the triple quadrupole (QqQ) providing high sensitivity and selectivity in MRM mode and tandem functions for identifications. The classical way of the identification of metabolites is the predictive MRM (pMRM) method. The MS fragmentation behavior of the parent drug molecule is identified in product ion scan mode and the two most intense fragment ions are selected for MRM experiments. These two MRM transitions are the input for the pMRM method. The molecular weight of the parent drug is changing during metabolism. This change in molecular weight can also be seen in the fragment mass if the metabolism takes place in the charged part of the molecule. However, when the modification occurs in the uncharged part of the molecule, the original fragment mass remains unchanged. Applying this methodology for both MRM transitions, the overall possibilities of one metabolite can be represented by four MRM transitions. Repeating this protocol for all metabolites, all of the possible metabolites of one parent drug molecule can be identified. By combining the created MRM method with HPLC conditions, we are ready for metabolite identification.

5F-cumylpegaclon (5FCP) is a gamma-carboline based synthetic cannabinoid molecule that has been marketed as a designer drug. We were studying the metabolic behavior of this molecule. The classical pMRM method was applied and several intense and minor metabolites could be observed in the MRM chromatogram. Intense peaks were detected on both MRM transitions of the parent drug, however, the retention time of this peak was unusual. Metabolic processes increase the polarity of the molecules, causing shorter retention on classical RP column. In our case all of the metabolites had longer retention times than the MRM transitions of parent drug. To rationalize this strange behavior the sample was reacquired on a high-resolution (QTOF) mass spectrometer. QTOF can measure the molecular weights of ions precisely to obtain elemental composition not only on the molecular but fragment ions as well. The mass of the ion eluting at the retention time of the parent drug (on the basis of RMR data!) had 130 ppm error regarding the elemental composition of 5FCP. On the basis of the exact mass we identified another possible elemental composition of that ion, having 3 additional oxygen atoms and C_2H_5F loss. The elemental compositions of the fragment ions were also identified and interestingly, the same nominal masses were observed. The details of this interesting observation, with identical precursor and fragment masses of parent drug and its metabolite are going to be discussed in my presentation.

This work was supported by the National Research, Development and Innovation Office in Hungary (TKP2021-EGA-31).

T-KC1-3 BEHIND THE SCENES: LIGHT ABSORPTION-BASED DETECTION ON IN VITRO ADME TEST SAMPLES.

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In vitro ADME studies play a crucial role in the drug development process. Besides the experimental methodologies and good practices, the proper bioanalysis of samples is essential to achieve the highest quality results. Rather complex samples should be analyzed, therefore (U)HPLC is essential for the separation of components. The most robust and cost-efficient detection methods use light absorption. My presentation will focus on this methodology. We will travel through the possible instrument configurations. Then, some method optimization strategies will be discussed, with particular attention to the detection process. Last, but not least, some real problems and solutions will be presented.

T-KC1-4 DIFFERENT DERIVATIZATION APPROACHES TO ENABLE TOXICOKINETIC BIOANALYSIS OF CURIOUS ANALYTES

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With the REACH-regulation coming into effect in the EU the demand for toxicological studies of a wide variety of chemicals increased tremendously in the past decade. From innocuous sugar molecules to hazardous industrial chemicals depending on their average annual use a standard array of studies for safety and risk assessment needs to be conducted. In a high number of study types analytical chemistry plays a crucial role in relevant data collection. Either we aim to check whether the proper dose is administered to laboratory animals, or to gain information about the systemic exposure of the test item in them, the toolbox of instrumental techniques needs to be duly opened and properly applied.

In the present work we would like to give an insight into two encounters with curious analytes posing bioanalytical challenges to analysts accustomed to 'nice' small-molecule drug candidates which typically obey the Lipinski's rule of five. Our number one example will be cyanate ion which called for a derivatization approach in order to construct a reliable, reverse-phase liquid chromatography tandem mass spectrometry (RP-LC-MS/MS) method suitable for the quantification of cyanate in rat plasma matrix. Contrary to previous research results which recommended using freshly prepared reagent anthranilic acid, we just came to the opposite conclusion and pointed out that ageing of the aqueous solution of the reagent is essential to provide the desired selectivity of the method. The plausible explanation for this finding was associated with the quality of ultrapure water.

As a second example, the bioanalytical method development for a high-production-volume chemical from the class of glycidyl ethers used in the manufacturing of epoxy resins is presented. The structure of the studied compound is still far away from the one a favoured drug molecule bears, yet closer than that of cyanate. Thus, the feasibility of a reverse-phase liquid chromatography method was not at stake but the sensitivity of the mass spectrometer raised some concerns in the analyst. In an early, tentative method we made a compromise with its sensitivity limitation by anticipating that the test item was expected to mostly remain in the bloodstream, reaching rather high plasma concentrations because of its hydrophilic character. After admitting by real sample analysis that this presumption was not correct at all, an entirely different derivatization approach from the one mentioned above was elaborated. In-source derivatization using the eluent acetonitrile provided a facile method with adequate sensitivity without tedious multiple sample preparation steps.

T-KA2 EXPLORING OPPORTUNITIES AND CHALLENGES OF HYDROGEN SULFIDE-RELEASING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS FOR EFFECTIVE PAIN CONTROL AND GASTRIC INTEGRITY

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The goal of pharmacological treatment is to elicit a favorable clinical response in patients, aiming to maximize efficacy while minimizing adverse events. In this presentation, I will discuss emerging opportunities and challenges in drug discovery, focusing on a promising class of compounds: hydrogen sulfide-releasing non-steroidal anti-inflammatory drugs, which exhibit potent analgesic effects with improved gastric safety profiles. Postoperative pain affects approximately 80% of surgical patients, often leading to suboptimal treatment outcomes and a diminished quality of life. While opioids and traditional NSAIDs are known for their analgesic effects, opioids carry the risk of addiction, and NSAIDs can cause gastrointestinal (GI) mucosa damage, limiting their utility. This discussion will encapsulate our preclinical research efforts and efficacy studies, elucidating the mechanisms of action of a hydrogen sulfide-releasing NSAID, ATB-352, in a murine model of postoperative pain induced by plantar incision surgery (PIS). Following the PIS procedure, mice exhibited prolonged mechanical allodynia and thermal hyperalgesia, effects correlated with decreased hydrogen sulfide (H2S) production and alterations in the expression of enzymes involved in its synthesis (cystathionine γ-lyase – CSE and cystathionine β-synthase - CBS) within the operated paw.

Selective or dual inhibition of CSE/CBS exacerbated nociceptive parameters in a dose-dependent manner. Oral treatment with ATB-352 (4.6, 15 and 46 mg/Kg) promoted superior analgesic efficacy compared to equimolar doses of KETO (3, 10 and 30 mg/Kg), with both compounds exhibiting a reduction in interleukin-1β release and an increase in superoxide dismutase activity. However, only ATB-352 reduced 3-nitrotyrosine expression. Pharmacological blockade of cannabinoid receptors 1 (CB1) and 2 (CB2) did not influence the analgesic properties of KETO, whereas CB1 antagonism reversed the anti-allodynic and anti-hyperalgesic effects to heat promoted by ATB-352, and CB2 antagonism reversed only the anti-allodynic effect. Importantly, ATB-352 did not induce gastric lesions and even increased gastric mucus levels, a phenomenon reversed by CB1 antagonism. Conversely, equimolar doses of KETO caused gastric damage, which was potentiated by CB1 antagonism. Neither KETO nor ATB-352 altered the exploratory behavior or spontaneous locomotion of the animals. ATB-352's enhanced properties may be attributed to its influence on endocannabinoid system via irreversible inhibition of the degradation enzyme fatty acid amid hydrolase (FAAH). This mechanism, supported by biochemical assays and molecular docking studies, underscores the potential of ATB-352 as a safer and more effective alternative to conventional NSAIDs for managing postoperative acute pain. In summary, ATB-352 emerges as a promising candidate with enhanced analgesic efficacy, antioxidant properties, and gastric safety compared to conventional NSAIDs, offering a multifaceted approach to pain management with potential therapeutic benefits in clinical practice.
Financial Support: FAPESP, CAPES (Finance Code 001), FAPESP and CNPq (142343/2020-0; 312514/2019-0).

T-KB2 PRECISION PHARMACOLOGY: TARGETING ENZYME ISOFORMS FOR TAILORED THERAPEUTICS

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If there is one pharmacological target that has stood the test of time, it is soluble guanylyl cyclase: nitric oxide-releasing glyceryl trinitrate has been the drug of choice for the treatment of acute angina pectoris for more than 150 years. However, soluble guanylyl cyclase is not a single pharmacological target, but rather two distinct targets. These are referred to as GC-1, consisting of the α_1 / β_1 heterodimer encoded by the GUCY1A1 and GUCY1B1 genes, and GC¬2, consisting of the α_2 / β_1 heterodimer encoded by the GUCY1A2 and GUCY1B1 genes. GC-1 is widely recognized as the predominant isoform in blood vessels and platelets. At the cellular level, it is distributed throughout the cytoplasm. In contrast, GC-2 accumulates at cell-cell interaction sites, such as the postsynaptic membrane of neurons, and appears to play a more specialized role in fewer cell types or tissues.

With classical drugs, the release of nitric oxide precedes the interaction with the two different isoforms, making tailored targeting impossible. This has changed with the new non-nitric oxide-releasing drugs: the stimulators such as riociguat used to treat pulmonary hypertension and the activators still in clinical development for chronic kidney disease. Recently, we discovered runcaciguat as the first isoform-specific activator of soluble guanylyl cyclase GC-1. In parallel, we discovered that a overactive GC-2 isoform in a patient with a heterozygous gain-of-function mutation in the GUCY1A2 gene leads to a syndromic neurodevelopmental disorder with intellectual disability, hearing loss and autonomous ovarian hyperfunction, supporting the importance of GC-2 in the central nervous system and suggesting an unexpected and novel regulatory role of GC-2 in ovarian hormone production.

It is clear that GC-1 and GC-2 have distinct physiological functions and that GC-1 is the predominant form in the cardiovascular system. We currently believe that GC-1-specific activation by runcaciguat may explain its unique clinical profile in the treatment of chronic kidney disease. Whether the activation of GC-2 by drugs mediates deleterious or beneficial effects, and whether the deleterious effects of overactive GC-2 are limited to an overactive GC-2 during fetal and early postnatal development, is currently the focus of work in our laboratory. To this end, we have generated a mouse model of overactive GC-2 and plan to generate an analogous mouse model of overactive GC-1.

T-KA3-1 ORKAMBI IS A POTENTIAL THERAPEUTIC OPTION FOR ACUTE PANCREATITIS

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Heavy alcohol intake is one of the most common causes of acute pancreatitis (AP). We have previously shown that ethanol (EtOH) decreases the expression and activity of the cystic fibrosis transmembrane conductance regulator (CFTR) which plays a key role in alcohol-induced AP development. Orkambi (Ivacaftor and Lumacaftor) is available to correct the impaired CFTR function and expression in cystic fibrosis patients. Our aim in this study was to investigate whether Orkambi could be also beneficial in pancreatitis. Intact guinea pig pancreatic ducts (PDs) were treated with different concentrations of EtOH (30; 50; 100 mM) alone and in combination with Ivacaftor and/or Lumacaftor (1; 3; 5 and 10 μM) for 3, 7, 9 and 12 hours and CFTR expression and activity were evaluated by immunostaining and patch clamp, respectively. Experimental AP was induced in Orkambi-treated guinea pigs and standard laboratory and histological parameters were measured. Ivacaftor (10 μM) and Lumacaftor (10 μM) alone or in combination dose-dependently restored the localization and activity of the EtOH-damaged CFTR channel, during 12 hours of treatment. Oral administration of Orkambi decreased the severity of EtOH-induced pancreatitis. We showed for the first time that Ivacaftor and Lumacaftor are able to restore the CFTR defect caused by alcohol and decreases the severity of pancreatitis. These results indicate that Orkambi may represent a novel therapeutic option in the treatment of AP.

The research was funded by the National Research, Development and Innovation Office (SNN134497 to VV, K135874 to ZR and K131996 to PH) and the Translational Medicine Foundation.

T-KA3-2 T-CELL SPECIFIC PARP-2 DOWNREGULATION IN LPS INDUCED INFLAMMATION OF THE LARGE INTESTINE.

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In inflammatory bowel disease (IBD), an immune response involving the activation of helper 17 T cells (Th17) and the suppression of regulatory T cells (Treg), along with elevated tumor necrosis factor alpha (TNF□) production, is commonly observed. This inflammation can lead to intestinal damage, highlighting the importance of modulating T cell responses. Poly(ADP-ribose) polymerase-2 (PARP2) is implicated in the development and function of T cell subpopulations. Our study aimed to explore the potential benefits of T cell-specific PARP2 downregulation during lipopolysaccharide (LPS)-induced inflammation in the cecum and colon.

Intraperitoneal administration of low-dose LPS was used to induce local inflammatory response, characterized by increased TNFα concentration in intestinal tissues. Experiments were conducted on control (CD4Cre; PARP2+/+) and T cell-specific conditional PARP2 knockout (CD4Cre; PARP2f/f) mice. We measured intestinal TNFα, IL-1β, and IL-17 levels using ELISA, assessed oxidative-nitrative stress via immunohistochemistry, and examined PARP1 activity, p38 MAPK, ERK phosphorylation, and NF-κB expression by Western blot. Additionally, we analyzed systemic and local T cell populations, including Th17 and Treg cells, using flow-cytometry and immunohistochemistry.

In contrast to control mice, LPS treatment did not increase TNFα production in T cell-specific PARP2 knockout mice. These knockout mice exhibited no significant elevation in IL-1β levels, and IL-17 levels were lower, indicating a reduced inflammatory response. Additionally, we observed no significant increase in oxidative-nitrative stress or PARP1 activation. However, altered activation of ERK and NF-κB was found. Furthermore, T cell-specific PARP2 knockout mice showed a higher number of anti-inflammatory Treg cells in the intestinal mucosa following LPS treatment, accompanied by a reduced Treg count in peripheral circulation.

Our findings suggest that T cell-specific PARP2 downregulation alleviates LPS-induced colitis. The suppressed production of TNFα, and IL-17 together with the increased number of intestinal regulatory T cell after LPS treatment may be also beneficial during inflammatory processes seen in IBD. Moreover, reducing oxidative-nitrative stress and PARP1 activation by T cell-specific PARP2 downregulation may also mitigate intestinal tissue damage during intestinal inflammation.

T-KA3-3 SEARCHING FOR NEW TARGETS FOR TREATMENT OF NSAID ENTEROPATHY

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Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) can cause damage in both the upper and lower segments of the gastrointestinal (GI) tract. The upper GI toxicity can be prevented and treated with antisecretory drugs, but these agents do not affect or may even aggravate the small intestinal damage. There is a need for identifying new targets for the treatment of both NSAID-induced gastro- and enteropathy. Over the years, we and other groups have characterized the gastroprotective effect of several drugs in different preclinical models. Our recent projects aim to determine whether these gastroprotective drugs can also ameliorate NSAID-induced small intestinal injury in mice and rats.

Methods: In order to induce enteropathy, C57BL/6 mice and Wistar rats were treated with indomethacin (IND, 20-30 mg/kg) via gastric gavage. Control groups received the vehicle (1% hydroxyethylcellulose). Gastroprotective drugs (i.e. agonists of lysophosphatidic acid type 2 receptor (LPAR2) and α2-adrenoceptor (α2-AR), as well as an inhibitor of fatty acid amide hydrolase (FAAH)) were administered by different protocols via gavage. Intestinal injury was assessed macroscopically, histologically and by measuring the levels of various inflammatory mediators.

Results: 1. Although LPAR2 activation by DBIBB reduced IND-induced tissue damage due to inhibiting epithelial apoptosis, it also aggravated the severity of intestinal inflammation associated with ulceration. 2. Treatment with different doses of α2-AR agonists (clonidine, dexmedetomidine) had either no effect on the severity of enteropathy, or they even aggravated it. This effect was associated with changes in gut bacteria and in the pharmacokinetics of IND. 3. FAAH inhibition by URB597 increased the severity of IND enteropathy in a dose-dependent manner.

Conclusions: We conclude that well-defined gastroprotective strategies do not necessarily provide protection against enteropathy. This can partly be explained by the substantially different pathogeneses of NSAID gastro- and enteropathy. It is important to evaluate in preclinical models the effects of different interventions on both gastro- and enteropathy in parallel.
Grant: NKFI FK 138842.

T-KA3-4 CONTROLLABLE GASEOUS MEDIATORS DELIVERY, PATHWAY-SPECIFIC PROTEINS PERSULFIDATION AND TRANSLATIONAL INSIGHTS INTO BARRETT'S ESOPHAGUS PATHOGENESIS

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Carbon monoxide (CO) and hydrogen sulfide (H_2S) signaling have shown promising potential in preventing gastrointestinal (GI) damage caused by non-steroidal anti-inflammatory drugs (NSAIDs) or ischemia/reperfusion. Moreover, pharmacological donors of CO or H_2S exhibited anti-inflammatory/oxidative properties vs experimental colitis. CO interacts with hemoproteins while H_2S regulates proteins functions under oxidative conditions via posttranslational persulfidation. However, their preventive potential against premalignant Barrett's esophagus (BE) remains unexplored. BE, characterized as an substitution of physiological squamous epithelium (SE) by intestinal metaplasia within the gastroesophageal junction, often results from chronic gastroesophageal reflux disease (GERD).

To address these gaps, we conducted animal and in vitro studies alongside clinical analyses of esophageal biopsies. We employed chemoproteomic method for the wide-scale analysis of persulfidated proteins in clinical BE and GERD-exposed SE. In animal model of chronic GERD/BE and in vitro models utilizing human esophageal cells representing BE or SE exposed or not to clinical GERD, we interventionally up-/downregulated H_2S-bioavailability through pharmacological treatments wit H_2S-donors (NaHS or GYY4137), CO-donors (e.g. BW-CO-111) or genetic knock out (by CRISPR/Cas9) of the CO/H_2S-producing enzymes.

We revealed pathway-specific proteomic and persulfidomic shifts induced by GERD, with significant alterations (>1000 proteins) observed in BE samples compared to GERD-exposed SE. In vitro and in vivo models demonstrated decreased CO or H_2S-bioavailability following chronic GERD exposure. Interventionally restored CO or H_2S signaling inhibited BE-associated molecular pathways and attenuated BE development.

We conclude that GERD-induced fall in CO or H_2S bioavailability activates metaplasia-specific molecular patterns, contributing to BE development. Additionally, GERD precedes BE with pathway-specific proteomic and persulfidomic shifts, initiated early in SE and enhanced in metaplasia. These findings indicate a potential therapeutic potential of H_2S- or CO-signaling modulation to prevent or treat chronic esophageal pathologies.
[Funding: National Science Centre (Poland): 2019/33/B/NZ4/00616, 2023/07/X/NZ4/01052]

T-KA3-5 NOVEL THERAPEUTIC TARGETS IN CHRONIC PANCREATITIS: PRECLINICAL FINDINGS AND TRANSLATIONAL POSSIBILITIES

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Patients with recurrent acute pancreatitis (RAP) are at significant risk of developing early chronic pancreatitis (CP), which progresses into irreversible, end-stage CP with severe symptoms and an increased risk of developing pancreatic cancer. There is no specific therapy in RAP or in early CP that may hinder disease progression. The pathogenesis of CP is complex and involves interactions among multiple cell types, including pancreatic acinar, ductal, and stellate cells (PSC). Therefore, it is pivotal to identify common pathogenic pathways in these cells that could be targeted pharmacologically. The Orai1-mediated store-operated Ca^{2+} entry (SOCE) is a ubiquitous signaling mechanism, which may become overactivated in pathological states resulting intracellular Ca^{2+} overload. In our previous study, we used *ex vivo* and *in vivo* preclinical disease models to demonstrate that Orai1 inhibition prevents progression of RAP and early CP. The selective Orai1 inhibitor CM5480 restored the expression of SOCE-associated regulatory factor in acinar cells, prevented uncontrolled Ca^{2+} elevation, protected acinar and ductal functions, mitigated immune cell infiltration, and diminished PSC activation, proliferation and migration. These results suggest that the overactivation of Orai1 is a crucial pathogenetic event in the progression of early CP, and inhibition of Orai1 could prevent the development of end-stage CP.

T-KB3-1 REPURPOSING PARP INHIBITORS FOR TREATING COVID-19-RELATED INFLAMMATION

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The current COVID-19 pandemic is a global health crisis that calls for the identification of novel drugs to combat COVID-19 disease, for which the swiftest way is drug repurposing. COVID-19 mortality is linked to lung inflammation following the acute viral infection and the subsequent macrophage overactivation syndrome. PARP enzymes play role in the life cycle of coronaviruses and were shown to be pro-inflammatory in models of lung diseases relevant for COVID-19. Based on these, we set out to assess the applicability of EMA/FDA approved PARP inhibitors (PARPi) in COVID-19.

In post-mortem lung tissue from patients who died of COVID-19 we observed markers of oxidative stress and strong PARylation, which varied between the stages of the disease, indicating the involvement of PARP activity in the pathogenesis. We therefore investigated the therapeutic potential of PARPi in models of the disease. First, we showed that in Vero E6 cells rucaparib, but not olaparib or talazoparib, effectively blocks the infection of the wild-type SARS-CoV-2 strain with the D614G mutation. The IC50 of rucaparib was 27.5 μM, higher than the relevant pharmacological concentrations, suggesting off-target effects. Rucaparib was active in neutralization experiments, which might be due to blocking SARS-CoV-2 binding on host cells. This was corroborated by *in vitro* experiments, showing direct target engagement of rucaparib against the SARS-CoV-2 Spike protein. Rucaparib does not inhibit the SARS-CoV-2 macrodomain and does not affect the interferon response directly, but interferon-induced ADP-ribosylation is reduced by high-dose rucaparib, suggesting inhibition of interferon-induced PARPs. Rucaparib was able to inhibit the expression of IL6 in human macrophages with the same efficiency as dexamethasone, the gold standard drug used in clinical settings.

Taken together, our data point for the applicability of rucaparib in blocking tissue damage and the inflammatory response in COVID-19 disease.

Our work was supported by the National Research, Development and Innovation Office of Hungary (K142141, SNN 135335), the University of Debrecen, Hungarian Academy of Sciences (POST-COVID2021-33) and by the Thematic Excellence Programme (TKP2021-TKP-19, TKP2021-TKP-20, TKP2021-TKP-13) of the Ministry for Innovation and Technology in Hungary. FAPESP grants 2018/18007-5 and 2020/05317-6 to NH and the Ministry for Innovation and Technology of Hungary (TUDFO/47138/2019-ITM) to HP and FJ. The research was performed in collaboration with Cell and Tissue Culture Core Facility at the Szentágothai Research Centre of the University of Pécs.

T-KB3-2 ANTINOCICEPTIVE ACTION OF BOTULINUM TOXIN A AND RECOMBINANT BOTULINUM TOXIN-BASED MOLECULES

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Botulinum toxin type A (BoNT-A), the most potent neuroparalytic toxin known, at the end of 20th century has been re-purposed into a valuable therapeutic protein based on its uniquely lasting effects and sustained efficacy after repeated use. Beyond its best-known cosmetic use for reduction of facial wrinkles, locally injected low-dose BoNT-A has become a first-choice therapy in a number of neurological indications, including several hyperkinetic movement and autonomic disorders. This was accompanied by its expanding off-label clinical use of the native toxin preparation in different chronic pain disorders, and its approval for chronic migraine prophylaxis. Driven by these promising clinical reports, a potential novel group of analgesic therapeutics based on recombinant botulinum toxins (BoNTs) has been explored by academia and pharmaceutical companies for more than 20 years. Different re-targeting strategies aiming to achieve a higher relative specificity for sensory neurons, with reduced action in autonomic or motor neurons, have been attempted to expand its relatively narrow therapeutic width and reduce the probability of local or systemic side-effects. These strategies have included recombinant constructs with altered or exchanged membrane acceptor binding moieties, and chimeric neurotoxins combining catalytic domains of different BoNT serotypes. However, despite these efforts, turning these preclinical candidates into a molecule superior to BoNT-A is proving to be difficult with more obstacles than expected. The most notable is a substantially lower potency of re-enginered molecules in experimental pain models, which may translate to higher immunogenicity compared to BoNT-A. Further question, with important implications for current molecular design and preclinical characterization strategies, are the yet uncertain sites and mechanisms of the antinociceptive action of parent molecule itself. While a peripheral site of toxin action has been widely assumed by the scientific community, our ongoing research proposed a necessary role of trans-ganglionic axonal transport and central trans-synaptic traffic for exertion of BoNT-A antinociceptive action. Thus, a better consensus regarding the relevant targeted synaptic sites/neuronal circuits, that, in turn, may lead to improvement of future development strategies of re-engineered BoNT-based analgesics, is needed.

Acknowledgements: The author's research is funded by Croatian Science Foundation (# UIP-2019-04-8277)

T-KB3-3 FRACTALKINE (CX3CR1) AND INTERLEUKIN-1 (IL-1) RECEPTORS MEDIATE NEUROINFLAMMATION AND RELATED HYPERSENSITIVITY IN MOUSE MODELS OF CHRONIC PRIMARY PAIN

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Chronic primary pain conditions, including Complex Regional Pain Syndrome (CRPS) and fibromyalgia (FM) are characterized by major distress, suffering and incapacity without direct underlying cause. CRPS is restricted to one limb after minor trauma, while FM is a widespread pain without injury, but both result in huge medical and socio-economic burdens. The pathophysiological mechanisms are unknown, but complex neuro-immune-vascular interactions and neuroinflammation are involved. Since their current therapies are unsatisfactory, it is crucial to unravel the pathophysiological processes, mediators and targets. The development of chronic pain is often underlain by neuroimmune interactions. Immune cells – microglia and astrocytes – release proinflammatory cytokines like interleukin-1 (IL-1) that modulate neurotransmission and elicit changes in pain sensation. Accumulating evidence highlights the importance of the chemokine fractalkine and its glial receptor (CX3CR1) as well as IL-1 in neuronal-microglial signalling during chronic pain. Therefore, we aimed to investigate the involvements of CX3CR1 and IL1 receptors in pain sensitivity of CRPS and chronic stress-induced FM-like models using gene-deficient mice or pharmacological blockade.

In CRPS, the injury was mimicked by plantar skin-muscle incision and purified plasma IgG of CRPS patients or healthy voluenteers was injected i.p. daily for 7 days. Chronic stress was induced by restrained the animals for 6h/day for 2 weeks. The effects of CX3CR1 antagonist, AZD8797and IL-1 blocker anakinra were used for pharmacological inhibition. Touch sensitivity was measured by aesthesiometry. Astrocyte and microglia markers in pain-related central nervous system (CNS) regions were detected by glial fibrillary acidic protein and Iba1 immunohistochemistry.

CRPS IgG significantly enhanced plantar incision-induced mechanonociceptive threshold decrease (hyperalgesia) by 40-50%. Chronic restraint stress induced 20-25% hypersensitivity. Microglia and astrocyte number/density in the d somatosensory cortex increased in both models demonstrating neuroinflammation. Genetic deletion of CX3CR1 and IL-1 as well as their blockade by the antagonists showed reduced mechanonociception, as well as micro- and astrogliosis in both models.

Our results suggest that CX3CR1 and IL-1 play crucial roles in the pathogenesis of CRPS and stress-related neuroinflammation leading to central sensitization and chronic pain, and might open novel analgesic perspectives in chronic pain therapy.

Affiliation: National Brain Research Program 3.0, NKFIH FK 146283, OTKA K-138046; TKP2021-EGA-16, RRF-2.3.1-21-2022-00015

T-KB3-4 IN VIVO INVESTIGATION OF COMBINED CAPSAICIN-DICLOFENAC CONTAINING TRANSDERMAL PATCH IN RAT MODELS OF ACUTE PAIN

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Pain is the most common symptom that patients complain about in health care settings across all ages. It stays in the background of decreased life quality, suffering and disability meaning a remarkable challenge for healthcare system, as well as a major socio-economic burden. The activation of capsaicin-sensitive sensory nerves via the Transient Receptor Potential Vanilloid 1 (TRPV1) receptor plays an important role in the pathogenesis of several pain conditions. In the medical practice, the TRPV1 agonist capsaicin is usually applied in form of ointment or high-concentration (8%) transdermal patch. However, these formulations cannot ensure precise dosing or cause temporary loss of nerve function, respectively. We have previously developed a silicone polymer matrix-based transdermal patch ensuring not only the precise dosing of the compound but also providing sustained release of that. Moreover, the capsaicin-induced increase of local microcirculation might facilitate the absorption of non-steroidal anti-inflammatory drugs (NSAIDs), e.g. diclofenac. Therefore, we aimed to investigate the analgesic effect of silicone-based polymer matrix transdermal patch containing capsaicin in low-concentration (<1%), or diclofenac or the combination of them in rat models of acute postoperative and inflammatory pain.

Acute postoperative pain was elicited with plantar skin-muscle incision and thermal hyperalgesia was assessed with increasing temperature water bath 18 h after surgery, as well as 2.5 h and 6 h after the application of the patch. Acute inflammatory pain was induced with carrageenan (3%, i.pl.) and mechanical hyperalgesia was determined with dynamic plantar aesthesiometer 3 h after carrageenan treatment, as well as 2.5 h and 6 h after the application of the patch.

Thermal hyperalgesia was decreased 2.5 h after the application of diclofenac-containing patch and 6 h after the application of capsaicin-containing patch. In the case of combined capsaicin-diclofenac containing patch, thermal hyperalgesia was reduced both 2.5 h and 6 h after patch application. Mechanical hyperalgesia was decreased 6 h after capsaicin patch, as well as 2.5 h and 6 h after diclofenac and combined patch.

Low-concentration capsaicin containing silicone-based polymer matrix transdermal patch can alleviate acute postoperative pain. Moreover, it is also able to prolong the short-term analgesic effect of diclofenac, if used in combination. Furthermore, combined capsaicin-diclofenac containing transdermal patch is also effective in the relief of acute inflammatory pain. The combined capsaicin-diclofenac containing transdermal patch might be a promising therapeutic tool in various pain states.

Patent pending: P2200237 (27.06.2022.), PCT/HU2023/050042 (27.06.2023.)

Funding: TKP2021-EGA-13, Proof of Concept "Kapszaicin-diklofenák kombinált hatóanyag tartalmú transzdermális tapasz kifejlesztése és preklinikai vizsgálata"; Kari Kutatási Alap (ÁOK-KA) Dr. Szolcsányi János kutatási alap: KA-2023-07

T-KB3-5 DEVELOPING SELECTIVE KV1.3 INHIBITORS FOR THE TREATMENT OF CHRONIC INFLAMMATORY DISEASES

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Despite significant advances in the development of therapeutic interventions targeting autoimmune diseases and chronic inflammatory conditions, the lack of effective treatment still poses a high unmet need. Modulating chronically activated T cells through the blockade of the Kv1.3 potassium channel is a promising therapeutic approach; however, developing selective Kv1.3 inhibitors remains an arduous task.

Chronically activated T cells play a significant role in the pathogenesis of immune inflammation and autoimmunity. The expression of Kv1.3 and KCa3.1 in T cells changes during activation and differentiation. Initially, T cells up-regulate KCa3.1 upon antigen activation, but with repeated antigen stimulation, they switch to up-regulating Kv1.3. For example, in patients with autoimmune diseases, pathogenic auto-reactive T cells that have been repeatedly stimulated by the relevant autoantigen during the disease exhibit a high expression of Kv1.3. This variation in potassium channel expression between acutely and chronically activated T cells underscores the importance of Kv1.3 as a therapeutic target in chronic inflammatory diseases.

VRG Tx's ISPE technology platform identified lead candidates with high affinity and selectivity for Kv1.3. Our screening system uses phage libraries based on animal toxins and deep sequencing to find potential drugs. VRGT's patentable lead molecule is a best-in-class Kv1.3 inhibitor, promising targeted therapy with minimal side effects. Ex vivo experiments demonstrated its effectiveness in preventing TEM cell activation, suggesting a safer profile than small molecules and lower immunogenicity than large biologics. Our lead compound also showed in vivo efficacy in a rat model of contact dermatitis with a high safety margin.

This research received funding from the National Research, Development, and Innovation (NRDI) Office under grant ID 2019-1.1.1-PIACI-KFI-2019-00127 and the Cooperative Doctoral Programme-2020 managed by the Ministry for Innovation and Technology (ITM).

T-KC3-1 CLINICAL NEED FOR RHYTHM OPTIMIZATION

Przemyslaw Leszek

Chronic heart failure (CHF) is a progressive condition with an uncertain prognosis. In stable, also advanced CHF, resting heart rate (HR) is a strong predictor of mortality and morbidity. In sinus rhythm HR rate reducing agents (β-blockers and/or ivabradine) improve prognosis of patients with CHF.

The cornerstone of CHF therapy are neurohormonal inhibitors (β-blockers, angiotensin converting enzyme inhibitors, aldosterone receptor antagonists).

However in CHF decompensation especially with hypoperfusion and systolic blood reduction, inotropic agents are wildly used. Inotropic agents act via various intracellular proteins ultimately promoting increased systolic release of Ca2+ from the sarcoplasmic reticulum, resulted in positive inotropic effect, but also increasing sinus node depolarization rate, resulting in positive chronotropic effects.

Based on the above observations, it is not clear whether, in a group of patients with decompensated HF, the use of inotropic-positive drugs has a direct positive effect (by increasing contractility) or a negative effect by accelerating HR. This information is especially important in the context of the possibility of for rhythm optimization.

T-KC3-2 EXPERIMENTAL STUDIES - WHAT THEY SUGGEST TO CLINICIANS

Michał Mączewski

Increased heart rate (HR) is a hallmark of a failing heart. On one hand it tends to preserve cardiac output in view of reduced stroke volume and may be regarded as a beneficial hemodynamic adjustment. On the other, though, epidemiological studies indicate that there is a direct relationship between HR and mortality. Furthermore, outcome of heart failure therapy seems to be related to HR change: interventions that increase HR tend to increase, while those that reduce HR tend to decrease mortality. Moreover, human studies indicate that positive force-frequency relation (i.e. a phenomenon in which myocardial contractility increases with increased HR) is reversed in heart failure (HF).

Both experimental and human studies clearly demonstrate that interventions that reduce heart rate (beta blockers, ivabradine) in the chronic treatment of heart failure prolong survival, reduce detrimental remodeling, improve calcium handling and reduce the incidence of arrhythmias. Furthermore, insufficient HR reduction during hospitalization despite beta-blocker treatment in acute decompensated heart failure is a poor prognostic marker.

In a rat model of post-myocardial infarction heart failure with reduced ejection fraction we show that HF shifts downward optimal HR for acute hemodynamic performance and that this phenomenon increases with the progression of HF. Thus HR-reducing interventions can offer immediate hemodynamic benefits additionally to an established long term beneficial anti-remodeling effect.

T-KC3-3 ANTIDIABETIC DRUGS REPURPOSED FOR HEART FAILURE

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The association between diabetes mellitus and heart failure represents a pressing clinical concern, given their shared pathophysiology and escalating prevalence rates. With a broad spectrum of anti-diabetic medications available, ranging from traditional to contemporary agents, understanding their cardiovascular effects and side effects is paramount in clinical practice. We comprehensively examine each drug class, elucidating their mechanisms of action and cardiovascular impacts, emphasizing findings from pivotal clinical trials. Notably, metformin and gliflozins demonstrate favorable effects, while thiazolidinediones and sulfanylureas pose risks in heart failure. Recent insights, such as those from the EMPA-REG OUTCOME trial, proved the therapeutic role for SGLT2 inhibitors in heart failure management, showing superiority in reducing hospitalizations for heart failure. However, the evidence regarding DPP-4 inhibitors is more varied, with certain agents like saxagliptin associated with an increased risk of heart failure hospitalizations. Similarly, two previous trials with GLP-1 analogs (FIGHT and LIVE) enrolled participants with HFrEF, and were neutral regarding their primary outcomes, but raised concern about potential harm. This synthesis underscores the evolving landscape of cardiovascular prevention and heart failure management within the realm of diabetes care, highlighting the potential for these medications to serve as preventive and therapeutic strategies for heart failure, but also raises the issue to look for potential cardiotoxic effect in this patient population.

T-KC3-4 ANTI-GOUT MEDICATIONS REPURPOSED FOR HEART FAILURE

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Inflammation and cytokine release is considered an important feature of cardiovascular diseases (CVD); thus, anti-inflammatory therapies are in the focus of drug development for CVD. Since inflammation is a common pathological feature in both gout and cardiac diseases, some anti-gout medications may exert clinical efficacy in the management of CVD.

Canakinumab, a monoclonal antibody against interleukin-1b (IL-1β) that is used for therapy-resistant gout, has been shown to provide benefit against cardiovascular events, suggesting that blockade of IL-1β secretion and signaling might be a promising therapeutic target in CVD such as heart failure (HF) as well. As inflammasome activation is the main contributor to IL-1β maturation, it was hypothesized that inflammasomes might be contributors to HF progression. In our previous study, we have provided evidence that inflammasome activity plays a pivotal role in HF. Moreover, we also found that probenecid, a well-known and approved uricosuric drug, shows anti-inflammatory properties partially by inhibiting inflammasomes.

Other anti-gout medications show promise as treatment options for CVD. Allopurinol has been believed to reduce CVD-related mortality among patients with ischemic heart disease; thus, it is recommended a first-choice option in patients with both CVD and gout. On the other hand, several clinical studies suggest that colchicine, a drug possessing marked anti-inflammatory activity, can be also beneficial; however, the cost-benefit of this drug should still be determined.

In conclusion, some anti-gout medications may be promising new therapeutic tools in the management of non-gout diseases with inflammatory pathomechanism; therefore, repurposing of anti-gout drugs could provide new strategies against CVDs in the future.

T-KC3-5 IRON DEFICIENCY - A CLINICIAN'S PERSPECTIVE

Przemysław Leszek

Heart failure (HF) is a disease associated with cardiac remodeling, involving many factors, including functional, metabolic myocyte remodeling, fibrosis, and inflammation leading to attenuated heart functionality. An adequate level of iron in the heart plays a vital role in the proper functioning of this organ. The human body contains 3.5 to 4.5 g of iron, depending on sex.

Iron is a crucial micronutrient necessary for many biological processes such as oxygen storage and transportation (as a component of hemoglobin, myoglobin, oxidative enzymes, and respiratory chain proteins) and the synthesis and degradation of proteins, lipids, or nucleic acids (Fe-S cluster formation). An adequate level of iron in the heart plays a vital role in the proper functioning of this organ. Both iron deficiency (ID) and iron overload may develop heart damage.

In HF ID exists in 35%–55% of patients (with a reduced ejection fraction–HFrEF), 59% of patients (with preserved ejection fraction), and 80% of patients (with acute HFrEF). Therefore, an intravenous iron replenishment is becoming a more frequent form of treatment. However still many knowledge gaps exist regarding the complex interplay between ID and HF. Therefore, from the clinical point of view, proper characterization of iron homeostasis in HF seems important.

T-KC3-6 WHAT BASIC RESEARCH TEACHES US ABOUT IRON DEFICIENCY

Aleksandra Paterek

Chronic heart failure (HF) is accompanied by systemic iron deficiency (ID) in as many as 50% of all patients. However, whether iron deficiency is just a marker of HF severity or whether it mediates heart failure progression and outcomes and therefore should be treated is not entirely clear. Several studies demonstrated that ID was associated with increased mortality, reduced exercise capacity and VO_2max and impaired quality of life of HF patients independently of other prognostic factors. However, it is unknown whether ID affects progression of HF or is merely an ominous sign of other contributing comorbidities.

The second crucial question is whether and how systemic ID affects cardiac iron status in HF, i.e. whether the failing heart is really iron deficient under conditions of systemic ID. Healthy mouse heart is remarkably resistant to iron deficiency under ID conditions. Human studies revealed either reduced, unchanged or even increased cardiac iron content in cardiac samples from HF patients.

We addressed these questions in a rat model of post-myocardial infarction heart failure with reduced ejection fraction. The animals were randomized to control, moderate ID and severe ID+anemia (IDA) groups by a combination of phlebotomy and low iron diet for 5 weeks. Serum and hepatic iron content were reduced by 55% and 70% (ID) and by 80% and 77% (IDA), respectively, while cardiac iron content was unchanged in HF rats. Changes in expression of all cardiomyocyte iron handling proteins indicated preserved cardiomyocytes iron status in HF and ID/IDA. Neither ID nor IDA affected left ventricular (LV) systolic or diastolic function or dimensions. Contractile function of LV cardiomyocytes, Ca^{2+} transient amplitude, sarcoplasmic reticulum Ca^{2+} release and SERCA2a function was augmented by ID and IDA as well as resulted in an increase in serum catecholamines.

Thus systemic ID does not result in cardiac ID and does not affect progression of HF and even improves contractile function of isolated LV cardiomyocytes, however, at the cost of increased catecholamine level. This suggests iron handling in HF is much more complicated than we previously thought.

T-KC3-7 EPICARDIAL FAT - HOW IT AFFECTS THE MYOCARDIUM

Michał Mączewski

Epicardial adipose tissue (EAT) is a fat depot covering the heart. No physical barrier separates EAT from the myocardium, so EAT can easily affect the underlying cardiac muscle.
EAT can participate in the development and progression of heart failure with preserved (HFpEF) and reduced ejection fraction (HFrEF). In healthy humans excess EAT is associated with impaired cardiac function and worse outcomes. In HFpEF this trend continues: EAT amount is usually increased and excess EAT correlates with worse function/outcomes. However, in HFrEF the opposite is true: reduced EAT amount correlates with worse cardiac function/outcomes. Surprisingly, while EAT has beneficial effects on cardiac function, it aggravates ventricular arrhythmias.

Dissection of these phenomena may explain these paradoxical findings and ultimately identify a target for novel heart failure therapies aimed at EAT rather than the myocardium itself. But success of this approach depends on thorough understanding of interactions between EAT and the myocardium.

T-KC3-8 INTRAMYOCARDIAL FAT - A NOVEL PROARRHYTHMIC FACTOR

Aleksandra Paterek

Heart failure is the major cause of death attributable to cardiovascular disease, and its prevalence is 1–2%. Patients with end-stage HF present an extremely poor prognosis and current HF therapies exhibit suboptimal efficacy. Thus, there is a need for novel treatment options for HF. As many as half of HF patients die of ventricular arrhythmias (VAs). However, the pathophysiology of VAs is poorly understood and their treatment options are limited.

Thus, there is an urgent need for a better understanding of VAs etiology to develop better preventive and therapeutic strategies.

Cardiac fat can affect the properties of the myocardium, including propensity to arrhythmias. The heart is surrounded by a layer of epicardial fat (epiFat) that is contiguous with intramyocardial fat (inFat) that penetrates between the cardiomyocytes and can affect local cardiomyocytes through secreted cyto-adipo-fibrokines and direct communication as well as generate mechanical barrier to efficient impulse conduction. Recent data indicates that (1) epiFat undergoes a pathological transformation in HF; no such data are available for inFat; (2) epiFat is highly correlated with the risk of VAs in HF; it is unknown if this is a causal relation and moreover no such data are available for inFat; (3) Fatty infiltrates are associated with slow conduction and arrhythmias in human atria and in animal models; (4) High fat diet in rats is associated with increased inFat, Cx43 downregulation, and slower conduction. Lateralization of Cx43 in myocytes adjacent to fibro-fatty infiltrations was also observed.

Since both cardiomyocytes and adipocytes express Cx43, adipocytes in inFat might electrically couple to cardiomyocytes; with their less negative potential, they could both trigger afterdepolarizations and impair conduction. Heterogeneous distribution of inFat inside the heart may generate electrophysiological heterogeneity, favoring arrhythmia development; (5) Several inflammatory adipokines downregulate the expression of connexin genes and repolarizing potassium currents.

Thus inFat is an emerging proarrhythmic factor. Its better understanding may lead to develop more effective antiarrhythmic therapies targeting metabolic factors rather than conventional ion channels.

T-KC3-9 MELANOMA SUBTYPE-DEPENDENT CARDIOTOXICITY TO IMMUNE CHECKPOINT INHIBITOR THERAPY

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Immune checkpoint inhibitors (ICIs) have been revolutionized cancer pharmacotherapy with unprecedented efficacy in the treatment of several malignancies. Despite the excellent therapeutic effects of ICIs, these drugs typically induce a broad spectrum of toxic reactions, mainly due to immune-related adverse events (irAEs). Out of the irAEs, the cardiovascular toxicities are the most severe form, they can be life-threatening complications. The pathomechanism of ICI-induced cardiotoxicity is mostly unknown so far. Therefore, identification of patients who are at risk to develop certain types of cardiotoxicities is currently not possible.

Our aim is to test the hypothesis, suggesting that T cells are targeting an antigen shared by the tumor and the heart in a preclinical model with two different melanoma cell lines. Immunologically "cold" B16F10 and human relevant mutations ($Braf^{V600E}$/wt, $Cdkn2a^{-/-}$ $Pten^{-/-}$) containing YUMM1.7 syngeneic melanoma cells were injected to the right flank of 8 weeks old C57Bl6 male mice. After initial tumor growth, we treated the the animals with anti-PD1 or corresponding isotype control. There is no significant difference in tumor growth between treatment groups. Based on echocardiography results, ICI treatment led to ejection fraction reduction in YUMM1.7 and no change in B16F10 group. Conversely, left ventricle mass increase was found in B16F10 and not in YUMM1.7 tumor group. We confirmed the hypertrophy with WGA and ILB4 lectin histochemistry and qPCR. Cardiac specific gene expression was measured from in vivo grown tumor tissues.

Our results show that anti-PD1 treatment of mice bearing different melanoma subtypes result in various phenotypes of ICI-induced cardiotoxicity. This phenotypic difference could be the consequence of different cardiac specific gene expression.

More thorough testing of ICI therapy in different tumor types with gene expression analysis is needed in preclinical and clinical studies in order to develop stratification score to predict cardiac irAEs development after ICI therapy based on shared antigen hypothesis.

T-KC3-10 EXPRESSION OF KEY IMMUNE CHECKPOINTS IN END-STAGE HEART FAILURE

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Introduction: The key regulatory molecules of the adaptive immune response (co-stimulatory and co-inhibitory) are also known as immune checkpoints (ICs). These ICs are the basis of novel immunotherapies in several devastating diseases, such as cancer, graft versus host disease, or rheumatoid arthritis. Nevertheless, the side effects of ICs-targeting therapies are not fully known. Certain IC inhibitors have cardiotoxic adverse effects and cause heart failure (HF), suggesting that ICs are essential for preserving cardiac homeostasis. However, there is a dearth of information about the expression of immune checkpoints in the healthy and failing human heart.

Methods and results: In this study, our goal was to characterize the expression of co-inhibitory immune checkpoints in patients with end-stage HF. At the time of heart transplantation, samples of myocardial tissue were obtained from patients who had dilated cardiomyopathy (DCM, n=7) and ischemic cardiomyopathy (ICM, n=7). Healthy organ donors whose hearts were not used for transplantation due to technical issues were our healthy controls (n=6). The level of expression of different co-inhibitory IC proteins (PD-L1, PD-1, CTLA-4, LAG3, and B7-H3) in the heart was characterized via Western blot analysis in a smaller cohort. Of the investigated checkpoints, PD-1 and LAG3 did not show differences between DCM and control groups. Meanwhile, in the ICM group, LAG3 significantly increased, and PD-1 was near the significance level. In the case of CTLA-4 and B7-H3, there was no significant differences between the control and HF groups. Furthermore, PD-L1 showed a significant increase in ICM and DCM samples compared with the control. Thus, we validated the results in the case of PD-L1 in a larger cohort of patients with end-stage HF (n=80). Myocardial PD-L1 expression showed a significant negative correlation with left ventricular ejection fraction, a marker of systolic cardiac function.

Conclusions: A negative correlation has been discovered between cardiac function and the upregulation of myocardial PD-L1 in failing hearts. In HF, PD-L1 is a promising target for both diagnosis and treatment and further investigation is required to understand its role in cardiac homeostasis.

T-KA4-1 FROM TANK TO BED IN PXE? USING ZEBRAFISH TO SEARCH FOR PSEUDOXANTHOMA ELASTICUM TREAMTMENTS

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Pseudoxanthoma elasticum (PXE) is a rare, monogenic disorder caused by the ectopic calcification of elastic fibers in connective tissues at various sites in the body. Symptoms generally appear in early adulthood and mostly affect the subcutaneous connective tissue, the vascular walls and the Bruch's membrane of the eye. The disease is caused by a biallelic mutation of the *ABCC6* gene, which encodes a cell membrane transporter protein. ABCC6 is an important player in the physiological calcification process, by regulating the transport of pyrophosphate into the blood. Its loss of function leads to the disruption of the inorganic phosphate (Pi) – pyrophosphate (PPi) ratio in the blood, resulting in the appearance of hydroxyapatite crystal deposits in essentially non-calcifying tissues.

Zebrafish has proved to be a particularly suitable model for this disease, as the mutant animals show a well-characterized phenotype that can be traced even at an early stage of development. Our primary goal is to use our *abcc6a* mutant zebrafish line for testing potential drug candidates, using mainly synthetic pyrophosphate analogues. In addition, we would like to create a humanized transgenic fish line in which the sequence of the zebrafish *abbc6a* gene is partially replaced by the human orthologous sequence. We plan to use this fish strain in the future to get a better understanding of the molecular and environmental background of the disease.

T-KA4-2 3D TISSUE PRINTING IN TOXICOLOGY RESEARCH

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One of the most important steps in drug research and development is establishing whether a potential active substance or drug molecule is effective and safe before clinical trials. Although development processes are becoming faster and more efficient with in-silico models, in vitro and preclinical systems are still essential and provide a wealth of important data early in development.

Classical two-dimensional toxicological tests, such as liver cell lines and primary hepatocytes in cell culture, have been used for many years. However, the models that predict drug effects do not accurately represent in vivo conditions. Drugs are metabolized differently in vivo, and this may affect their effects. A solution to this problem is using three-dimensional systems such as hydrogel cell cultures, magnetic spheroid systems, (3D) tissue, and organ printing, which is a suitable method for creating tissue and organ models that approximate the original tissues' spatial structure and biochemical properties. Artificial tissues produced so far have shown better results than conventional 2D systems. Artificial liver tissues created with modern technology can also be used for high-throughput measurements and lab-on-a-chip applications. Other cell types that make up liver tissue, such as Kupffer cells, endothelial cells, stroma, and stem cells, can also be used in these systems, which increases their complexity. Our preliminary results demonstrate that 3D spheroid systems made from HepG2 cells are suitable for long-term maintenance of liver cells, allowing viability and toxicity studies based on CYP expression patterns.

In addition to liver tissue, 3D-printed skin, kidney, heart, lung, and tumor models, and "disease model tissues" containing cells specific to a certain disease type have appeared in recent toxicology tests. It is important to be aware that creating artificial tissues is not a standardized process. The structure of tissues printed using different printing technologies may vary depending on the type of cells present and the hydrogels used. While 3D printing technology significantly impacts drug discovery and manufacturing, there is no doubt that it will continue to revolutionize these areas in the years to come. Artificial tissues have already been tested in clinical trials, and many 3D-printed medicines have been on the market for some time.

T-KA4-3 „TOP-DOWN" EFFECTS OF PSYCHOACTIVE COMPOUNDS ON A DEFINED SIMPLER NERVOUS SYSTEM ENCODING ASSOCIATIVE MEMORY

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Due to the agricultural, industrial, residential, and recreational activities, many pharmacologically active compounds are being released into the surface waters. One group of these compounds, the psychoactive drug residues, receives special attention as they can potentially affect the neuronal processes of non-target aquatic invertebrate and vertebrate animals.

Previously, our research group detected various psychoactive compounds in Lake Balaton and its catchment area in the concentration range of a few ng/L – µg/L. Besides revealing the spatio-temporal variations of these compounds, we investigate their possible long-term effects on the widely used model species of invertebrate neuroscience and ecotoxicology, the great pond snail (*Lymnaea stagnalis*). This snail exhibits defined behaviours similar to the ones in vertebrates, and the underlying neuronal mechanisms are not fundamentally different from those of vertebrates either. However, due to the simpler nervous system, many neuronal processes can be studied easily in *Lymnaea* than in classic vertebrate models. The aim of the present work was to investigate the „top-down" effects of three psychoactive compounds – carbamazepine (antiepileptics), citalopram (SSRI), tramadol (opioid painkiller and SSRI) – on *Lymnaea* with a characteristically integrative approach. To do so, adult snails were treated with the compounds individually and in a mixture at an environmentally relevant concentration of 1 µg/L for 21 days. After the chronic exposure, behavioural alterations (feeding, locomotion, learning and memory) and their neuronal underpinnings were investigated.

We have showed that both carbamazepine and tramadol treatments significantly decreased the locomotor activity and learning ability. After a 21-day cleaning period, locomotion but not learning recovered. Citalopram or the mixture of the compounds did not cause significant changes in the behavioural patterns investigated. We identified the homologous sequences of the vertebrate $GABA_A$ receptor subunits and showed that they are expressed in the whole central nervous system (CNS) and in an identified key serotonergic interneuron of implicit learning, the Cerebral Giant Cell (CGC). Using electrophysiological recordings, we demonstrated that carbamazepine can block the voltage-gated sodium channel, which plays a role in learning, of the CGC. We identified the homologous sequence of the vertebrate serotonin transporter and showed that it is expressed in both the CNS and the CGC. Using HPLC-MS measurements, we found that the amount of serotonin in the CNS does not change during tramadol treatment, hence we suppose that tramadol affects the reuptake of serotonin.

Our results highlighted that psychoactive drug residues can affect the physiology of aquatic animals even at an environmentally relevant concentration, hence they can lead to a decrease in fitness of members of the ecosystem.

This work was supported by the National Brain Project (#NAP2022-I-10/2022), the Hungarian Scientific Research Fund (#138039), and the Bolyai Foundation (#BO/00646/21/8).

T-KA4-4 THE USE OF IN VITRO MODELS IN TOXICOLOGY STUDIES

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Animal welfare and the 3Rs principles are increasingly being taken into account during the conduct of regulatory toxicology studies, both nationally and internationally. One of the most important tools to facilitate animal welfare is the use of i*n vitro* toxicology models, as significant reductions can be achieved in the number of animals used for toxicology assessment by using those alternative models. In particular, in vitro 3D reconstructed human tissue models which have an OECD guideline became especially important tools in the modern toxicology studies. This presentation will give an overview of these alternative methods.

T-KA4-5 THE POWER OF ZEBRAFISH: SWIMMING TO SUCCESS TO FIND PLANT-BASED ANTIEPILEPTIC DRUG CANDIDATES

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Zebrafish (*Danio rerio*) are commonly used as model organisms in neuroscience studies. Despite their relatively simple nervous system, it shows similarities with the mammalian nervous system. They can lay hundreds of eggs and their development is very fast, therefore a lot of information can be gained relatively quickly. The 5–7-day old zebrafish larvae show behaviors like escaping, hunting, and negative thigmotaxis by swimming. Zebrafish have successfully been used to model different human neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease. It is also a common model to investigate epilepsy and the effect of antiepileptic pharmaceuticals. It is based on analysis of the movement patterns of zebrafish larvae treated with convulsants like pentylenetetrazol (PTZ). In proper concentration, the PTZ-induced epilepsy causes more active swimming, which can be easily measured. With zebrafish larvae large-scale movement analysis can be performed because 96 larvae can be examined at the same time. In addition, EEG can be performed on a larvae 5-day post fertilization and the epileptic signals, or their absence can be observed.

Anti-epileptic drugs are not effective in one-third of epileptic patients therefore new therapeutic approaches are needed. The zebrafish larvae can be treated by adding experimental substances directly into their medium because they can absorb it from water. Our research focused on polyphenolic molecule groups of different plants. We did experiments with curcuminoids, one of the most important active ingredients of turmeric (*Curcuma longa*) and with different flavonoids extracted from the pepper plant (*Capsicum annuum*). The anti-epileptic effect of curcumin and ferulic acid has not been confirmed, but other curcuminoids have great potential. Among the flavonoids luteolin and apigenin did not change the distance moved by zebrafish larvae, while the chlorogenic acid significantly reduced the effects of PTZ.

With the use of zebrafish larvae, numerous novel antiepileptic drug candidates can be examined relatively quickly and cheaply, filtered out thereby the less effective or toxic ones. Pre-screening with zebrafish experiments allows only the most promising drugs to be tested on rats and mice, which is a more expensive, complicated, slower process and means more suffering for the animals. This model paves the way toward finding new anti-epileptic drugs, with the potential to develop new independent or combined therapeutic approaches, which may be even effective in treating drug-resistant patients.

T-KA4-6 HUMAN INDUCED PLURIPOTENT STEM CELL-DERIVED ORGANOIDS FOR DISEASE MODELLING

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For long neurons were considered to be the only cell type affected by neurodegenerative diseases. More recently, astrocytes, a major population of glial cells have been shown to be involved in most neurological diseases too. Two-dimensional (2D) co-culture models of neuron-glia interactions were soon replaced by brain organoids which can model neurodegenerative diseases more accurately, due to their complex cell composition and tissue-like structure. Organoids can be differentiated from induced pluripotent stem cells (iPSCs), which are either reprogrammed from healthy individuals or patients, or can be gene edited to carry disease-relevant mutations or treated by certain factors to induce a disease phenotype.

We have previously shown that air-liquid interface cultures of sliced cerebral organoids (ALI-COs) promote formation of neuronal circuits and the output of functional neurons as well as allowing longer culturing times, leading to the generation of more mature cells. Using this technique, we were able to develop a patient-specific model of amyotrophic lateral sclerosis with overlapping frontotemporal dementia (ALS/FTD), a disease that rapidly leads to muscle paralysis and death with no effective treatment. Developing treatments for ALS/FTD is hindered by the complexity of the disease, since it is not only affecting the cerebral cortex but the spinal cord too. To be able to study early pathomechanisms of ALS/FTD in the spinal cord a novel reproducible patient-derived spinal cord organoid protocol was developed. We demonstrate early disturbances in protein homeostasis, which are becoming more severe over the curse of the *in vitro* culture.

This novel human translational model provides a platform for discovering early pathomechanisms of ALS/FTD in the spinal cord and can be used for drug-screening.

T-KB4-1 TRPV1: BACK ON THE LIST OF TARGETS FOR ANALGESIA

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The transient receptor potential ion channel vanilloid subfamily member 1 (TRPV1) is a noxious heat transducer expressed in a major proportion of nociceptive primary sensory neurons (nPSN). TRPV1 is engaged in three major functions in nPSN; it contributes to the detection of noxious heat by healthy tissues, provides input for thermoregulatory mechanisms, and is essential for the development and persistence of burning pain (heat hyperalgesia) associated with inflammatory process that follows tissue injury. Due to TRPV1's essential role in the development and persistence of inflammatory heat hyperalgesia, a series of TRPV1 inhibitors has been developed as putative novel analgesics. However, while the compounds effectively control inflammatory heat hyperalgesia, they all failed because they induce hyperthermia and increase the pain threshold for noxious heat. which increases the risk of burn injury. Here, we provide evidence that the three functions of TRPV1 in nPSN are segregated both at the cellular and regulatory levels. Hence, only a sub-population of TRPV1-expressing nPSN are involved in the development and persistence of inflammatory heat hyperalgesia. These findings open novel possibilities to target TRPV1 for analgesia.

T-KB4-2 MECHANISMS OF THE THERMOREGULATORY EFFECTS OF TRPV1 ANTAGONISTS

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Antagonists of the transient receptor potential vanilloid-1 (TRPV1) channel often cause thermoregulatory effects in experimental animals and humans, which can be hyperthermia (more frequently) or hypothermia.

Polymodal TRPV1 antagonists, which block all three major modes of TRPV1 channel activation (viz., vanilloid, proton, and heat), cause hyperthermia. Mode-selective TRPV1 antagonists, which inhibit the vanilloid activation mode, but potentiate the proton activation mode, cause hypothermia. Both thermoregulatory effects are produced when the antagonist blocks (hyperthermia) or potentiates (hypothermia) the tonic activation of TRPV1 by protons in the abdomen, thereby altering thermogenesis and skin vasoconstriction. In humans, the development of a hyperthermic effect depends on the potency of the antagonist in inhibiting not only the proton mode but also the heat-activated mode, whereas the vanilloid mode has no role.

The neural pathway of the hyperthermic response to TRPV1 antagonists was identified recently. The TRPV1 antagonist-induced hyperthermia was not attenuated by either bilateral vagotomy or bilateral transection of the greater splanchnic nerve. However, the hyperthermia was attenuated by bilateral cervical transection of the spinal dorsolateral funiculus. The non-splanchnic, but spinal mediation of TRPV1 antagonist-induced hyperthermia was proposed to be triggered by abdominal signals that originate in skeletal muscles (and not the viscera). Within the brain, the central projections of the TRPV1-expressing abdominal afferents impinge on the thermoregulatory pathways driving autonomic cold defenses.

The identified neural pathway can serve as the mechanism of the previously proposed acido-antithermogenic and acido-antivasoconstrictor reflexes, which may play an important role in the regulation of deep body temperure during physical activity.

The research summarized herein was supported, in part, by the National Research, Development and Innovation Office of Hungary (grant FK 138722).

T-KB4-3 SENSORY TRP CHANNELS IN THE HUMAN DENTAL PULP AND THEIR ROLE IN PULPITIS

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Pulpitis, the most common inflammatory disease of the teeth, is often associated to hyperalgesia toward various physical stimuli, especially cold. Transient receptor potential (TRP) ion channels expressed in somamtosensory fibers are primary sensors of the external temperature and changes in their expression and sensitivity crucially contribute to the development of various forms of hyperalgesia or allodynia. However, TRP channels expressed in non-neuronal cells in the tissues can also shape sensory and inflammatory processes. In this study, we investigated sensory TRP channels in human dental pulp derived cells isolated from healthy molar teeth.

We identified the expression of various warm- and cold-sensitive TRP channels in primary human dental pulp cells (hDPCs). The functionality of TRP channels was investigated by using specific agonists. In some experiments, hDPCs were differentiated into odontoblast-like cells (OBLCs) and the role of TRP channels in mechanosensation of OBLCs was also investigated.

To investigate the role of TRP channels in pulpal inflammation, we established inflammatory conditions in hDPC cultures by applying various ligands as pathogen and damage associated molecular patterns activating Toll-like receptors. We found that poly(I:C), a ligand of Toll-like receptor 3 (TLR3), induced robust inflammatory responses, whereas other ligands were less effective. Poly(I:C) stimulated production of pro-inflammatory cytokines, induced oxidative stress and highly upregulated TRPA1, a cold sensitive nociceptor in the TRP family. In poly(I:C) induced inflammatory conditions, intracellular Ca^{2+} signals evoked by TRPA1 ligands were highly potentiated. Poly(I:C)-treated cells displayed increased Ca^{2+} responses to H_2O_2 which was abolished by TRPA1 antagonism. Moreover, poly(I:C) resulted in mitochondrial disfunctions and diminished cellular viability which was alleviated by antioxidant glutathione and partly by TRPA1 antagonism or silencing. In our current experiments we are investigating novel pharmacological tools to alleviate poly(I:C) induced inflammation.

Our results suggest that TRP channels in the dental pulp are promising pharmacological targets to combat pulpitis and alleviate inflammation related pulpal tissue damage.

The research was supported by NKFI 134725, 134791, and ÚNKP-23-3-II-DE-430.

T-KB4-4 TRPM4 IN HILAR MOSSY CELLS, A ROLE IN EPILEPSY

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Mossy cells comprise a large fraction of excitatory neurons in the hippocampal dentate gyrus, and their loss is one of the major hallmarks of temporal lobe epilepsy (TLE). The vulnerability of mossy cells in TLE is well known in animal models as well as in patients; however, the mechanisms leading to cellular death is unclear.

Transient receptor potential melastatin 4 (TRPM4) is a Ca^{2+}-activated non-selective cation channel regulating diverse physiological functions of excitable cells. Here, we identified that TRPM4 is present in hilar mossy cells and regulates their intrinsic electrophysiological properties including spontaneous activity and action potential dynamics. Furthermore, we showed that TRPM4 contributes to mossy cells death following status epilepticus and therefore modulates seizure susceptibility and epilepsy-related memory deficits. Finally, we demonstrated that *in vivo* application of meclofenamate a novel antagonist of TRPM4 before the induction of status epilepticus reduces the frequency and duration of seizures in mice.

Our results provide evidence for the role of TRPM4 in MC excitability both in physiological and pathological conditions.

T-KB4-5 DIFFERENT CONTRIBUTIONS OF PRIMARY SENSORY NEURON SUBPOPULATIONS TO THE INITIATION OF NERVE INJURY INDUCED SPINAL MICROGLIA ACTIVATION

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Peripheral nerve injuries induce spinal microgliosis which plays a decisive role in the development of neuropathic pain behaviour. The ensuing neuro-glial interactions have been demonstrated to contribute to the altered nociceptive processing in the spinal dorsal horn. Several agents of primary afferent origin causing the microglial reaction have been identified, but the type(s) of primary afferents that release these mediators and activate the microglial cells are still unclear. To identify the type(s) of primary afferents involved in the microglial response selective chemodenervation by capsaicin or transection of peripheral nerves having different peripheral target specificities were applied.

Comparative quantitative morphometric evaluation of the microglial reaction in the central projection territories of intact and injured peripheral nerves in the superficial (laminae I and II) and deep (laminae III and IV) spinal dorsal horn revealed a significant, about three-fold increase in microglial density after transection of the sciatic or the saphenous nerve. Prior perineural treatment of these nerves with capsaicin, resulting in a selective defunctionalization of TRPV1 expressing C-fiber afferents failed to affect spinal microgliosis. Similarly, peripheral nerve injury-induced increase in microglial density was unaffected in rats treated neonatally with capsaicin known to result in a near-total loss of C-fiber dorsal root fibres. Perineural treatment with capsaicin per se did not evoke a significant increase in microglial density.

We also demonstrated that while axotomy of a low number of cutaneous afferents (partial transection of the saphenous nerve) produced detectable microglial reaction in the spinal dorsal horn, transection of multiple branches of the tibial nerve damaging high number of muscle afferents failed to induce significant microglial reaction in their spinal projection territories.

These observations indicate that injury-induced spinal microgliosis may be attributed to phenotypic changes in injured myelinated primary afferent neurons, mainly belonging to the subpopulation of cutaneous afferents, whereas the contribution of C-fibre primary sensory neurons to this neuroimmune response is negligible. Spinal myelinated primary afferents may play a hitherto unrecognized role in regulation of neuroimmune interactions. These observations may bear of importance for the mechanisms of neuropathic pain brought about by damage to peripheral nerves.

Acknowledgement: This work was in part supported by the research grant of NRDIO K138568

T-KC4-1 MODULATION OF AQUAPORIN-4 EXPRESSION BY TRIFLUOPERAZINE AUGMENTS FUNCTIONAL RECOVERY AFTER EXPERIMENTAL ISCHEMIC STROKE

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Astrocytic aquaporin-4 water channels (AQP4) are central to edema evolution after acute ischemic stroke (AIS). The efficacy of AQP4 inhibition is inconclusive because AQP4 is implicated in cytotoxic edema formation early after AIS but facilitates edema resolution days later. Trifuloperazine (TFP) has recently been suggested to attenuate edema formation in the injured spinal cord by the modulation of calmodulin dependent AQP4 trafficking. Here, we have explored the impact of TFP on functional outcomes after experimental AIS.

Three sets of experiments were performed on male isoflurane anesthetized C57BL/6 mice (n=37). In the first set, AIS was induced by transient (60 min) middle cerebral artery occlusion (MCAO). Neurovascular coupling (NVC) was tested 3 days post-AIS. Infarct volume and edema formation were estimated with MRI. In the second set, recurrent spreading depolarizations (SDs) were elicited in the optimally perfused cortex with 1M KCl. CBF was measured with laser-Doppler flowmetry or laser speckle contrast imaging. Neuronal activity was monitored with extracellular field potential recording. In the third set, brains were harvested 3 days after MCAO for Western-blot analysis of AQP4 expression. TFP or its vehicle were administered for calmodulin inhibition or as control.

TFP decreased infarct size measured in T2 MRI sequences (23.87±17.65 vs. 45.95±22.90 mm^3, TFP vs. Control) and restored the impairment of functional hyperemia with NVC after AIS (20.7±8.8% vs. 4.0±3.8%; TFP vs. Control), although did not change edema volume (21.1±6.9 vs. 18.8±16.1 %HSE, TFP vs. Control). Likewise, TFP-treated mice displayed greater hyperemia coupled to SD (37.39±17.07 vs. 46.95±25.22%; TFP vs. Control). SD frequency was unaltered (8 and 9 SDs; TFP and Control), but SD amplitude gradually increased after TFP treatment (18.3±3.4 vs. 14.3±6.2 mV; TFP vs. Control). TFP administration reduced the protein level of the AQP4 tetramer complex.

Astrocyte end-foot swelling due to water influx has been implicated in the narrowing of the microvascular lumen and the reduction of CBF. We propose that TFP might temper the anchoring of AQP4 complexes to the astroglial cell membrane and thus restore NVC after AIS and augment the hyperemic response to SD. Based on these data, the pharmacologic modulation of AQP4 assembly in the astrocyte cell membrane is a therapeutic target to improve CBF after AIS.

Funding: H2020 No. 739593, ERANET JTC2022 IMATRIX, NKFIH K134377 and FK142218, NAP3.0, TKP2021-EGA-28, SZAOK Research Fund.

T-KC4-2 PREDICTING DRUG RESPONSE USING GENE EXPRESSION SIGNATURES IN CELL LINE MODELS

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Introduction: Recent advancements in sequencing technologies have empowered researchers to comprehensively analyze entire transcriptomes across extensive sample cohorts. These have unveiled the potential for identifying distinct subgroups by concurrently examining hundreds to thousands of genes. While the standard therapeutic approach for Small Cell Lung Cancer (SCLC) remains confined to chemotherapy and radiotherapy, recent breakthroughs have pinpointed a subset of patients exhibiting enhanced responsiveness to therapies. This progress underscores the criticality of pinpointing gene expressions indicative of drug response. Our objective was to develop a framework capable of assessing drug responsiveness based on gene expression patterns within cell lines.

Methods and Results: Cell line gene expression and drug response datasets were aquired from the SCLC-CellMinerCDB, a database that aggregates genomic and drug response information from various sources (NCI, GDSC, CCLE, and UTSW). We tested novel and known gene signatures utilizing diverse metrics such as variability, mean expression, and bimodality. Various clustering techniques (including k-means, NMF, and DBscan) were explored, both with and without prior dimensionality reduction (e.g., tSNE, UMAP, and neural networks), alongside gene set enrichment analysis methods. Our analyses successfully predicted cell line drug responses using multiple gene signatures, validating the feasibility of a system enabling researchers to evaluate drug responsiveness in cell lines utilizing novel gene expression patterns.

T-KC4-3 THE CLINICAL SIGNIFICANCE OF EPIGENETIC, RNAPII AND TRANSCRIPTIONAL VARIABILITIES OCCURRING IN CLEAR CELL RENAL CELL CARCINOMA AS A POTENTIAL PROGNOSTIC MARKER

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Patients diagnosed with clear cell renal cell carcinoma (ccRCC) have poor prognosis for recurrence and approximately 30-40% of them will later develop metastases. For this reason, the appropriate diagnosis and the more detailed molecular characterisation of the primary tumour, including its susceptibility to metastasis, are crucial to select the proper adjuvant therapy by which the most prosperous outcome can be achieved. Nowadays, clinicopathological variables are used for classification of the tumours. Apart from these, molecular biomarkers are also necessary to improve risk classification, which would be the most beneficial amongst modern adjuvant therapies. As a potential molecular biomarker, to follow the transcriptional kinetics in ccRCC patients (n=30), we analysed epigenetic changes (γH2A.X, H3K4me3, and H3K9me3) and the alterations in the level of RNA polymerase II (RNAPII) by immunohistochemical staining on dissected tissue sections. The variabilities between the tumorous and non-tumorous parts of the tissue were detected using quantitative image analysis by monitoring 30 cells from different positions of either the tumorous or the non-tumorous part of the tissue sections. Data obtained from the analyses were used to identify potential prognostic features and to associate them with the progression. These markers might have a value to predict patient outcomes based on their individual cellular background. These results also support that detection of any alteration in the level of H3K4me3, H3K9me3, and γH2AX can account for valuable information for presuming the progression of ccRCC and the clinical benefits to select the most efficient personalized therapy.

T-KC4-4 HOW NEURONS IN HUMAN BRAIN ARE DIFFERENT FROM ANIMAL MODEL CELLS, AND WHY THIS IS IMPORTANT?

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All mammalian species have largely similar brain comprising analogous neuron types which develop, function, and exhibit pathological processes in quite similar way. However, as a product of evolution there are tiny differences between mammalian species in the neurons' basic function and structure. Human neurons exhibit various physiological features different from the corresponding cell types in the rodent brain. During the past decade, increasing amounts of neuronal features specific to human have been reported, yet their significance is still poorly understood. I highlight here some differences between specific neuron types in the human and rodent brain, and suggest teleological explanations to their existence.

T-KC4-5 ADRENAL INFLAMMATION IN HEART FAILURE

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Inflammatory mediators such as interleukin-1β (IL-1β) are key triggers of the progression of cardiovascular diseases and it is now increasingly recognised that IL-1β-driven inflammation is an important factor in the pathophysiology of heart failure as well. This is supported by recent clinical trials (e.g. CANTOS) highlighting that IL-1β is a promising new target for the therapy of cardiovascular diseases. The adrenal gland is part of the neuroendocrine maladaptive responses involved in heart failure pathophysiology and established therapeutic approaches such as beta blockers and RAAS inhibitors are targeting these processes. However, despite the fundamental importance of the adrenal gland in heart failure, the exact local changes in the tissue (i.e. inflammatory processes) are not characterized. Here, we show, using a rat left anterior descending artery ligation induced heart failure model system, that IL-1β and NLRP3 inflammasome expression is elevated in the adrenal gland of these animals. We also demonstrate that colony stimulating factor 1 receptor (Csf1r)- and CX3C motif chemokine receptor 1 (Cx3cr1)-positive macrophage populations are major sources of IL-1β in the adrenal gland. Finally, we show that a subset of steroidogenic cells in the adrenal cortex also express IL-1β during heart failure. Adrenal gland inflammation may therefore contribute to pathological inflammatory and neuroendocrine events in heart failure and represent a potential new therapeutic target to slow down the progression of the disease.

T-KC4-6 AGING-ASSOCIATED WEAKENING OF THE ACTION POTENTIAL IN FAST-SPIKING INTERNEURONS IN THE HUMAN NEOCORTEX

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Aging is associated with the slowdown of neuronal processing and cognitive performance in the brain; however, the exact cellular mechanisms behind this deterioration in humans are poorly elucidated. Recordings in human acute brain slices prepared from tissue resected during brain surgery enable the investigation of neuronal changes with age. Although neocortical fast-spiking cells are widely implicated in neuronal network activities underlying cognitive processes, their high metabolic demand makes them vulnerable to neurodegeneration. Herein, we analyzed the electrical properties of 166 fast-spiking cells in neocortex samples from individuals aged 11–84 years. By studying the electrophysiological features of action potentials and intrinsic excitability, we report that action potential overshoot significantly decreases and spike half-width increases with age. Moreover, the action potential maximum-rise speed (but not the repolarization speed or the afterhyperpolarization amplitude) significantly changed with age, suggesting a particular weakening of the sodium channel current generated in the soma. Cell intrinsic excitability, which is measured as the input resistance, membrane time constant, and cell capacitance remained unaffected by senescence. Thus, we conclude that the action potential in fast-spiking interneurons shows a significant weakening in the human neocortex with age. This may contribute to the deterioration of cortical functions with age.

T-KA5-1 THE POWER OF MULTIOMICS

Ágnes Angyal

GeneTiCA Kft.

Nowdays, combining several technologies in multiomics is becoming standard. Several studies show that the greatest benefit in such approaches is proteomics and transcriptomics. That is why we are already introducing such technologies that facilitate access to the plasma proteome as well as transcriptomics in space that is full of unexplored mysteries.

T-KA5-2 BIOTECH – A KEY PARTNER IN STRUCTURAL BIOLOGY SOLUTIONS FOR A PHARMACEUTICAL INDUSTRY

Josef Uskoba

BioTech a.s.

„BioTech is a supplier of an alternative solutions for structural biology - complete/physico-chemical characterization of interaction kinetics under defined conditions at the molecular or cellular level and in multiplex mode, without the need for labeling"

T-KA5-3 DEEPER INSIGHTS INTO CELL MODELS WITH AGILENT CELL ANALYSIS INSTRUMENTS

Péter Keresztúri

BioTech Hungary Kft.

Advances in automated cell imaging, real-time cell analysis, and flow cytometry are powering scientific discovery and advancing groundbreaking therapeutics. For unlocking the true potential of immuno-oncology and drug discovery research by understanding crucial biological insights and changes in cells in real-time Agilent invented the only platform on the market, which capable of collecting live-cell imaging and real-time impedance data at the same time .The xCELLigence RTCA eSight measures and monitors the health and viability of a cell population to fully understand the effects of a new drug, cell or gene therapy. We would like to present the benefits of the equipment by maintain the integrity of experiments and the cellular environment by analyzing viable, living cells, geting information-rich analysis by multiple readouts and the power of dual technology.

FRIDAY
7 JUNE 2024

F-KA OXIDATIVE STRESS AND CELL DEATH: ROLES OF PARP1, AMPK AND CASK

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AMPK is among the most potent regulators of cellular energy balance and is a key player in a variety of physiological processes. AMPK might be considered one of the most promising targets for both prevention and treatment of diseases. PARP1 is a multifunctional enzyme involving in DNA repair, gene regulation and cell viability control. Calcium-calmodulin dependent protein serine kinase (CASK) is a multidomain scaffold protein and its major identified function is most in the brain. In this talk, I will mention how these proteins regulate various types of cell death (apoptosis, autophagic death and parthanatos) under oxidative stress. AMPK pre-activation protects retinal pigment epithelial cells and Müller cells against UVA and methylglyoxal.

The protection major results from the reduction of mitochondrial ROS production and in turn mitochondrial fission. On the other hand, in retinal pigment epithelial cells UVA induces ROS-PARP-1-AMPK-autophagy axis, but decreases lysosomal mass, leading to autophagolysosome accumulation. Finally I will show the role of CASK in mediating H2O2-induced apoptosis and parthanatos in various cell types.

F-KA1-1 ANTIVIRAL THERAPIES: CYCLODEXTRINS IN DUAL FUNCTION

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Cyclodextrins (CDs) as safe and efficient enabling excipients have long been used in pharmaceuticals. Currently, over 100 human drug products are approved that contain CDs.
The presentation deals with the application of CD technology addressing to the challenge of viral infections. The utility of CD technology serves antiviral therapies in two major ways:
CDs, as enabling excipients and CDs, themselves as drug actives.

1.**Cyclodextrins as excipients:** Cyclodextrins (CDs) as enabling excipients can improve physicochemical properties, e.g. the aqueous solubility, stability, bioavailability, antiviral efficacy of different synthetic drug actives. The presentation will review by listing examples of antiviral drug-CD complexes (some of them already approved), how molecular encapsulation improves the pharmaceutical utility and therapeutic potency of antiviral drugs.
An example where CD-technology has clearly proven its utility and potency in antiviral therapy: the case COVID-19 pandemy and role of Veklury® (remdesivir-sulfobutylether-beta-CD) infusion treaments.

2.**CDs as pharmaceutical actives:** One of the typical functional propensitiy of CDs is their affinity to entrap lipids, such as cholesterol, phospholipids, etc). CDs selectively complex biologically important lipids.

The lipid affinity of CDs are utilized in the removal and mobilization of lipids of envelop-type viral coatings, which results in the viricidal effect of envelop viruses (corona viruses, HIV, influenza, West Nile virus, Dengue, etc)

Another lipid-CD interaction occurs at cell membrane lipid rich domains (rafts), where certain viruses will land and infect the target cells. This unique antiviral mechanism of action of CDs will be demonstrated by showing marketed products for prevention of viral infections. (CD-coated facial masks and CD-containing throat gargle and CD-based antiviral skin and nasal sprays).

F-KA1-2 APPLICATION OF CYCLODEXTRINS IN TRADITIONAL AND ALTERNATIVE DRUG FORMULATIONS; CASE STUDIES

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Cylodextirns (CDs) and their derivatives have received considerable attention in the pharmaceutical field. Various physiochemical properties of drugs can be altered through CD complexation, especially drug solubility in aqueous biological media. In aqueous media, drug molecules of appropriate size and structure will enter into the central cavity of CD molecules to form water-soluble complexes and, frequently, enhanced total drug solubility is observed. Efforts to innovate existing medication include the development of medicines with higher selectivity of action, less toxicity and side-effects, higher stability, a more favourable pharmacokinetic profile and improved patient compliance.

Our research interest focused on the development of preparation protocols using CDs combined with other additives to produce suitable formulations (to reach local or systemic effect) to get effective therapies in different diseases. Meloxicam-potassium (MXP) and ibuprofen (IBU) were used for preparation of samples which could be used by intranasal and pulmonary applications.

Differently charged – neutral (BCD), cationic (QABCD) or anionic (SBECD) – β-cyclodextrins were applied as permeation-enhancers, and (polyvinyl)alcohol (PVA) was used as a viscosity increasing excipient. Polymer-containing and polymer-free samples were prepared by nano spray-drying. The particle size distribution, morphology, crystallinity of the formulations, interactions between the materials, in vitro mucoadhesion, dissolution and permeation of the drug were examined. The charged cyclodextrin-containing formulations had higher in vitro mucoadhesion than BCD containing formulations, the dissolution of MXP was fast from all samples. The permeation of the drug was the highest in the case of powders containing QABCD, the diffusion of MXP was improved in all cases in the presence of PVA.

Cyclodextrin-based metal-organic frameworks (also known as CD-MOFs) are highly porous materials with non-toxic characteristics that are formed by coordinate bonding between alkali metal cations and cyclodextrins. Research on CD-MOFs is still in its infancy, but their potential for use in drug delivery-particularly in the lung seems promising. Our aim was to use the freeze drying (FD) method in one step to create a novel approach for the preparation of CD-MOFs. MOFs consisting of γ-cyclodextrin (γCD) and potassium cations were employed to encapsulate the poorly water-soluble model drug Ibuprofen (IBU). In summary, IBU/CD-MOF complexes were prepared, in addition to the simplicity of the method, it takes less time compared to traditional methods, with the use of lower amount of organic solvents and more efficient conversion. The complexes have tunable crystallinity and morphological properties, and increased IBU's solubility, and achieved rapid active ingredient release. Their use can be beneficial in dry powder inhalation preparations due to their micrometer size, morphology and nanoporous structure.

Acknowledgement: This work was supported by the NKFI OTKA K_146148 project

F-KA1-3 TARGETING CANCER CELLS BY CYCLODEXTRINS VIA ENDOCYTOSIS

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Cyclodextrins have various biological effects which are based on their interaction with biomolecules, and it was also revealed, that cyclodextrins can enter the cells by endocytosis. The cellular delivery of cyclodextrins established their therapeutic application in cholesterol storage disorders to eliminate the intracellularly accumulated cholesterol from the cells. The endocytosis of cyclodextrin derivatives has a great potential to develop new targeting strategies, however it has not been studied and compared yet on different cell lines. Our aim was to characterize the physicochemical properties of fluorescein-, and rhodamine-labelled derivatives and test the endocytosis of fluorescein-labelled hydroxypropyl-beta-cyclodextrin (FITC-HPBCD) and random methyl-beta-cyclodextrin (FITC-RAMEB) in different cancer cell lines. An attempt was made to find physicochemical and biological attributes, that affect their endocytosis. Octanol-water partition coefficient and molecular association was determined, and significant differences were revealed among the fluorescent derivatives of HPBCD and RAMEB. Rhodamine labeling had more pronounced effect on the molecular association, and lipophilicity of the molecules. Cancer cell lines showed major differences in the endocytosis of the cyclodextrins and interestingly the uptake of HPBCD and RAMEB was different in the tested cell lines. The expression of KRAS protein and its role in the endocytosis of cyclodextrins was investigated as well as the in vivo tumor uptake of the radiolabeled derivatives in tumor models. The results showed the applicability of cyclodextrins in targeting various types of cancer via the endocytic pathway.

F-KA1-4 METHYLATED CYCLODEXTRINS: UNDERSTANDING QUALITY – BIOACTIVITY RELATIONSHIPS

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Some neurodegenerative diseases, such as Alzheimer's disease, Huntington'disease, Parkinson's disease are characterized by alterations of lipid metabolism and homeostasis in the brain. The rare lysosomal storage disease, Niemann Pick disease type C is also characterized by disturbed cholesterol metabolism.

Cyclodextrins are cyclic oligosaccharides which interact with various lipid components of cell membranes, such as cholesterol, phospholipids and sphingolipids. While fatty acids, phospholipids and sphingolipids prefer the alpha-cyclodextrin cavity, cholesterol is complexed first of all by the beta-cyclodextrin and its derivatives. Methyl beta-cyclodextrins have extreme affinity to all of these lipids, which are common constituents of cell membranes of animals and plants.

Various brands of methylated beta-cyclodextrins have been marketed differing in the degree of substitution that is the number of methyl substituents in a cyclodextrin ring. They are randomly substituted derivatives consisting of a large number of isomers. The distribution of these isomers is also a key factor determining the affinity toward lipids. The detailed characterization of the structure helps in understanding the structure – bioactivity relationships.

Several examples will be shown on how the structure of methylated cyclodextrins, e.g. degree and pattern of substitution as well as isomeric purity influence the interaction with cholesterol, fatty acids and phospholipids. The potential pharmacological effects will be envisaged, too.

F-KA1-5 ANALGESIA VIA LIPID RAFT DISRUPTION BY CYCLODEXTRINS

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Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) are nonselective cation channels highly expressed on nociceptive sensory nerve terminals and primary sensory neurons. Their involvement in pain integration and inflammation is well described. It is well-known that their activation is facilitated by cholesterol-rich lipid microdomains (lipid rafts) located in the membrane. Previous experiments demonstrated that cyclodextrin (CD) derivatives forming an inclusion complex with cholesterol thus depleting it from membrane raft regions can reduce receptor activation *in vitro*, potentially exerting analgesic effect *in vivo*. Our aim is to further investigate the effect of these lipid-protein hydrophobic interactions on TRPA1 and TRPV1 activation and to identify CD derivatives as analgesic and anti-inflammatory agents with novel mechanisms of action.

We compared three different CD derivatives selected on basis of our previous results: random methylated β-cyclodextrin (RAMEB, 3 mM), (2-hydroxypropyl)-β-cyclodextrin (HPBCD, 10 mM) and sulfobutylether-β-cyclodextrin (SBECD, 10 mM) (CycloLab Cyclodextrin Research and Development, Ltd.). Analgesic effect of the compounds was evaluated in different acute pain models after CD pretreatment applied 30 minutes before selective TRPV1 or TRPA1 receptor agonist treatment in 12-16-week-old male NMRI mice. Acute somatic chemonociception was induced by intraplantar (i.pl.) injection of formalin (2.5 %, 10 µL) to the hind paw of mice and nocifensive behavior was measured. Resiniferatoxin (RTX, 0.1 ug/mL, 10 uL, i.pl.)-induced acute thermal allodynia and mechanical hyperalgesia were measured by increasing temperature hot plate and dynamic plantar aesthesiometer, respectively. The cholesterol depleting effect of 30-minute i.pl. CD treatment was measured from mouse plantar skin homogenate by colorimetry using Abcam Cholesterol Assay kit. Mustard oil (MO, 1%)-induced acute skin inflammation was investigated on mouse ear based on the changes in cutaneous blood perfusion by laser speckle contrast analysis (PeriCam System) in a 0-20 minutes period after MO smearing (10-10 µL on both sides).

CD pretreatment significantly reduced the duration of nocifensive behavior during the second phase of formalin-induced acute inflammatory pain evoked by the release of inflammatory mediators, as well as RTX-induced mechanical hyperalgesia 30 and 90 minutes following RTX administration. However, thermal allodynia was not affected by CD pretreatment. CD treatment reduced the total cholesterol content in the plantar skin of mice compared to the cholesterol content measured in control animals treated with physiological saline revealed by Abcam Cholesterol Assay kit. In MO-induced acute skin inflammation model cutaneous blood perfusion was found to be significantly lower in CD-pretreated animals compared to the control group.

Based on our findings we conclude that the investigated CD derivatives are promising agents for exerting peripheral analgesia and anti-inflammatory actions via a novel mechanism.Our future plan is to reveal the mechanism and investigate these derivatives in chronic inflammatory and pain models.

Funding: TKP2021-EGA-16 and TKP2021-EGA-13 (Development and Innovation Fund of Hungary); RRF-2.3.1-21-2022-00015 (Pharmalab); ELKH-PTE, Hungarian Research Network (HUN-REN PTE Chronic Pain Research Group), Pécs, Hungary; NKFIH K 138936

F-KB1-1 CHALCOGEN-CONTAINING 1,3,5-TRIAZINE COMPOUNDS IN SEARCH OF BREAKTHROUGH THERAPY FOR NEURODEGENERATIVE DISEASES

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In search for an innovative therapy of neurodegenerative diseases G-protein coupled receptors (GPCRs) are an important protein target. Among them, the serotonin 5-HT_6 receptor (5-HT_6R), one of the latest identified members of serotonin receptors' family, is under special interest for over 25 years. Due to special location almost exclusively in CNS - in the brain areas responsible for memory and cognitive functions, the 5-HT_6R ligands are promising for the treatment of cognitive dysfunctions associated with Alzheimer's disease (AD). Although their activity was confirmed in animal models and several 5-HT_6R antagonists entered clinical trials, none has passed them to reach pharmaceutical market to date. A narrow chemical space of 5-HT_6R antagonists containing predominantly sulfone and/or indole moieties, is considered one of the causes of these failures. In this context, we started to explore a new series of non-indole and non-sulfone 1,3,5-triazine derivatives, which allowed us to find a population of active 5-HT_6R agents with nanomolar affinities in radioligand binding assays. After subsequent pharmacomodulations, the compounds with chalcogen-containing linkers turned out "hits" that displayed high selectivity, strong antagonistic action in functional assays, profitable CNS-drugability *in vitro*, procognitive effects and satisfying pharmacokinetics *in vivo* in rats. In particular, Se- and thioether-containing compounds, seem to be promising in search for breakthrough therapy of AD as, in addition to a highly favorable receptor profile, satisfactory "druglikeness" and pharmacokinetics, they showed beneficial neuroprotective properties, including a desired regulation of genes expression, related to neurodegeneration and inflammation in neuroblastoma cells. These results translated into significant procognitive effects in behavioral tests in rats, after acute administration, and in the case of the thioether compound, also after chronic administration. Here, the rational stages of pharmacomodulations as well as synthesis pathways and comprehensive biological screening results for selected the most active chalcogen-containing 1,3,5-traizine compounds will be shown, and discussed in the context of potential new therapy of neurodegenerative diseases, with an accent on cognitive disorders.

Partly supported by Jagiellonian University, Medical College project N42/DBS/000331.

F-KB1-2 COULD THE TRPA1 BE A PROMISING TARGET IN THE TREATMENT OF CNS DISEASES?

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The Transient Receptor Potential Ankyrin 1 (TRPA1) non-selective cation channel expressed by the primary sensory neurons participates in mediation of pain and inflammation. However, there are only few data about its localization and function in the central nervous system.

In earlier studies we found, that Aβ1-42-injected WT mice showed significant cholinergic cell loss and cholinergic fiber loss, which was significantly attenuated in TRPA1-/- animals. In NOR and RAM tests significant memory loss was detected in Aβ1-42-injected TRPA1+/+ mice, but not in TRPA1-/- group. Old KO mice showed significantly milder memory loss, which could be seen as higher discrimination index in the NOR and less exploration time in the RAM.

RNAscope in situ hybridization technique showed the expression of Trpa1 mRNA in the mouse EWcp-UCN1 neurons and in the piriform cortex. We recently demonstrated that the EWcp area is the site of the strongest Trpa1 mRNA expression in the mouse central nervous system that is localized to the peptidergic, UCN1-containing neurons. Considering that the EW is affected by AD, and the TRPA1 is highly expressed here, we presumed that TRPA1 plays a role in the AD-associated neurodegeneration of the peptidergic neurons of EW. We have also presented evidence, that TRPA1 might participate in stress adaptation, mood regulation, as well as in the smell loss which is an early sign of several neurodegenerative disorders.

In the present study 3xTg (amyloid precursor protein, presenilin-1 and tau protein overexpressing) mice were used as a model of AD. Two, 12 and 18 months old 3xTg mice and C57BL6 mice of the same age as controls were studied. Trpa1 RNAscope in situ hybridization was combined with UCN1 immunofluorescence labeling in the EWcp to measure Trpa1 mRNA expression and UCN1 peptide content. Significantly higher Trpa1 expression was observed in 2-months-old controls than in age-matched 3xTg mice. Trpa1 expression decreased by age in the C57BL6 strain. 3xTg mice showed lower Trpa1 expression that was not affected by the course of aging. The UCN1 peptide level peaked at 12 months of age in both genotypes compared to their 2-month-old counterparts, followed by a trend of decline by 18 months of age.

Altered age-related dynamics of Trpa1 expression in the urocortinergic neurons of AD mice suggest that the peptidergic neurons may also be affected by AD. Our data demonstrate that TRPA1 might play a crucial role in neurotoxicity- and aging-induced dementia.

Funding: Hungarian Brain Research Programme (NAP3) 2022-2025. Thematic Excellence Program 2021 Health Sub-programme of the Ministry for Innovation and Technology in Hungary, EGA-16. Research grant of Medical School, University of Pécs (KA-2022-29).

F-KB1-3 ASSESSMENT OF THE EFFECTS OF A HYDROXAMIC ACID DERIVATIVE DRUG CANDIDATE ON COGNITIVE FUNCTION OF AGED RATS

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The escalating worldwide concern surrounding age-related cognitive decline and the concomitant onset of neurodegenerative disorders, notably dementia, underscores a pressing challenge. The pharmaceutical candidate BGP-15, a hydroxamic acid derivative, has exhibited cytoprotective and mitochondrial protective properties, alongside a commendable safety profile.

Our goal is to explore the potential neuroprotective effects of the BGP-15 compound in an aging rat model, employing AI-assisted Morris water maze (MWM) testing, alongside histological analysis of the frontal lobe and hippocampus, aimed at uncovering its underlying mechanism of action.

Aged male SD rats were divided into three groups: (I) control: oral vehicle treatment; (II) nasal BGP-15 treatment (40 mg/kg; nano-dispersed liquid, 40 µL/kg nasal drop); (III) oral BGP-15 treatment (40 mg/kg; gavage), for 2 months. At the endpoint, MWM test was conducted to assess spatial memory, supplemented with a self-developed AI-based classification system. At the endpoint, tissue sections were prepared from the prefrontal cortex and the hippocampus (vibratome, sagittal orientation). Immunohistochemical staining and Western blot analyses were conducted on the samples to assess the expression levels of vesicular glutamate transporter 1 (VGLUT1), silent information regulator transcript-1 (SIRT1), cAMP response element-binding protein (CREB), and glial fibrillary acidic protein (GFAP). Furthermore, morphological alterations in microglial cells were examined using a specific Iba1-staining.

During the AI-assisted Morris water maze (MWM) test, the treatment cohorts exhibited discriminative accuracy ranging from 86% to 96%. Notably, animals subjected to both oral and nasal administration of BGP-15 displayed enhanced performance in the MWM task. Immunohistochemical analysis revealed comparable levels of VGLUT1 expression across experimental groups, whereas an upregulation of SIRT1 was observed in the treated animals. Administration of BGP-15 induced alterations in the morphological characteristics of microglial cells within the hippocampus and cortex (Iba-1 staining), including changes in density, shape factor, and diameter, suggestive of microglial activation. Furthermore, protein expression profiling demonstrated elevated levels of hippocampal SIRT1 and CREB levels, alongside decreased expression of GFAP relative to the control group.

BGP-15 treatment exerts a preventative effect against cognitive decline in aged rat models through modulation of the SIRT1-CREB pathway and microglial activation. Nonetheless, more comprehensive investigations are requisite to ascertain these mechanisms with greater precision.

The work is supported by the GINOP-2.3.4-15-2020-00008 project. The project is co-financed by the European Union and the European Regional Development Fund. TKP2021-EGA-18. ÚNKP-23-3-II-DE-252.

F-KB1-4 MACHINE LEARNING METHODS IN THE SERVE OF NEW DRUGS DEVELOPMENT – CASE STUDY OF BIASED AGONISTS OF MU OPIOID RECEPTOR

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In the current era of information explosion, machine learning methods have become indispensable tools for handling large-scale, high-dimensional data. As the predictions made by machine learning methods can often reduce laboratory experiments, their application in drug design campaigns can lower the costs of the search for new potential medicines. Machine learning methods are frequently employed in drug design pipelines, not just for the identification of new active ligands, but also for the optimization of their physicochemical and pharmacokinetic properties [1].
In the study, we used the machine learning and statistical analysis to achieve two main goals. At first, we performed virtual screening to identify new ligands of the mu opioid receptor (MOR). It involved the multi-step computational protocol in which the potential candidates identified by machine learning in the ligand-based approach were docked to the selected MOR crystal structures which was followed by the automatic analysis of the interactions present in the obtained ligand-receptor complexes. Several hit compounds which were identified via this procedure are now under the further development.
Within the second task, the molecular modeling methods were used to identify molecular determinants of the functional bias of selected compounds. This effect is related to the selective activation of particular signaling pathways (signaling via G protein or beta-arrestin-mediated signal transduction) and can help in eliminating the side effects of opioid drugs related to the activation of the beta-arrestin path. In the study, we performed a series of molecular dynamics simulations at a microsecond scale to uncover the molecular determinants for the selective signaling of particular ligands. We correlated the observed ligand-protein interaction patterns with the outcome of the in vitro and in vivo tests, indicating amino acid positions which should be taken into particular consideration when designing new G protein-biased MOR ligands [2].
Acknowledgments: The study was supported by the project OPUS 2018/31/B/NZ7/03954 financed by the National Science Centre (www.ncn.gov.pl).
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[2] Podlewska, S.; Bugno, R.; Kudla, L.; Bojarski, A. J.; Przewlocki, R. Molecular Modeling of μ Opioid Receptor Ligands with Various Functional Properties: PZM21, SR-17018, Morphine, and Fentanyl–Simulated Interaction Patterns Confronted with Experimental Data Molecules, **2020**, *25*, 4636.

F-KB1-5 STUDYING THE EFFECT OF CARIPRAZINE IN INDUCED NEURONS DIRECTLY REPROGRAMMED FROM HUNTINGTON'S DISEASE'S PATIENT'S FIBROBLASTS

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Huntington's disease (HD) is an uncurable autosomal dominant progressive neurodegenerative disorder. The role of the dopaminergic system in developing HD symptoms is crucial, as the central dopaminergic pathways are overactivated in HD. The dopaminergic overactivity can be reduced by several drugs. However, their effectivity on psychiatric symptoms is limited. Moreover, the treatment of apathy and cognitive symptoms still remains challenging in HD. Cariprazine, a third-generation antipsychotic is acting as a dopamine D3 and D2 receptor agonist. Previous results showed a positive effect in HD patients after cariprazine treatment. Clinical studies indicated positive effects in early-stage HD patients after cariprazine treatment in some psychiatric symptoms such as depressed mood, apathy and cognitive function in patients. Moreover, cariprazine also improved dopamine imbalance in the prefrontal cortex.

Aims: In this project, we aim to study the effect of cariprazine in a novel in vitro model system of HD using donor-derived aged-induced neurons. Our goal is to understand the putative beneficial effects of cariprazine in HD patients and to better understand its mechanism of action by focusing on autophagy. Using reverse translational strategy, we use cariprazine treatment in induced neurons directly reprogrammed from ctrl, HD drug-naive and cariprazine-treated HD patients' fibroblasts. For detection, we use immunocytochemistry (ICC) followed by high-content automated microscopy (HCS). We suppose that the described abnormal neurite morphology and the neurite-specific impairment of subcellular autophagy are positively altered following cariprazine treatment.

F-KB1-6 FELODIPINE EFFICIENCY ANALYSIS ON INDUCED NEURONS DERIVED FROM HUNTINGTON'S DISEASE FELL-HD CLINICAL TRIAL PATIENTS

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder, caused by CAG expansions in the huntingtin gene (*HTT*), which results in the aggregation of the mutated huntingtin protein (mHtt). HD is uncurable, and after disease onset around 30-40 years of age patients die within the next 10-20 years. Autophagy, a lysosomal degradation pathway ensuring cytoplasmic homeostasis is dysfunctional in HD, contributing to insufficient mHTT protein removal. The Fell-HD clinical trial is based on repurposing Felodipine, an already licensed L-type calcium channel blocker and antihypertensive drug with a low chance of side effects. Felodipine significantly increases autophagy in animal models of HD and subsequently reduces the level of toxic mHTT, neurodegeneration, and disease symptoms, like tremors and loss of motor coordination.

In this current project, in parallel with the FELL-HD trial, we are testing Felodipine drug efficacy in induced neurons (iN) directly reprogrammed from the FELL-HD cohort's skin fibroblasts. Transdifferentiated iNs keep the genetic and aging signatures of the donor bypassing any stem cell or neuroprogenitor phase during conversion. We converted 7 control and 18 HD patients' fibroblasts with mild symptoms to iNs with the same conversion efficiency and purity. Our previous results indicated an accelerated aging in HD-iNs defined by DNA methylation. Therefore, in the current cohort, we are using the Horvath epigenetic clock to investigate the presence of accelerated aging.

HD-iNs showed a less elaborated neuronal morphology. We used 0.1 μM and 1 μM felodipine treatment for 24h and 72h. After 28 days of conversion followed by Felodipine treatment iNs were fixed and counterstained using neuronal (TAU, MAP2) and autophagy markers (p62, LC3, LAMP1) to determine neuronal morphology and subcellular autophagy changes using high-content automated screening microscopy. Our preliminary results showed that Felodipine treatment enhances autophagy in a subset of patients while having no adverse effect on other HD-iNs.

These preclinical results will be directly compared and correlated with FELL-HD trial outcomes and the patient's cognitive and motor scores. This project using an in vitro preclinical iN model can provide predictive information about drug effectiveness, opening a new dimension in clinical trial optimization and personalized medicine.

F-KC1-1 QUALITY REQUIREMENTS FOR BIOLOGICALS IN CLINICAL TRIALS

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The presentation of the Hungarian National Center for Public Health and Pharmacy summarizes the quality requirements for the development of biologicals with appropriate quality, safety and efficacy profile.

Setting up CMC (chemistry, manufacture, control) regulatory compliance strategy for biopharmaceuticals has unique features due to several reasons: the use of living source material; increased complexity of biologic manufacturing processes and increased complexity of the biologic molecules themselves.

The regulatory guidelines, including several EMA and ICH Q guidelines, give the framework of quality requirements for the development of biological drug products. Scientific guidelines for biopharmaceuticals reflect a harmonised approach of the EU Member States and the agencies on how to interpret and apply the requirements for the demonstration of quality, safety and efficacy set out in the Community directives.

The main elements of the „Guideline on the requirements for quality documentation concerning biological investigational medicinal products in clinical trials" (EMA/CHMP/BWP/534898/2008 Rev. 2; 27 January 2022) are presented which focus on the characterisation, manufacture, control, reference standard, container closure system and stability of the active substance and the investigational medicinal product under test.

The PRIME scheme of EMA is discussed, that enhance support for the development of medicines that target an unmet medical need. The PRIME strategy is based on enhanced interaction and early dialogue with developers of promising medicines to optimise development plans and speed up evaluation process.

On the field of biopharmaceuticals, the continuous communication between the authorities and the companies is crucious and helps the efficient translation of developments. To achieve this goal, the possibilities of Scientific Advice with the relevant national authorities and/ or EMA are also discussed.

F-KC1-2 THE VALUE AND IMPORTANCE OF A PROFESSIONAL ETHICAL CODE FOR MEDICINES DEVELOPMENT

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Individuals doing similar work usually describe their aims and services in a professional code. Ethics is an important, mandatory part of each professional code. It summarizes the moral standards guiding their working quality. In addition, the ethical codes describe the expected behavior of their members within the society they serve and towards their professional organization as well as its members. The International Federation of Associations of Pharmaceutical Physicians and Pharmaceutical Medicine (IFAPP) developed an ethical framework addressing moral issues faced by the rapidly increasing number of pharmaceutical physicians and other variously trained scientists working together in modern medicines development. The conceptual organization of the document and the moral comments primarily addressing problems arising at the interface between medicine and pharmaceutical industry will be presented.

F-KC1-3 CLINICAL TRIALS IN HUNGARY FROM THE PERSPECTIVE OF THE COMPETENT AUTHORITY

Krisztina Szabone Nemesy
National Center for Public Health and Pharmacy

The Eu clinical trial environment has been influenced by several factors in recent years.

The introduction of Clinical Trials Regulation (Regulation (EU) No 536/2014) and consequently the launch of Clinical Trials Information System (CTIS) on 31 Jan 2022, have fundamentally changed the regulatory landscape. Instead of a national assessment, authorisation is now based on a cooperation between Member States, and adverse reaction monitoring and assessment is also carried out in a framework of European cooperation. However not only the new regulatory environment, but also other factors have had a significant impact on this field.

The experience of the COVID pandemic has led to the increasing use of decentralisation elements in clinical trials, complex and adaptive clinical trial design is now widespread, shortening the development time. All these factors are challenges for competent authorities, but also for developers.

This presentation will discuss the impact of these factors on clinical trials in Europe and especially in Hungary.

F-KC1-4 THE ROLE OF MEDICAL AFFAIRS IN PHARMA - FOCUS ON REAL WORLD EVIDENCE GENERATION

Szabó Lilla
AstraZeneca Kft., Budapest,

In the dynamic landscape of pharmaceuticals, the role of Medical Affairs has evolved into a pivotal function, particularly in the realm of real-world evidence (RWE) generation.

Medical Affairs serves as the bridge between pharmaceutical companies and healthcare stakeholders, as a trusted source of medical education and scientific exchange, fostering collaborations with key external experts and healthcare professionals. Through educational initiatives, Medical Affairs professionals disseminate evidence-based information, empowering healthcare providers to make informed treatment decisions based on the latest clinical evidence.

Moreover Medical Affairs plays a crucial role in generating, interpreting, and disseminating real-world evidence. Through collaborations with healthcare providers, payers, regulators, and patients, Medical Affairs professionals facilitate the collection of data from diverse sources, including electronic health records, payers databases and patient registries.

One of the primary objectives of RWE generation is to complement traditional clinical trial data with insights derived from real-world clinical practice. Medical Affairs professionals play a key role in designing and executing observational research initiatives by which prospectively monitoring the long-term safety and effectiveness of medications. Medical Affairs teams also employ advanced analytics and data science techniques to analyze large datasets, uncovering valuable insights into the epidemiology of specific diseases states and unmet need. This type of study was delivered in chronic kidney disease under the umbrella of the partnership between AstraZeneca and the Universty of Pécs-the CKD Epi HUN.These type of datasets could play a strategic role in policy decisions. RWE generated by Medical Affairs informs health economic and outcomes research (HEOR) assessments, demonstrating the value proposition of pharmaceutical products in improving patient outcomes and reducing overall healthcare costs

In conclusion, the evolving landscape of healthcare demands a proactive and strategic approach to evidence generation and dissemination. Medical Affairs, with its unique blend of scientific expertise, strategic insights, and collaborative partnerships, is well-positioned to drive the generation and leveraging of real-world evidence, to enhance decision-making processes and drive innovation and ultimately advancing patient care and shaping the future of healthcare.

F-KC1-5 MODALITIES OF EARLY AND SUPPORTED PATIENT ACCESS TO MEDICINES

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Access to new medicines are essential for patients especially in diseases with a high unmet medical need, when treatment options are scarce and where reimbursement is slow and/or limited. Pharmaceutical companies can offer different access strategies to provide a new treatment possibility to patients and healthcare professionals according to the development stage of the medicine and to the local legislation of a certain country to address the need. This can include early access modalities, before the marketing authorization is given, under special conditions or a logistical or financial aid for patients where reimbursement is not available. In this presentation international examples are shown through the lens of the company Boehringer Ingelheim.

F-KA3-1 CARDIOVASCULAR BENEFITS OF SGLT2I

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An abundance of clinical and preclinical evidence suggests that sodium–glucose co-transporter 2 inhibitors (SGLT2i) beyond the anti-diabetic effect, mitigate the symptoms of heart failure irrespective of left ventricle ejection fraction and all causes of mortality. Nevertheless, a more comprehensive understanding of patient-specific factors for long-term administration of SGLT2i therapy is imperative to guide their optimal utilization in diverse clinical scenarios. In addition, an ongoing clinical trial aims to clarify whether the administration of SGLT2i acutely in patients with ST-elevation myocardial infarction (MI) protects the heart against myocardial damage. Preclinical data suggest that both chronic and acute administration of SGLT2i have cardioprotective benefits in the setting of MI. However, Beyond the salt-dependent effects of SGLT2i on renal hemodynamics, SGLT2i inhibited several key aspects of macrophage-mediated cardiac inflammation and fibrosis, including inhibiting the differentiation of monocytes to macrophages, promoting the polarization of macrophages from a proinflammatory M1 phenotype to an anti-inflammatory M2 phenotype, and suppressing the activation of inflammasomes and major proinflammatory factors. Interestingly, SGLT2 inhibitors can profoundly enhance myocardial energetic, using ketone bodies or may limit lipids toxicity in the myocardium. In our study, we aimed to clarify and discuss the cardiovascular benefits of SGLT2i in setting of 1) acute myocardial ischemia/reperfusion injury after cardioplegic arrest, 2) in pressure overload induced left ventricular hypertrophy and 3) the efficacy of SGLT2i in cardiac complications in Duchenne Muscular Dystrophy. In addition, our data also demonstrate whether chronic SGLT2i affect cardiac proteomic and protein posttranslational modification, and cardiac energetics in various preclinical models of heart failure.

F-KA3-2 CARDIOPROTECTION BY EXOGENOUS MICRORNA-125B* MIMIC IN A MOUSE MODEL OF ACUTE MYOCARDIAL INFARCTION

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Cardioprotection against myocardial ischemia/reperfusion injury (MIRI) is still challenging, however, recently, non-coding RNAs including microRNAs, have opened up a new pharmacological approach. We have previously shown that cardiac microRNA-125b* (microRNA-125b-2-3p; miR-125b*) increased cell viability in isolated cardiac myocytes subjected to simulated IRI, therefore, it is termed as a ProtectomiR. Now, we aimed to characterize the pharmacokinetics and pharmacodynamics, as well as the effect of miR-125b* mimic on infarct size in an *in vivo* mouse model of acute myocardial infarction.

To characterize pharmacokinetics of miR-125b*, a single intravenous (i.v.) bolus of 10 μg miR-125b* mimic or its scramble miRNA control or vehicle (a neutral lipid emulsion) was administered to male C57BL/6 mice, and 1, 2, 4, 8 and 24 hours later miR-125b* expression was determined from plasma and heart samples. The effect of miR-125b* on myocardial infarct size was assessed after single iv. bolus of 10 μg miR-125b* mimic administered at the 10th min of a 45-min coronary occlusion followed by 24-hour reperfusion. For negative controls, 10 μg non-targeting miRNA or vehicle were administered the same way. To assess the molecular mechanism of cardioprotection, ventricular expression of selected mRNA targets of miR-125b* were also measured using identical sampling as pharmacokinetics.

Circulating and cardiac expression of miR-125b* was significantly increased at 1 hour after miR-125b* mimic treatment. Infarct size was significantly reduced after i.v. administration of miR-125b* mimic (20.7±2.62%, n=25) as compared to the vehicle (32.2±2.67%, n=25, $p<0.01$). The expression of Ccna2, Eef2k and CaCnb2 target mRNAs were significantly reduced 8 hours after injection of miR-125b* mimic.

This is the first demonstration of the pharmacokinetics of miR-125b* mimic in mice. Furthermore, we have shown the first time that miR-125b* mimic provides cardioprotection in a well-translatable manner when administered as an i.v. bolus before recanalization in an *in vivo* mouse model of MIRI.

F-KA3-3 BENEFICIAL APD/QT NORMALIZING EFFECTS OF L-CARNITINE IN TRANSGENIC SQT1 RABBIT MODEL

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Introduction: Short-QT syndrome 1 (SQT1) is a genetic cardiac channelopathy caused by gain-of-function mutations (HERG-N588K) in HERG/I_{Kr}, that leads to shortened QT-interval, increased risk for arrhythmias and sudden cardiac death (SCD). Data on efficient pharmaco-therapy for SQT1 are scarce. In patients with primary carnitine-deficiency, acquired-SQTS has been observed and rescued by carnitine-supplementation, indicating that carnitine might affect cardiac repolarization. We aimed to investigate potential beneficial (APD/QT-prolonging) effect of L-Carnitine in (genetic) SQTS using transgenic SQT1 rabbits that mimic the human disease phenotype.

Methods: Effects of L-Carnitine on cardiac repolarisation were assessed in adult wild-type (WT) and transgenic SQT1 (HERG-N588K, gain of I_{Kr}) rabbits using *in vivo* ECG and *ex vivo* Langendorff-perfused whole-heart or isolated ventricular cardiomyocyte action potential (AP) recordings. Effects on ion currents were assessed by whole-cell patch-clamping.

Results: *In vivo*, the heart-rate corrected QT index (QTi) was prolonged significantly by L-Carnitine both in WT (QTi, baseline 102.7%±4.9 vs. L-Carnitine 106.9%±6.2, $p<0.05$, n=12) and SQT1 (QTi, baseline 94.8%±7.4 vs. L-Carnitine 99.5%±8.2, $p<0.05$, n=13), leading to normalisation of QTi in SQT1. Ex vivo, whole-heart monophasic and cellular APs were also significantly prolonged by L-Carnitine in WT and STQ1 (change in monophasic APD75, ms, WT +13.9±4.4, SQT1 +9.9±7.0; change in cellular APD90, %, WT +10.4%, SQT1 +10.0%, all $p<0.05$). As underlying mechanisms, we identified acute effects on the main repolarizing ion currents: I_{Kr}-steady, which is pathologically increased in SQT1, was reduced by L-Carnitine/C16-Carnitine and deactivation kinetics were accelerated. Moreover, L-Carnitine/C16-Carnitine decreased I_{Ks}-steady and I_{K1}.

Conclusion: L-Carnitine/C16-Carnitine prolong/normalize QT and whole heart/cellular APD in SQT1 rabbits. These beneficial effects are mediated by acute effects on I_{Kr}. L-Carnitine may serve as potential future QT-normalizing, anti-arrhythmic therapy in SQT1.

F-KA3-4 HIDDEN CARDIOTOXICITY AND CARDIOPROTECTION: DEVELOPMENT OF PRECLINICAL TEST PLATFORMS FROM IN VITRO TO IN VIVO MODELS

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Drug-induced cardiotoxicity is 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 hyperlipidemia, diabetes, etc) and their medications (e.g. nitrates, antidiabetic drugs, statins, etc) may interfere with cardiac ischemic tolerance and molecular signalling of endogenous cardioprotection. 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.

F-KB3-1 ROLE OF MICROGLIA IN MODULATION OF CEREBRAL CIRCULATION AND NEUROVASCULAR COUPLING

Ádám Dénes
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Microglia are key regulators of inflammatory processes in the CNS. Microglial activity is altered in common brain diseases and changes in microglial function have major impact on outcome in experimental models of neurological disorders. However, the underlying mechanisms are not well understood. We have recently identified a novel form of microglia-neuron interaction, which is present in the majority of neurons in mouse and human brain. Somatic microglia-neuron junctions possess specialized nanoarchitecture optimized for purinergic signaling. We show that activity of neuronal mitochondria is linked with microglial junction formation, which is induced rapidly in response to neuronal activation and blocked by inhibition of P2Y12 receptors. Microglia also shape vascular responses via purinergic, compartment-specific actions through which microglia modulate cerebral blood flow, neurovascular coupling and cerebral hypoperfusion. Recent data also show that microglia-neuron-vascular interactions are markedly altered under inflammatory conditions, leading to perfusion changes and modulation of vascular responses to different challenges.

Understanding the mechanisms of microglia-neuron-vascular interactions is likely to help the identification of novel therapeutic targets in common neurological disorders.

F-KB3-2 TARGETING THE BRAIN METASTATIC ENVIRONMENT

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Brain metastases are life-threatening complications of triple negative breast cancer, melanoma and a few other tumour types. Poor outcome of cerebral secondary tumours largely depends on cells of the neurovascular unit, which have a Janus-faced attitude towards the tumour cells being both destructive and protective at the same time. Here we aimed to identify tumour-supportive mechanisms related to brain pericytes and astrocytes, with the final goal of determining potential future therapeutic targets.

We observed that pericytes secreted large amounts of insulin-like growth factor 2 (IGF2), which had a very significant pro-proliferative effect on mammary carcinoma, but not on melanoma cells. By inhibiting IGF2 signalling using silencing or picropodophyllin (PPP), we could block the proliferation increasing effect of pericytes on breast cancer cells. Administration of PPP (a blood-brain barrier-permeable substance) significantly decreased the size of brain tumours in mice inoculated with triple negative breast cancer cells.

In addition, we found NLRP3 inflammasome components and IL-1β to be highly and specifically expressed in peritumoural astrocytes. Soluble factors from triple negative breast cancer cells induced upregulation and activation of NLRP3 and IL-1β in astrocytes, while astrocyte-derived mediators augmented the proliferation of metastatic cells. Moreover, inhibition of NLRP3 inflammasome activity using MCC950 or dampening the downstream effect of IL-1β prevented the proliferation increase in cancer cells. In vivo, MCC950 reduced IL-1β expression in peritumoural astrocytes, as well as the levels of inflammasome components and active IL-1β. Most importantly, significantly retarded growth of brain metastatic tumours was observed in mice treated with MCC950.

Altogether, we identified pericytes and the IGF axis, as well as astrocytes and the NLRP3 inflammasome as potential novel therapeutic targets in brain metastatic disease.

F-KB3-3 PROTECTION OF BRAIN ENDOTHELIAL CELLS AS A THERAPEUTIC TARGET IN CENTRAL NERVOUS SYSTEM DISEASES

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Blood–brain barrier (BBB) provides a special compartment necessary for the proper functioning of the CNS by actively controlling cellular and molecular trafficking between the blood and the nervous system. The continuous layer of cerebral endothelial cells attached to each other by tight intercellular junctions (TJs) constitutes the morphological basis of the BBB. The phenotype of the BBB including TJs, the lack of fenestrae, specific transendothelial transport systems and active efflux pumps regulates the entry of solutes and cells into the brain, and performs metabolic and detoxifying functions to protect the CNS.

The structural and functional integrity of the BBB is one of the most critical factors for maintaining the normal brain homeostasis. Emerging evidence supports the crucial role of BBB disruption in the early stages of various brain disorders, and BBB dysfunction has therefore been identified as a potential new therapeutic target for a number of brain disorders.

The degree of BBB disruption is tightly correlated with cognitive dysfunction in neurodegenerative disorders, especially in Alzheimer's disease (AD). Enhancing amiloid beta clearance across the BBB, as well as protection of the BBB from injury are among the proposed new strategies for the therapy of AD. In our experiments, pentosan polysulfate (PPS), a polyanionic polysaccharide decreased the toxic effects of amiloid beta peptides in brain endothelial cells and protected the function of the BBB. This new observation may suggest a potential therapeutical application of PPS in AD.

The intake of the polyunsaturated fatty acid docosahexaenoic acid (DHA) has been associated with decreased amyloid deposition and reduced risk in AD. Our results proved that DHA may protect not only neurons but also the other elements of the neurovascular unit from the toxic effects of amiloid and this effect may be beneficial in the therapy of AD.

BBB damage is also a major feature of neurovascular diseases, such as stroke. During and after ischemic stroke, BBB disruption facilitates injury progression and increases the risk of hemorrhage predicting high mortality. Since junctional proteins are key in the regulation of BBB function, we targeted three different signaling pathways of the BBB with small molecular inducers using paralelly which resulted in tighter junctions and improved BBB functions after oxygen glucose deprivation in a stroke model.

Our results demonstrated that protecting BBB functions as a therapeutic target in brain disorders may lead to the development of new approaches for the treatment of CNS diseases.

F-KB3-4 ALANINE AND GLUTATHIONE TARGETING OF DOPAMINE- OR IBUPROFEN-COUPLED POLYPEPTIDE NANOCARRIERS ELEVATES CROSSING ACROSS THE BLOOD-BRAIN BARRIER AND PROTECTIVE EFFECTS

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Targeting the blood-brain barrier (BBB) is the key to the effective brain delivery of nanocarriers. We have previously discovered that ligand combinations of BBB nutrient transporters, especially alanine and glutathione, increase the permeability of vesicular and polypeptide nanocarriers across the BBB. Polypeptides are versatile nanoplatforms to combine high functionality with excellent biocompatibility.

Our aim here was to investigate whether the alanine and glutathione targeting molecules can also promote the efficient transfer of 3-armed poly(L-glutamic acid)-coupled dopamine or ibuprofen across a novel human co-culture model with induced BBB properties.

The dual-targeted nanoformulations of both drugs showed elevated cellular uptake in a time-dependent, active manner via endocytic mechanisms. Free alanine and glutathione inhibited the cellular internalization of targeted nanocarriers suggesting the crucial role of ligands in the uptake processes. The targeted nanocarriers had a higher permeability across the BBB model. After crossing the BBB, the targeted dopamine nanocarriers could subsequently enter midbrain-like organoids derived from healthy and Parkinson's disease patient-specific stem cells. The ibuprofen-coupled targeted nanocarriers had protective effects against cytokine-induced toxicity and BBB opening.

These results indicate that coupling dopamine and ibuprofen to BBB-targeted poly(L-glutamic acid) can be used as nanocarriers for nervous system applications.

F-KB3-5 DASATINIB AND QUERCETIN ARE PROTECTIVE IN FOCAL CEREBRAL ISCHEMIA IN AGED RATS

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In addition to aging, atherosclerosis and carotid stenosis are significant risk factors for acute ischemic stroke. Stroke is substantial burden in our aging population and there is an increasing need to investigate new and effective therapeutic strategies. Our aim was to explore the potential neuroprotective effects of the combination of dasatinib (an anticancer drug) and quercetin (an antioxidant flavonoid) in a rat model of acute ischemic stroke.

In young (6-7 weeks) and old (18-24 months) isoflurane anesthetized Wistar rats, carotid stenosis common in the elderly was mimicked by the unilateral ligation of right common carotid artery. Over the next 2 weeks, a cocktail of dasatinib (5 mg/kg body weight)-quercetin (50 mg/kg body weight) or vehicle (0.1 % DMSO in saline) was administered i.p. every second day. Then focal cerebral ischemia was induced by the occlusion of the distal branch of the middle cerebral artery (dMCAO) ipsilateral to carotid ligation. Spontaneous spreading depolarizations (SD) in the parietal region were recorded for one hour using a glass capillary microelectrode and coupled changes in cerebral blood flow (CBF) were assessed by laser speckle contrast imaging. The brains were harvested for further immunocytochemical analysis of cellular senescence and neuronal injury.

The aged brain was more prone to SD as compared to the young. Treatment with dasatinib and quercetin had no significant effect on physiological parameters (baseline CBF, blood pressure). Induction of acute ischemic stroke resulted in a significant CBF drop in all groups. In the aged animals, SD occurrence was reduced in the treated compared to the control group (SD number 1.0±0 vs. 3.3±1.5, treated vs. control). The slope of repolarization (0.34±0.35 vs. 0.04±0.02 mV/sec, treated vs. control) and SD duration (42±16 vs. 196±186 sec, treated vs. control), both indicative of the ability of the tissue to recover were also smaller in the treated compared to the untreated group.

The recurrence of SDs in the ischemic penumbra has been recognized to exacerbate neuronal injury. The treatment with dasatinib-quercetin after carotid stenosis but before acute ischemic stroke inhibited SD in the ischemic penumbra, which suggests a neuroprotective effect. The combined administration of dasatinib-quercetin guided by the diagnosis of carotid stenosis offers a personalized therapeutic opportunity as a preventive measure to limit the consequences of acute ischemic stroke.

Funding: H2020 No. 739593, NKFIH K134377, NAP3.0, TKP2021-EGA-28, SZAOK Research Fund.

F-KC3-1 TRANSLATIONAL MEDICINE – FROM BEDSIDE TO BENCH AND TO BEDSIDE AGAIN

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The primary objective of translational medicine (TM) is to convert scientific discoveries into tangible societal advantages. Regrettably, data from EUROSTAT indicate that Europe witnesses an average of 1.7 million deaths annually, out of which 1.2 million could have been averted with more effective primary prevention and public health initiatives. The Academia Europaea has recently crafted a translational cycle model to streamline and expedite the application of scientific insights for the betterment of society[1]. This model places equal emphasis on healthcare provision, the generation of new scientific findings, the creation of clear and comprehensible summaries of these findings, and their dissemination to all stakeholders. A crucial aspect of this model is the imperative for healthcare professionals to stay abreast of the latest scientific developments. Unfortunately, the current medical education curriculum predominantly focuses on theoretical knowledge and clinical practice, offering scant opportunities for students to familiarize themselves with the knowledge needed to apply the latest scientific discoveries for the benefit of patients.

In response to this gap, we have pioneered a hybrid Ph.D./healthcare TM program that allows students to immerse themselves in both healthcare and scientific research simultaneously in an extremely efficient way[2]. Within this program's structure, students hone their clinical research skills through a "learning by doing" way. Among the methodologies taught, meta-analysis stands out as a powerful tool. Based on the evidence-based pyramid, it provides the strongest evidence in medicine. It's essential to note that, like all scientific methodologies, meta-analysis begins with a focused question, proceeds with data collection, then organizes the data followed by biostatistical analysis, culminating in the articulation of new scientific findings and drawing conclusions. This methodology is fundamental in ensuring that scientific results can be effectively translated back into everyday clinical practice, enhancing patient care with evidence-backed strategies.

[1]Hegyi P et al. J Clin Med. 2020 May 19;9(5):1532.
[2]Hegyi P et al. Nature Medicine. 2021 Aug;27(8):1317-1319.

F-KC3-2 TRANSLATIONAL MEDICINE – CLINICAL INVESTIGATIONS OF NATURAL PRODUCTS INITIATED BY META-ANALYSES

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Herbal products have been part of medicine for centuries. For some of these products, information on indication and dosage is derived from experience of traditional use. In case of some of the plants, clinical trial data also support the efficacy.

The Hungarian Phytotherapy Working Group was established in 2018 to promote the development of evidence-based herbal medicine by initiating and coordinating scientific research. The primary objective of the working group is to analyse clinical trials on herbal medicinal products by preparing meta-analyses and systematic reviews.

Throughout its history, the working group has been involved in the preparation of a number of meta-analyses. We have analysed the antidepressant effect of saffron, the antihypotensive effect of a combination of hawthorn and camphor, the antidiabetic effect of *Momordica charantia*, and the muscle pain-relieving effect of essential oils, the weight-loss effects and safety of synephrine, the safety of dronabinol and nabilone, the antihyperlipidemic effects of berberol, the effects of chasteberry on premenstrual syndrome symptoms, and the anti-emetic effects of ginger. In several cases, our research was the first to provide scientific evidence for the rational use of a natural product.

The collection, review and critical appraisal of scientific evidence is not only essential for rationalising patient care. Analysing the data can reveal important discrepancies and gaps that can stimulate further research. The heterogeneity of the data on the efficacy of ginger has highlighted problems with the stability of the active substance. This became the starting point for an analytical research project, which also yielded interesting results from a scientific and industrial point of view, suitable for publication. The meta-analysis of the antidepressant effects of saffron stimulated research that led to the identification of a more economical raw material. In several cases, the lack of clinical data has led to the design and initiation of clinical trials.

In this way, translation has been achieved in the research work in several ways: between different research methodologies and objectives, and in the practical application of scientific research results.

F-KC3-3 TRANSLATIONAL MEDICINE – FROM MOLECULAR PHYSIOLOGY TO META-ANALYSES TO MOLECULAR PHYSIOLOGY OF EPITHELIAL ION TRANSPORT PROCESSES AND TRANSPORT PRODUCTS

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Using molecular physiological method investigations, our group revealed high level of similarities between the dental enamel producing ameloblasts, the pancreas and the salivary glands. However, we have to admit that recent basic science discoveries have not been translated into clinical applications and there is still a long way to go into that direction successfully. Therefore, besides using basic science modelling of the pathophysiology and pharmacology of enamel formation and salivary secretion, we also started research on the other end of science: making available clinical results regarding enamel and salivary function damage for looking optimal procedures to prevent and to restore the damaged function.

As an example, there is a growing need for effective methods in the management of early-stage dental enamel lesions, particularly in relation to fluoride. Therefore, we evaluated the efficacy of combined casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and fluoride on remineralizing damaged enamel lesions (WSLs) compared to fluoride-only interventions. Our meta-analysis results revealed the combination of the clinically widely used CPP-ACP and fluoride did not overcome the effect of fluoride given alone. Therefore, more efficient materials than CPP-ACP are needed to achieve robust enamel remineralization clinically.

We also had extensive experience in characterizing the molecular physiology of salivary secretion. During the outbreak of COVID-19 infection in Hungary, we aimed to utilize our expertise regarding the diagnosis of this serious and potentially deadly disease. Early diagnosis of infected individuals plays an important role in stopping the disease from further escalating. The gold standard was the nasopharyngeal swab sampling method. Our aim was to conduct a meta-analysis on the reliability and consistency of SARS-CoV-2 viral RNA detection in saliva specimens. In our systematic search and meta-analysis we found 91% (CI 80-99%) sensitivity for saliva tests and 98% (CI 89-100%) sensitivity for nasopharyngeal swab (NPS) tests among previously confirmed COVID-19 patients, with moderate heterogeneity among the studies. These results revealed that saliva tests offer a promising alternative to NPS for COVID-19 diagnosis. However, further diagnostic accuracy studies and test kit optimalizations were needed to improve their specificity and sensitivity. Thus, with an industrial partner we jointly developed a test kit optimized for salivary samples and identified the nucleic acid isolation method which is optimal to decrease the viscosity salivary samples.

Taken together, these studies not only provided enhanced level of evidence for best clinical practices, but also opened new perspectives for both basic molecular pharmacology research and their potential for clinical applications.

Supported by NKFIH K-147107 and NIH NIDCR 1R01DE027971.

F-KA4-1 PROGRESS REPORT ON DEVELOPMENTS TOWARDS HUMAN CELL AND GENE THERAPY AND XENOORGAN TRANSPLANTATION

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International clinical developments in the field of gene and cell therapies for regenerative medicine and genetic mutation corrections are progressing at an increasing speed. Overview of the current situation in clinical trials and approved therapies will be presented with special emphasis on Type-1 diabetes, cardiac failure and Parkinson's disease trials. Furthermore, recent developments of compassionate trials of xenoorgan transplantations using genetically modified pig heart, kidney and liver into patients will be presented, and the potential roadblocks and main challenges towards clinical applications in the EU, US and Asia will be reviewed.

Our own projects and progress in the development of human induced pluripotent stem cell (hiPSC)-derived beta-cell organoids and maturing hiPSC-cardiomyocytes, creating and testing patient-specific antisense oligonucleotide treatments for ultra-rare diseases and the potential for xenoorgan developments will be reported, including an overview what role Hungary might play in the fierce international competition towards clinical translation of advanced therapy medicinal products (ATMPs).

The presented projects received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 760986 (iNanoBIT) and No. No. 953138 (EMAPS-Cardio), from EU Horizon Europe Marie Sklodowska-Curie Action ITN No. 101120256 (MMM), COST Action CA21151 (HAPLO-iPS), COST Action CA21113 (GenE-Humdi), CA22170 (TENET) and 2020-1.1.5.-GYORSÍTÓSÁV-2021-00016 from the National Research, Development and Innovation Fund.

F-KA4-2 A MULTIPURPOSE ANTI-INFLAMMATORY THERAPEUTIC AGENT: MESENCHYMAL STEM CELLS

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Inflammation, although one of the most general signs/symptoms of many diseases, is a rather unspecific phenomenon; it comprises hundreds of partially different molecular and cellular processes with various outcomes. Therefore, inhibiting the actual inflammation may also be a complicated task, especially when a single therapeutic target is utilized. Mesenchymal stem cells (MSCs), besides their regenerative capacity, are a potent anti-inflammatory cell type acting on T cells, NK cells, macrophages, dendritic cells, B cells and other inflammation-related cell types. MSCs use multiple mechanisms to achieve their effects: cell-cell contact, soluble mediators and extracellular vesicles. Immunosuppressive attributes of MSCs are accompanied by their antimicrobial, revascularizing, and re-epithelializing properties.

Utilizing MSCs as an Advanced Therapy Medicinal Product (ATMP) raises the question of how the immunosuppressive capacity of different MSC lines/batches can be standardized. Our main goal is to set up a scoring algorithm based on a multiplatform test system to rank the potency of individual MSC lines. This approach would allow us to use a uniform set of MSCs for a given indication – e.g., the most immunosuppressive but moderately revascularizing ones could be dedicated to patients with graft-versus-host disease, whereas moderately immunosuppressive MSCs with strong re-epithelializing and antimicrobial properties could be used to treat diabetic foot ulcer patients.

We isolate MSCs from healthy umbilical cord Wharton jelly, propagate and characterize them *in vitro* according to the International Society for Cellular Therapy criteria. We have bio-banked more than 30 individual MSC lines, providing a statistically sufficient basis for the potency assay. To set up such a multiplatform test system, we use flow cytometry to measure the cell surface immunosuppressive molecules expressed on MSCs, a proliferation assay to determine T cell suppression, a colorimetric assay to test kinurenin production and a bead-based multiplex cytokine assay to assess the anti-inflammatory, antimicrobial and proangiogenic cytokine/growth factor profile of MSCs, as well as the suppression of proinflammatory cytokines produced by macrophages.

In conclusion, our ongoing project will hopefully result in an applicable platform to characterize and classify MSC lines as potent immunosuppressive ATMPs.

Support: Semmelweis University "STIA-KFI 2022" R&D Grant; Project no. RRF-2.3.1-21-2022-00015 TKP2021-EGA-24 (MOLORKIV) NKFIH; Project no. 2022-1.1.1-KK-2022-00005 NKFIH.

F-KA4-3 PRE-CLINICAL DEVELOPMENT OF A CELL THERAPY PRODUCT

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Mesenchymal stem cells derived from adipose tissue hold significant promise in regenerative medicine. They demonstrate the capacity to regenerate tissues and regulate the immune system, making them particularly valuable for treating chronic inflammatory ulcers and wounds. Despite their inherent abilities, research indicates that pretreatment enhances their therapeutic efficacy.

In our study, we exposed adipose-derived mesenchymal stem cells to inflammatory factors and TLR ligands, such as IL1b, TNFa, IFNy, PolyI:C, and LPS, for 24 hours as part of our experimental design in vitro. Wound healing was measured by in vitro assays. Subsequently, we assessed alterations in gene expression and proteome profiles and observed the rate of wound closure post-treatment.

Our results show that specific pretreatments, such as IL-1β, notably expedited the wound-healing process. Analysis of gene and protein expression profiles unveiled changes in pathways associated with tissue regeneration. These findings suggest that primed cells exhibit potentially superior therapeutic efficacy compared to untreated cells, offering insights into optimizing regenerative strategies utilizing adipose tissue-derived stem cells. in clinical trials, and many 3D-printed medicines have been on the market for some time.

F-KA4-4 NETWORK THEORETICAL AND MACHINE-LEARNING-BASED ANALYSIS OF THE INTERACTOME FOR THE DEVELOPMENT OF OLIGONUCLEOTIDE THERAPIES IN CARDIOVASCULAR DISEASES

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In the treatment of ischemic heart disease pharmaceutical approaches explored using classical, hypothesis-driven research methodologies all failed so far to be translated into the clinical practice. These repeated failures are most likely due to the biased experimental design and neglecting the modifying effects of comorbidities.
To facilitate the application of hypothesis-free approaches we aimed to develop and utilize software tools that enable the discovery of new drug targets for oligonucleotide therapies through network theoretical and machine-learning-based analysis of omics datasets.
We developed a software (https://github.com/semmelweis-pharmacology/ppi_pred) based on the conditional generative adversarial network (cGAN) architecture for the prediction of protein-protein interactions. Our software has been validated *in silico* on protein-protein interaction networks from the STRING and the BioGRID databases and experimentally by yeast-two-hybrid method, as part of a collaboration organized by the International Network Medicine Consortium. To investigate the role of small non-coding RNAs, including microRNAs, in the pathomechanism of various diseases, we developed miRNAtarget™ (Pharmahungary, Szeged, https://mirnatarget.com), a user-friendly web-based software tool for the integrative analysis of microRNA-target networks. Targets identified by the miRNAtarget software, which relies on predicted and experimentally validated microRNA-target interaction databases (microrna.org, miRDB, miRTarBase), were successfully validated at the mRNA- and protein-level in several experimental models including cardiovascular diseases and related comorbidities.
We have first demonstrated that a cGAN-based machine learning algorithm, which relies only on the analysis of network topology without requiring molecular information can efficiently predict protein-protein interactions. In addition, as part of a cost-effective approach, by analyzing small RNA transcriptomic profiles, several potential oligonucleotide drug targets were identified with the use of our successfully validated miRNAtarget software.

F-KA4-5 DEVELOPMENT OF SMALL NON-CODING RNA THERAPEUTICS: THE EXAMPLE OF PROTECTOMIR MIR-450A MIMIC

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Background and aims: Cardioprotection against myocardial infarction is still an unmet clinical need. Due to the complexity of cardioprotective signaling, multitarget drugs modifying several pathways may provide advance in this field. The fine-tuning regulators gene expression microRNAs are promising multitarget drug candidates. Previously we have identified cardioprotective microRNAs, termed protectomiRs, by a systematic analysis of microRNA expression pattern in myocardial infarction and cardioprotection induced by ischemic conditioning in rats. Here we will show some aspects of further development of miRNA therapeutics via an example of a recent project where we aimed to identify protectomiRs in a translational porcine model of acute myocardial infarction (AMI).

Methods: Tissue samples from the infarcted region of the left ventricles from our previous study in closed-chest AMI model in domestic pigs, subjected to sham operation (Sham), ischemia-reperfusion to induce AMI (AMI) or preconditioning (IPreC), postconditioning (IPostC), and remote perconditioning (RIPerC). MiRNA expression pattern was detected by high-throughput qRT-PCR. Potential protectomiRs were selected by systematic comparison of significant expression changes due to different conditioning stimuli vs. AMI. To validate the cardiocytoprotective effect of potential protectomiRs, isolated rat cardiomyocytes were transfected with specific miRNA mimics or inhibitors (antagomiRs) of the selected protectomiRs with cross-species sequence homology, and the survival of cells was measured after simulated ischemia-reperfusion injury. **Key Results:** Expression of 220 miRNAs was assessed and 57 microRNAs were changed by IPreC, 54 by IPostC and 68 by RIPerC as compared to AMI. Expression of 14 microRNAs changed significantly due to all three conditionings vs. AMI (four miRNAs were upregulated and ten downregulated). Rat homologs of these 14 protectomiR candidates were identified and 12 showed 100% sequence homology with the original pig miRNAs. Out of the selected 4 miRNA mimics and 8 antagomiRs, transfection of miR-450a and miR-451 mimics improved the survival of isolated rat cardiomyocytes after ischemia-reperfusion injury.

Conclusion and Implications: This is the first demonstration that miR-450a and miR-451 are associated with cardioprotection by ischemic pre-, post- and remote conditioning in a clinically relevant porcine model and also show their direct cardiocytoprotective effect. These protectomiRs are potential therapeutics for cardioprotection.

F-KB4-1 ECOSYSTEM NETWORK AROUND GEDEON RICHTER PLC.

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Innovation and ecosystems – fashion words Today but they have significant meaning when new industry trends are setting. At Richter a project was initiated to explore the still unexploited possibilities of building a novel innovative ecosystem around the company. There are necessary ingredients to build such system including the establishment an effective unit that has the main focus on the ecosystem work. Another important element is the organization of an active „ecosystem life" while keeping the focus on the original aims. Beyond that there are many other details of the organization work when building and maintaining a truly effective network on the long term.

F-KB4-2 OPTIMIZATION OF NOVEL α7 NICOTINIC ACETYLCHOLINE RECEPTOR POSITIVE ALLOSTERIC MODULATORS AND THE DISCOVERY OF A PRECLINICAL DEVELOPMENT CANDIDATE MOLECULE

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Scaffold hopping and HTS-based approaches for the development of α7 nAChR positive allosteric modulators with significant procognitive potential were utilized. HTS campaign of the corporate compound collection resulted in a novel, oxalic acid diamide scaffold of a7 nACh receptor positive allosteric modulators with good physicochemical and in vitro parameters. During the hit expansion, several derivatives demonstrated not only high in vitro potency, but also in vivo efficacy in the mouse place recognition test. During the hit-to-lead optimization campaign, the elevated risk of mutagenicity was successfully reduced by the elimination of the aromatic amine building block that resulted in a novel, aminomethylindole compound family. Subsequently, attempts were made to optimize the reactive indole structural element, the suboptimal metabolic stability, as well as the low kinetic solubility and found that the indole was important for in vitro activity. As a result of further refinements, a new chemotype (the azetidinespirochromone family) was identified, which proved to be one order of magnitude less lipophilic, while retaining the same high level of in vitro potency, improved metabolic stability and kinetic solubility. The most advanced compound with the most balanced physicochemical and pharmacological profile with significant in vivo efficacy in the scopolamine-induced amnesia test (Compound 53) was selected to be a novel preclinical development candidate (as RGH-560).

F-KB4-3 INTRACELLULAR PATTERN RECOGNITION NOD-LIKE RECEPTORS (NLR) IN DIFFERENT MACROPHAGE SUBPOPULATIONS

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Nod-like receptors (NLRs) are intracellular pattern recognition receptors (PRRs) that form protein complexes upon recognition of pathogen- or danger-associated patterns, PAMPs or DAMPs, respectively. NLRs regulate the production of key inflammatory cytokines (e.g. IL-1b via inflammasomes) or signal transduction pathways that modulate a variety of cellular processes, such as cell division or cell death. While inflammation is beneficial in the defense against pathogens, prolonged, chronic inflammation (e.g. sterile inflammation) or sudden high levels of inflammation (e.g. cytokine storm) can lead to severe tissue and cell damage. For this reason, NLRs and NLR-mediated pathways may serve as ideal targets in therapeutic interventions in various diseases.

The main mediators of inflammatory processes are macrophages, which have different functions depending on their origin (e.g. yolk sac, liver, bone marrow), localization (tissue resident macrophages) or activation (altered microenvironment). Our results show that, upon activation, NLR family members are expressed with different dynamics in different monocyte-derived macrophage subpopulations, and the outcome of the NLR-mediated responses is highly dependent on the microenvironment and the macrophage subpopulation. We will review the cell-specific properties of NLRs through literature and our own research, and their potential usage in therapeutic interventions.

Funding: NKFIH-OTKA K131844 and NFKIH OTKA 147109.

F-KB4-4 DEVELOPMENT OF A COMPLEX TRANSLATIONAL TEST BATTERY FOR THE INVESTIGATION OF CORTICAL EXCITABILITY IN NON-HUMAN PRIMATES

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Cortical excitability (CE) refers to the general responsiveness of neurons to discrete stimulation events in the cerebral cortex that can be considered a prerequisite of various cognitive processes including attention, memory, and decision-making, and is altered in neurological and psychiatric conditions. Various therapeutic interventions may also alter CE, leading to adverse events such as sedation, seizures or cognitive impairment. Non-human primates (NHPs) are considered the most relevant preclinical models of cortical functions and are ideal models to determine whether a drug candidate alters cognition, or, conversely, normalizes altered cortical function in pathological states. In the present series of experiments, we separately used non-invasive electroencephalography (EEG), transcranial magnetic stimulation (TMS) and cognitive testing in arrangements that are similarly applicable in humans and NHPs, aiming to establish and validate a new non-invasive experimental battery for the assessment of CE and its downstream consequences on cognition and behaviour in rhesus macaques.

In the TMS experiments, we stimulated the primary motor cortex at the hand area of awake monkeys using frameless neuronavigation and measured the resulting motor evoked potentials (MEPs) via electromyography. We recorded individual motor thresholds (MT) as per traditional guidelines and also assessed the degree of neuronal recruitment on input-output (I/O) curves from MEP responses to stimulation at various intensities. In the EEG experiments NHPs performed a simple eye-fixation task, during which we recorded ongoing spontaneous and task-evoked scalp-EEG activity using a high-performance telemetric EEG system. For cognitive testing, pre-trained animals performed an object-location working memory task (paired associates learning, PAL) in a touch screen apparatus. To validate the measurement systems by intentional modulation of CE, diazepam (DIAZ), a positive allosteric modulator to the $GABA_A$ receptors with a potent sedative effect was used in 3 doses: 0.1, 0.3 and 1 mg/kg.

In TMS, DIAZ exerted a pronounced increase of MT, and a rightward shift in the I/O curve. In scalp EEG, a profound increase of low-frequency (alpha-beta) and a decrease of high-frequency (gamma) oscillatory power was observed with a strong frontal focus. In the PAL test, animals showed a marked dose-dependent decrease in memory performance accompanied by slower response times. Thus, for the same treatments that had sedative and/or amnestic behavioural effects, TMS and EEG reliably and convergently indicated a pronounced shift of CE towards inhibition, showing a remarkable potential towards their complementary use to assess CE. The presently introduced preclinical battery for NHPs may also be a great tool to understand the downstream effects of CE on cognition and behaviour, and for the validation of novel drug targets and treatment avenues in psychiatric indications that are thought to involve abnormal CE.

ANIMALAB

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- Anesztézia, eutanázia berendezések
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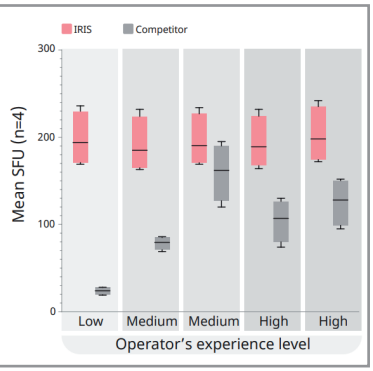

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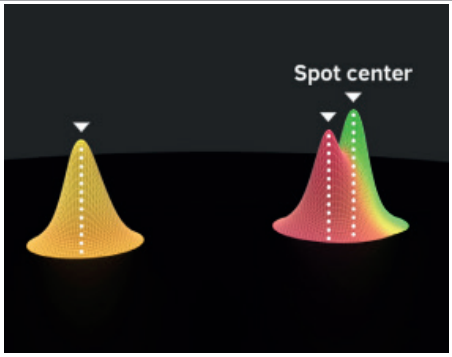

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POSTER ABSTRACTS

Conference on Pharmacology, Pharmacokinetics & Innovation

HUPHAR 2024

MATRAHAZA **5-7, JUNE**

1. LOW ACTION POTENTIAL THRESHOLD SHORTENS INPUT-TO-OUTPUT DELAY IN ELECTRICALLY SLOW PARVALBUMIN INTERNEURONS IN THE HUMAN NEOCORTEX

Emoke Bakos[#1,2], Ádám Tiszlavicz[#1], Viktor Szegedi[1,2], Abdennour Douida[1], Pal Barzo[3], Gabor Tamas[4], Attila Szucs[5], Karri Lamsa*[1,2]

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In human brain, high energy-demanding neurons such as fast-spiking interneurons in the neocortex need reduced ion fluxes for action- and synaptic potentials because they exhibit increased somatic input resistance compared to rodent cells. This has to be an evolutionarily important adaptation in human neurons because it also slows down electrical reactivity of a neuron making cell soma slower in processing electrical inputs and generating action potential. Slowness is not beneficial to fast-spiking neuron computation, and therefore compensatory mechanisms have evolved in human neurons to boost their electrical rapidity. We report that fast-spiking interneurons in the human neocortex exhibit lowered action potential firing threshold, and this shortens the delay to generate action potential in response to excitation in soma. We show with anatomical analysis and demonstrate with realistic single-neuron computational model that the firing threshold is regulated through axon initial segment (AIS) length and proximity to soma whereas sodium channel localization pattern in the AIS is similar across fast-spiking cells.

2. TOWARD MACHINE-ASSISTED CAUSALITY ASSESSMENT IN PHARMACOVIGILANCE: USING CONTRASTIVE LEARNING TO CREATE VECTOR REPRESENTATIONS OF ADVERSE EVENTS FROM SPONTANEOUS REPORTS

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In the pursuit of drug safety, post-marketing drug surveillance, also known as pharmacovigilance, plays a crucial part, yet it faces challenges as its methods rely on text-based data which are unsuitable for contemporary machine learning approaches. Recent innovations in artificial intelligence are spearheaded by natural language processing (NLP) and computer vision (CV), that offer a plethora of sophisticated tools to be adapted onto other scientific fields.

Here, we investigate how to best utilize contrastive learning algorithms, one from NLP (skip-gram negative

sampling) and one from CV (NT-xent), to generate vector representations of adverse events from structured spontaneous reports to be used in drug-event causality assessment.

We present comprehensive analyses of the resulting representation spaces through qualitative and statistical evaluation methods, revealing compelling patterns that reflect both functional and causal relations of the adverse events. We demonstrate how they capture drug-safety related information better than existing taxonomies, by using recorded adverse reactions from text-mined drug labels, and support our visualization of the results with cluster analysis. Furthermore, we demonstrate their applicability in practice by our downstream predictive model, performing causality assessment, for which we created a new, thoroughly standardized training dataset compatible with several biomedical databases. Model performance was tested on independent benchmarks and summarized by area under the receiver operating characteristic (AUROC) values, which showed on-pair and outperforming results (AUROC: 0.75-0.92) as compared to previous methods.

As such, we advance machine-assisted pharmacovigilance by providing adverse event representations that are interpretable and information rich on their own, but also flexible and effective as feature vectors for training arbitrary models. This enables the application of a wide array of machine learning approaches to aid and accelerate the decision making of experts during drug-event causality assessment, increasing cost effectiveness and leading to improved drug safety.

3. STUDY OF THE INTRACELLULAR PATTERN RECOGNITION NOD-LIKE RECEPTORS IN SKELETAL MUSCLE

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Besides locomotion, skeletal muscle may act as an endocrine organ and produces myokines in response to environmental and internal stimuli as well as following physical activity. These myokines (e.g. IL-6, IL-15) play a critical role in many physiological processes, including muscle development and regeneration, and also they affect the function of other organs. Furthermore, skeletal muscle is also able to respond to cytokines produced by other organs. One such cytokine is IFNγ, the presence of which is important for proper skeletal muscle differentiation. However, increased IFNγ production in chronic inflammatory diseases has a negative effect on muscle function. The largest family of intracellular pattern recognition receptors (PRRs) are the NOD-like receptors (NLRs), which have been the focus of intense research for many years. Members of the protein family play important roles in cytokine production, cell division, inflammation or various cell death processes. While the function of NLRs are highly studied in immune competent cells, the potential role of NLRs in skeletal muscle is almost completely unknown.

To study the expression and function of NLRs, we did *in vitro* experiments (C2C12 immortalized mouse skeletal muscle cell lines), *ex vivo* experiments (primary skeletal muscle myoblasts isolated from C57BL/6 mouse) with undifferentiated (Myoblast) or differentiated (Myotube) state, while the *in vivo* measurements were performed on mouse T*ibialis anterior* (TA) muscles. The expression of PRRs was determined at baseline and after specific stimuli using RT-qPCR and RNASeq techniques, and the results were validated by Western blot. Myokine secretion was measured by ELISA.

Our results show that many PRRs are expressed in skeletal muscle and their expression changes dynamically during differentiation. We observed increased expression of several PRRs (e.g. NOD1) following IFNγ treatment of C2C12 and intramuscular injection of the T*ibialis anterior*. Importantly, in the presence of IFNγ, NOD1 specific agonist administration strongly induced IL-6 myokine secretion, and response was mediated via NF-κB signaling pathway. Furthermore, the co-expression of IFNγ and NOD1 agonist inhibited C2C12 differentiation.

Our results show for the first time the dynamic expression of several NLRs in skeletal muscle, and that targeted manipulation of NLRs affects myokine secretion and differentiation in muscle cells.

Financial support: NKFIH OTKA K131844 and NFKIH OTKA 147109 (B.S.). B.E. holds ÚNKP-23-3 scholarship. H.A. is a receiver of Stipendium Hungaricum Scholarship.

4. THE STEROID RGH-235, A SUBTYPE SELECTIVE NEGATIVE MODULATOR OF HISTAMINE H3 RECEPTOR SHOWS EFFICACY IN ANIMAL MODELS OF COGNITIVE IMPAIRMENT

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Histamine H3 receptor is a G-protein coupled receptor activated by the endogenous ligand histamine. It shows highest expression levels in the basal ganglia, cortex, and hippocampus; and considered as a favourable molecular target for the treatment of cognitive deficits. Here we present the *in vitro* and *in vivo* profile of the steroid (15alpha)-N-ethyl-3-{3-[(2R)-2-methylpyrrolidin-1-yl]propoxy}-17-oxoestra-1,3,5(10)-triene-15-carboxamide (RGH-235), a potent, selective, and orally active H3 receptor antagonist/inverse agonist.

RGH-235 displayed high affinity to H3 receptors in radioligand displacement assays (Ki = 3.0–9.2 nM, depending on species), without significant affinity to H1, H2 or H4 receptors and >100 other targets. RGH-235 proved to be an inverse agonist in [35S] GTPgammaS binding assay and antagonized imetit-induced ERK1/2 phophorylation by ELISA assay. Unlike most neuroactive steroids RGH-235 showed no effect on the function of GABA-A receptors. With favourable kinetics after oral administration, RGH-235 inhibited the imetit-induced dipsogenia (a pharmacodynamic test). RGH-235 was active at 1 mg/kg in spontaneously hypertensive rats (SHR), generally considered as a model of ADHD, and revealed pro-cognitive profile in mice place recognition and rat novel object recognition tests from 0.3 mg/kg. RGH-235 also showed procognitive efficacy in models of high translational value as at 0.05 mg/kg it reversed MK-801 induced cognitive decline in rat touch screen visual discrimination test; and at 0.3 mg/ kg it reversed scopolamine induced performance decline in rhesus monkeys in a visual reaction time task assessing the attention domain of cognitive performance.

In conclusion, the centrally acting steroidal RGH-235 effectively inhibits the function of H3 receptor. The multiple and convergent procognitive effects support the view that beneficial cognitive effects can be linked to antagonism/inverse agonism of H3 receptors.

5. ACUTE AND CHRONIC EFFECTS OF CARIPRAZINE ON PRIMARY HIPPOCAMPAL NEURONS

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Cariprazine is a novel antipsychotic drug which is used to treat patients with psychiatric disorders like schizophrenia, bipolar (type I) and major depressive disorder. An intriguing feature of this compound is that it is affecting not only positive symptoms, such as hallucinations, but also modulating negative symptoms of schizophrenic patients. In our research, we aim to investigate the acute and chronic effects of cariprazine on neuronal function. For this, we investigate the action of the drug on the firing properties and synaptic communication of neurons in primary hippocampal cultures using the whole-cell patch clamp method. Our aim is to understand the underlying mechanism of cariprazine on mouse primary hippocampal neurons, which are relatively straightforward to obtain and already thoroughly characterized by our laboratory. For this, we are recording the neurons both in current clamp and voltage clamp mode to identify potential cellular and synaptic targets of cariprazine. Neurons under total synaptic isolation (bath solution containing CNQX, bicuculline and AP-5 receptor antagonists) are stimulated with incrementing levels of intracellular current to elicit subthreshold and suprathreshold voltage responses. We determine passive and active membrane properties of the neurons including the membrane resistance, voltage sag, rheobase, spike amplitude and slope among others. On the other hand, we investigate the effects of cariprazine on glutamatergic and GABAergic spontaneous neurotransmission using tetrodotoxin and voltage clamp recording of postsynaptic membrane currents.

6. UNRAVELING THE IMPACT OF NSAIDS ON KIDNEY FUNCTION: AUTOPHAGY, FIBROSIS, AND DOSE-DEPENDENT MORPHOLOGICAL INSIGHTS

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Background: NSAIDs, commonly used to manage inflammation, pain, and fever, work by inhibiting COX enzymes and reducing prostaglandin synthesis. Despite their benefits, NSAIDs can impact renal health, potentially causing nephrotoxic effects. Additionally, NSAID use can cause mitochondrial dysfunction and oxidative stress, contributing to renal damage and fibrosis–processes involving pro-fibrotic factors such as TGF-β and transcription factor EGR1. Kidney injury might alter autophagy processes leading to reduced cellular repair and contributing to kidney fibrosis. Yet, the effects of NSAID on renal autohpagy has not been investigated.

Aim: Our study endeavors to elucidate how NSAIDs affect pro-fibrotic signaling and autophagy pathways and to examine the associated histological damage to uncover the underlying pathology.

Methods: Male Wistar rats were orally administered various NSAIDs twice daily for two weeks: indomethacin (2 mg/kg), naproxen (10 or 20 mg/kg), celecoxib (10 and 30 mg/kg), or a vehicle (1% hydroxyethylcellulose). Left kidneys underwent histological evaluation using PAS stained slides. Right kidney cortex and medulla samples were snap-frozen for qPCR and immunoblotting analyses.

Results: Our research reveals that NSAIDs, specifically Celecoxib and Naproxen, induce dose-dependent changes in renal morphology, such as increased tubular dilatation and atrophy. High doses of these NSAIDs significantly elevate *Egr1* expression in both the renal cortex and medulla, highlighting a drug and dose-specific impact. In the renal cortex, pro-fibrotic *Tgfb1* gene expression was generally reduced, while Naproxen and Celecoxib increased *Egr1* and *Timp1*, respectively. Contrastingly, in the medulla, these NSAIDs elevate *Egr1*, *Timp1*, *cJun*, and *Tgfb1*, illustrating a nuanced interaction between NSAID concentration and the modulation of pro-fibrotic genes. Immunoblot analyses indicate enhanced autophagy initiation, as shown by raised LC3-I levels and the LC3-II/I ratio, but the concurrent increase in SQSTM1 (p62) levels suggest disrupted autophagic degradation. Together, these findings highlight the complex role of NSAIDs in modulating kidney damage through distinct changes in

pro-fibrotic signaling and autophagy pathways.

Conclusion: NSAIDs, especially Celecoxib and Naproxen, exhibit dose-dependent effects on renal morphology and key biological markers, underscoring a delicate balance between therapeutic benefits and renal risks. Our findings advocate for cautious NSAID usage, emphasizing the need for further research to optimize NSAID therapy while minimizing renal adverse effects.

7. POTENTIAL AND LIMITATIONS OF INFLAMMATORY AND NEUROPATHIC RAT MODELS OF TRIGEMINAL ACTIVATION

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Primary headache disorders such as migraine are prevalent and debilitating pain conditions affecting a large population. While the precise underlying pathophysiological mechanisms are not fully understood, activation and sensitization of trigeminal sensory neurons and neurogenic inflammation via the release of pro-inflammatory neuropeptides in the dura mater play important roles. Given the lack of optimal translational models, it is important to characterize and compare different *in vivo* paradigms to identify key mediators and novel therapeutic targets. Inflammatory and neuropathic orofacial or periorbital pain models are accepted surrogate models for investigating pain mechanisms related to primary headaches. Here we performed functional studies in three rat models of different origin.

Chronic orofacial inflammation and consequent allodynia was induced by subcutaneous injection of Complete Freund's Adjuvant (CFA) into the right whisker pad of adult male Sprague-Dawley rats. Meningeal inflammation and periorbital allodynia were evoked by supradural infusions of an "inflammatory soup" consisting of 2 mM histamine, bradykinin, serotonin, and 0.2 mM prostaglandin E2 (Oshinsky model). Neuropathic allodynia was induced by partial ligation of the infraorbital nerve (pIONL model). The mechanonociceptive threshold values were measured using von Frey filaments.

In the CFA model approximately 60% of all rats exhibited allodynia (n = 29), with threshold values decreasing from 18.30 g to approximately 5 g, persisting for 9 days. In the Oshinsky model around 45% of the rats displayed periorbital allodynia (n = 13) shown by mechanonociceptive threshold decrease from 18.30 g to around 12 g, with small variations throughout the 27-day experiments. In the pIONL model roughly 30% of the animals developed orofacial allodynia (n = 36), with threshold decrease from 18.30 g to 13 g, the degree of sensitisation varied considerably between measurements, lasting for 16 days.

All three models are suitable for testing trigeminovascular activation-induced allodynia, so they can all be used for investigating the pathophysiological mechanisms behind primary headache diseases. Since CFA induced the most severe and stable allodynia and in the highest proportion of animals, this model seems to be appropriate for testing the effects of potential novel antimigraine drugs.

Acknowledgements: Supported by the Gedeon Richter's Talentum Foundation; the New National Excellence Program (ÚNKP-23-4-II), the National Brain Research Program 3.0 (NAP-3; Chronic Pain Research Group); the National Research, Development, and Innovation Fund of Hungary, under the EGA 16 funding scheme (TKP 2021-EGA-16) and by the European Union (Project no. RRF-2.3.1-21-2022-00015).

8. CARDIOPROTECTIVE MICRORNAS (PROTECTOMIRS) IN A PIG MODEL OF ACUTE MYOCARDIAL INFARCTION AND CARDIOPROTECTION BY ISCHEMIC CONDITIONING: MIR-450A AND MIR-451

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MiRNAs are promising therapeutic tools for cardioprotection. In this study, we aimed to identify cardioprotective miRNAs (protectomiRs) in a porcine model of acute myocardial infarction (AMI), in which ischemic conditioning was applied for cardioprotection, and to validate their cardiocytoprotective effects in isolated rat cardiac myocytes.

In our previous study, domestic pigs were subjected to sham operation, ischemia-reperfusion to induce AMI (AMI), or AMI and ischemic preconditioning (IPreC), postconditioning (IPostC), or remote perconditioning (RIPerC). Tissue samples were collected from the infarcted region of the left ventricles. To select protectomiR candidates, we performed a systematic comparison of miRNA expression changes after different conditioning stimuli versus AMI using high-throughput qPCR analysis. By cross-species sequence comparison, we identified rat orthologs of the protectomiR candidates identified in pigs. To validate their cytoprotective effects, mimics or inhibitors (antagomiRs) of the selected protectomiRs or negative control miRNAs were transfected into isolated rat cardiac myocytes at 25, 50, or 100 nM concentrations. Cell survival was measured after 6 hours of simulated ischemia and 2 hours of reperfusion (sI/R).

In total, 220 miRNAs were detected in porcine hearts. Of these, 57 miRNAs were altered by IPreC, 54 by IPostC, and 68 by RIPerC compared to AMI. Four miRNAs were upregulated, and ten were downregulated by all three conditioning stimuli compared to AMI. 12 protectomiR candidates showed 100% sequence orthology with the identified porcine miRNAs in rats. The survival of rat cardiac myocytes was significantly improved by miR-451 and miR-450a mimics at 25 nM after sI/R compared to the negative control miRNA transfection. However, the cardiocytoprotective effects of the other protectomiR candidates could not be validated.

This is the first demonstration that miR-451 and miR-450a are associated with cardioprotection by ischemic conditioning in a clinically relevant pig model and show cardiocytoprotective effect. These protectomiRs could serve as a basis for developing cardioprotective therapeutics.

9. DECREASED LEVEL OF THE VIRAL RESTRICTION PROTEIN IFITM3 IN HEART FAILURE

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Introduction: Viral infections are key risk factors for heart failure exacerbations patients and are often causes of death in heart failure patients. Interferon-induced transmembrane protein 3 (IFITM3) is an antiviral protein, part of the innate immunity, and is able to restrict virus infection by the inhibition of virus entry into the cells. IFITM3 deficiency may predispose to severe complications upon infection with viruses including influenza or COVID-19. Recently it was also discovered that IFITM3 has a protective role in the heart tissue during influenza virus, as it is associated with limiting cardiac fibrosis and electrical dysfunction during infection. In our current study, we aimed to investigate the expression level of IFITM3 in human heart tissue samples from different types of heart failure (heart failure with reduced ejection fraction [HFrEF], heart failure with preserved ejection fraction [HFpEF]), and from patients with different comorbidities (diabetic (DM) – non-diabetic).

Methods: IFITM3 mRNA expression levels were investigated in the following groups: HFrEF (with ischemic [ICM, n=8] and non-ischemic [DCM, n=8] etiology), and heart failure with preserved ejection fraction (HFpEF) (n=6) samples, compared to healthy control samples (n=14). Comorbid HFrEF+DM (n=7) and HFpEF + DM (n=8) samples were also used to compare IFITM3 levels. Protein level expression was measured by Western Blot (WB) in the DCM and ICM sample groups.

Results: According to our results, in both DCM and ICM groups, the mRNA level of IFITM3 was significantly downregulated compared to the healthy control samples, which was validated at the protein level by WB measurement. Similarly, in both HFrEF and HFpEF/HFpEF+DM sample groups, the expression level was significantly lower compared to the control group.

Conclusion: In conclusion, we hypothesise that the reduced expression level of IFITM3 in different heart failure groups may play a role in the higher susceptibility of viral infections and may contribute to the higher mortality of these patients.

10. GLYCINE TRANSPORTER 1 INHIBITORS DELAY MORPHINE ANALGESIC TOLERANCE IN RATS

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Introduction: Opioid analgesics are a mainstay in the management of mild to severe acute and chronic pain types, yet their long term-application raises concerns related to central side effects such as addiction, respiratory depression, and analgesic tolerance. Three types of opioid receptors have been identified and named as μ-opioid receptors (MORs), δ-opioid receptors (DORs), and κ-opioid receptors (KORs). The majority of current prescribed opioid analgesics target MORs. The long-term use of MOR agonists develops analgesic tolerance, dose escalation is required to retain the opioid analgesic efficacy, increasing the side effects too. Despite numerous efforts being done to delay tolerance development, none of them have clinical relevance. Thus, exploring new treatment avenues is crucial for chronic pain patients. Recent research suggests that glycine transporter-1 (GlyT1) inhibitors have potential in halting neuropathic pain, which shares certain spinal mechanisms with opioid tolerance such as ionotropic N-methyl-D-aspartate receptors' (iNMDARs) activation [1], [2].

Aims: This study aims to investigate the involvement of the spinal glycinergic system in morphine analgesic tolerance development and to assess the impact of the selective GlyT1 inhibitor NFPS on opioid antinociceptive tolerance following repeated morphine administration.

Methods: In vivo and in vitro studies were applied to measure tolerance and NFPS effect. The rat tail-flick assay, a thermal pain model was employed, where male Wistar rats (170-250g) received subcutaneous morphine, NFPS or their combination for 10 days. Pain threshold was measured before and after treatment (30, 60, 120, 180 min). After 10 day-treatment, cerebrospinal fluid (CSF) glycine levels were measured using capillary electrophoresis. Motor function was assessed using the rotarod test. Statistical analysis was performed using appropriate tests.

Results: Acute morphine treatment (10 mg/kg) resulted in significant antinociceptive effects, but high degree of tolerance was measured after 10 days. NFPS failed to show antinociception alone but significantly delayed morphine tolerance when co-administered chronically at 0.6 mg/kg. CSF glycine levels increased following NFPS treatment, significantly in combination with morphine at 0.6 mg/kg. Chronic treatment with either NFPS doses failed to affect the motor function of the animals at the tested time points.

Conclusions: The reduction in morphine antinociceptive efficacy over 10 days aligns with previous reports on opioid analgesic tolerance development. Simultaneous administration of GlyT1 inhibitors with opioid analgesics maintains antinociceptive effect of opioids. The observed effect could be related to the regulation of glycine at the vicinity of spinal iNMDARs, in particular the extrasynaptic GluN2B which is implicated in the mechanism for opioid analgesic tolerance.

Fund: FK_138389

[1] M. Al-Khrasani *et al.*, 2019
[2] A. Mohammadzadeh *et al., 2021*

11. TOLPERISONE/PREGABALIN COMBINATION PRODUCES ACUTE ANTIALLODYNIC ACTION IN RAT MONONEUROPATHIC PAIN

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Introduction: Neuropathic pain (NP) is a chronic condition that results from a disease or damage to the somatosensory neurons. Treatment of NP has not been solved thus far owing to the diversity of etiologies

and underlying mechanisms. The current treatment of NP is unsatisfactory; therefore, effective novel agents or combination-based analgesic therapies are needed. Pregabalin (P) is considered a first-line medication for the treatment of NP, and its effect is mediated by calcium channels. On the other hand, our recent data showed that tolperisone (T) displays antiallodynic effect in rats with partial sciatic nerve ligation (pSNL) whereas morphine (M) effect was only achieved in high doses.

Aims: The present work aimed to investigate the antiallodynic effect of T, P, and M alone or in combinations in pSNL-induced NP in rats. In addition, to assess the test drugs or combinations in mouse vas deferens (MVD) experiments.

Methods: The antiallodynic effect of oral T and P (both at 25 mg/kg), M at 3.22 mg/kg alone and in combination, was investigated in male Wistar rats with mono-neuropathy evoked by pSNL. Tactile allodynia is indicated by a decrease in the paw withdrawal threshold measured by a dynamic plantar aesthesiometer. The acute drug effect was assessed 2 weeks post-operation. In vitro assay, MVD experiments used male NMRI mice (35–45 g, 6–10 weeks of age) to assess the efficacy (Emax) of T and P compared to that of reference compounds, DAMGO and M. Then, the T, P, or M concentration that produced 20% inhibition was tested in drug combinations.

Results: The single dose of either T or P alone failed to produce an acute antiallodynic effect even in doses up to 100 mg/kg. M in a small dose failed to produce an effect, but a dose of 6.4 mg/kg produced an effect on operated and non-operated paws. Only the T/P combination acutely alleviated allodynia. However, the T/M combination failed to show an antiallodynic effect. In vitro, T, P, M, or DAMGO, in a concentration-dependent manner, inhibited the MVD smooth muscle contractions. The measured Emax for T, P, M, or DAMGO was 84.85, 36.57, 74.01, or 91.06 %, respectively. T/P in submaximal concentrations produced 26 % vs 16% when added separately. This character was not seen when T or P was combined with M.

Conclusion: Our study highlights the limited efficacy of single-agent therapies for NP, with respect to T, P, or M even when administered for a long period of time. On the other hand, once combined T with P but not with M could produce higher antiallodynic efficacy of fast onset. Mechanistically, our study demonstrated that simultaneous blockage of the sodium and calcium channels is worth further investigation in the context of NP management.

Funding: The present work was supported by the 2018-1.3.1-VKE-2018-00030 project.

12. THE EFFECT OF LOCAL ADMINISTRATION OF BGP-15 ON THE REGENERATION OF ARTERIAL MICROVASCULAR ANASTOMOSIS IN RATS

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Regeneration of microvascular anastomosis is highly dependent on fibroblast proliferation and persistence, as well as the deposition of new extracellular matrix that provides biomechanical stability. BGP-15 is a multifaceted drug candidate that shows exceptional promise in cellular integrity and reducing oxidative stress in a variety of diseases. Additional effects on the local postoperative inflammation or following systemic absorption of the agent can supposedly be beneficial. The aim of this research was to investigate whether BGP-15 has a beneficial effect on the regeneration of microvascular anastomoses. This study was extended to include not only mechanical variables, but also hematological and hemorheological factors and their potential implications.

Each of the four defined animal groups contained eight male Wistar rats (330.2g±17.5; permission

registration number: 21/2022/UDCAW). Three groups of rats underwent microvascular anastomosis on their right femoral arteries; two of these groups additionally got a subcutaneous cannula with local administration of either saline or BGP-15 (15 mg). We applied the 10/0 polyamide-6 eight-stitch end-to-end microsurgical anastomosis technique. To evaluate vascular patency, blood flow (Transonic T206) of the vessels was monitored prior to and after surgery, and on the 21th postoperative day. Subcutaneous BGP-15 was given to the remaining animal group that did not undergo any surgical procedures. We used lateral tail venous samples to investigate weekly changes in the general hematological and micro-rheological variables. The animals were over-anesthetized on day 21 in order to collect tissue samples, to measure tensile strength, and to examine the arteries histologically.

Every anastomosis was succesfully performed, with little variations in blood flow and without any aneurysm or stenosis. Anastomoses treated with BGP-15 had comparable mechanics to newly created anastomoses (0.91±0.14 N), with a considerably higher tensile strength (0.84±0.21 N) than those treated with saline (0.64±0.07 N), or control (0.44±0.04 N). BGP-15 also significantly reduced white blood cell count, which attenuated the inflammatory response. Moreover, during the first two weeks following surgery, it increased erythrocyte aggregation and somewhat decreased the quantity of red blood cells. However, when the medication was administered on its own, these modifications were not noticed.

Our findings indicated that artery anastomoses' mechanical strength was enhanced by local BGP-15 treatment. Following absorption, this agent had potent anti-inflammatory effects, which helped to foster the vascular regeneration. We also demonstrated the safety profile of BGP-15 on hematology and hemorheology, and so on microcirculation.

13. CX3CR1 FRACTALKINE RECEPTOR ACTIVATION REDUCES CHRONIC ARTHRITIC PAIN: IN VIVO STUDY USING GENE-DEFICIENT MICE AND SELECTIVE RECEPTOR ANTAGONIST

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The fractalkine chemokine receptor 1 (CX3CR1) is primarily expressed on monocytes/macrophages, T lymphocytes, osteoclast precursors and microglial cells. It was described to mediate inflammatory mechanisms both in the periphery and the central nervous system. Although the role of neuroinflammation has been described in some pain conditions, little is known about the role of CX3CR1 in chronic joint pain. Therefore, we investigated the involvement of CX3CR1 in the adjuvant-induced chronic mouse arthritis model.

Complete Freund's adjuvant (CFA) was injected intraplantarly and into the tail root in C57BL6/J wild-type (WT) and CX3CR1 gene-deficient (CX3CR1$^{-/-}$) mice. WT mice were treated with the small molecule CX3CR1 antagonist AZD8797 (2x1 mg/kg/day, i.p.) or its vehicle every day during the 21-day experimental period. Mechanonociception was measured by aesthesiometry, thermonociception by constant temperature hot plate, paw volume by plethysmometry, neutrophil myeloperoxidase (MPO) activity by luminescence, plasma extravasation by fluorescence in vivo imaging, and histopathological alterations by semiquantitative scoring.

Approximately 20-40% mechanical hyperalgesia, 40-60% latency decrease in nocifensive behaviors on the hot plate, 80-100% paw edema, increased neutrophil MPO activity and plasma extravasation, and histopathological damage (mononuclear cell infiltration, synovial hyperplasia, cartilage destruction) were detected in CFA-injected WT and vehicle-treated mice. Mechanical hyperalgesia was lower in CX3CR1$^{-/-}$ mice

between days 10 and 21, and in AZD8797-treated WT mice between days 3 and 15 with moderate/large effect size (g>0.5). Thermal hypersensitivity was lower in CX3CR1$^{-/-}$ mice between days 12 and 15, and in AZD8797-treated WT mice between days 15 and 21 also with moderate/large effect size (g>0.5). However, no differences were found in any inflammatory or tissue damage parameters compared to the WT and vehicle-treated controls.

CX3CR1 activation mediates chronic arthritic pain, mainly independently of the peripheral inflammatory processes. Therefore, its inhibition offers promising novel analgesic perspectives.

Acknowledgement: OTKA K-138046, RRF-2.3.1-21-2022-00015, TKP2021-EGA-13, TKP2021-EGA-16, Hungarian Research Network (Chronic Pain Research Group), National Brain Research Program 3.0

14. PHARMACOLOGICAL CHARACTERIZATION OF THE CHRONIC STRESS-INDUCED PAIN MODEL IN MICE: GABAPENTIN, BUT NOT TRAMADOL REDUCES BOTH COLD AND MECHANICAL HYPERALGESIA

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Background and aims: Chronic psychosocial distress induces and aggravates pain conditions such as fibromyalgia. This is a widespread musculoskeletal primary chronic pain, in which stress is the only known etiological factor. The opioid tramadol and the adjuvant analgesic anticonvulsant gabapentin are among its treatment options, but they have limited efficacy and broad side effect profiles. The chronic restraint stress (CRS)-induced mouse pain model is often used to study fibromyalgia-like mechanisms, but its pharmacological sensitivity has not been described. Therefore, here we investigated the effects of tramadol and gabapentin on CRS-induced hyperalgesia.

Methods: Female and male 12- to 14-week-old C57BL/6 mice were exposed to CRS in well-ventilated tubes for 6 h/day for 2 weeks, non-stressed mice served as controls (n=8-10/group). Cold sensitivity of the paw was measured by paw withdrawal latency from icy water and mechanonociceptive threshold with dynamic plantar aesthesiometry before the stress paradigm and at the end of both weeks. Gabapentin (1 mg/ml), tramadol (2 mg/ml) or saline (vehicle) was injected i.p daily from day 6, 30 min prior to measurements. Open field test was performed on day 13 to detect spontaneous locomotion and anxiety.

Results**:** CRS induced approximately 50-60% cold and 25% mechanical hyperalgesia of the paw already after 1 week, which was maintained until the end of the second week without remarkable anxiety and locomotor disturbances. Gabapentin, but not tramadol reduced cold hyperalgesia on day 13 with large effect size values (Cohen's d=1.185 for gabapentin, 0.772 for tramadol), while neither drug had effect after acute administration on day 6. Both compounds exerted inhibitory actions on mechanical hyperalgesia after acute (day 7) and chronic (day 14) administrations, but the effect did not increase after the 8-day-treatment. Neither treatment altered behavioural parameters in the open field test.

Conclusions**:** The CRS model is appropriate to study cold and mechanical pain observed in fibromyalgia patients. Gabapentin acting via inhibiting voltage-gated Ca^{2+} channels and consequent glutamate release, simultaneously decreases cold and mechanical hyperalgesia indicating inhibitory potentials on both peripheral and central sensitization. Meanwhile, the opioid tramadol only reduces mechanical hyperalgesia suggesting its mainly central mechanism of action. These results provide pharmacological validation of the CRS model for

fibromyalgia research.

Funding: NKFIH K 138936, NKFIH K 138046 and NKFIH FK 146283 (OTKA) as well as Project no. RRF-2.3.1-21-2022-00011, titled National Laboratory of Translational Neuroscience has been implemented with the support provided by the Recovery and Resilience Facility of the Europian Union within the framework of Programme Széchenyi Plan Plus.

15. TOPIRAMATE REVERSED THE ADJUVANT-INDUCED INFLAMMATORY OROFACIAL ALLODYNIA IN RAT

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Orofacial pain is a common debilitating condition, affecting the face, head, and the neck. The pain is usually caused by the hypersensitization of extra- and intracranial trigeminal primary afferents [1, 2]. It has been described that orofacial inflammation can activate the peptidergic, trigeminal, sensory neurons, inducing mechanical allodynia or hyperalgesia [3]. As a large proportion of patients remain without adequate therapy, it is important to find new therapeutic approaches in different animal models. Therefore, we aimed to characterize the Complete Freund's Adjuvant (CFA)-induced orofacial allodynia model with drugs (e.g. sumatriptan, topiramate), which are widely used in disorders connected with the the trigeminovascular system. Topiramate inhibits mainly the voltage-gated Na^+ and Ca^{2+} -channels, and also modulates other targets (e.g., $GABA_A$ receptors, glutamate receptors), while sumatriptan acts as an agonist on the 5-HT1B/1D receptors. Our results may prove the relevance of the model and enables the testing of new drug candidates.

In order to induce orofacial allodynia, CFA was injected (0.5 mg/ml, 50 µl subcutaneous) into the right whisker pad of adult male Sprague-Dawley rats, then mechanonociceptive thresholds were measured using von Frey filaments. Mechanical allodynia was tested 3, 5, and 7 days after CFA injection, during which the effects of sumatriptan (1 mg/kg s.c.) and topiramate (30 mg/kg p.o.) were evaluated 60, 120 and 180 minutes after treatments. Data were analysed using GraphPad Prism 9 software.

Three days after CFA administration, robust orofacial inflammatory allodynia was developed in about 60% of the animals, with mechanonociceptive thresholds decreasing from 18.30 g to about 5 g. Topiramate produced a statistically significant antiallodynic effect on all experimental days compared to the vehicle-treated group. Moreover, significant differences in mechanonociceptive threshold values were observed pre- and post-topiramate treatment within the same group. Although sumatriptan appeared to reduce CFA-induced allodynia, no statistically significant difference was observed compared to the vehicle-treated group.

This model is appropriate to investigate chronic orofacial allodynia related to trigeminovascular activation and to evaluate the effect of new drug candidates in comparison with topiramate, as the reference compound.

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Acknowledgment: Supported by TKP 2021-EGA-16, NAP-3, PTE-ÁOK-KA-2023-23, ÚNKP-23-4-II.

16. IDENTIFICATION AND CHARACTERIZATION OF ORGANIC POLYSULFIDE BINDING SITE ON HUMAN TRPA1 RECEPTOR

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Inorganic polysulfides are endogenous compounds that are mainly generated at the site of inflammation and exert analgesic and anti-inflammatory effects through direct activation of the TRPA1 receptor and indirect activation of the SST_4 receptor. These effects would be beneficial in the treatment of persistent pain and chronic inflammation. However, inorganic polysulfides are too reactive and unstable compounds to be suitable as drugs. Instead, we have turned our attention to exogenous organic polysulfides such as the garlic derived dimethyl trisulfide (DMTS) from garlic, diallyl disulfide (DADS) and diallyl trisulfide (DATS), which have similar effects to inorganic polysulfides but are much more stable compounds.

To better understand the mechanism of action of organic polysulfides, we wanted to identify their binding site on the human TRPA1 receptor. We first performed *in silico* molecular docking, which revealed the key role of C621 in TRPA1 activation by covalent binding of organic polysulfides. Using site-directed mutagenesis, we replaced cysteines with alanines at known binding sites of electrophilic agonists (C621, C641 and C665) and at putative binding sites in the transmembrane region (C727 and C834). The effects of organic polysulfides on TRPA1 and its mutant variants were investigated in receptor expressing CHO cells and compared with the non-electrophilic agonist carvacrol, which has a different binding site on TRPA1.

Activation of TRPA1 was measured using Fluo-4 calcium-sensitive fluorescent dye by flow cytometry, radioactive calcium-45 liquid scintillation counting and whole-cell patch-clamp method. The overlapping results showed that C621 is the most important, C665 the second and C641 the least but still important cysteine in the binding of organic polysulfides. Only their combined triple mutation could completely eliminate the effect of organic polysulfides on TRPA1. C727 and C834 in the transmembrane region, which were predicted to bind hydrophobic agonists, did not contribute to the activation of TRPA1 by organic polysulfides.

Identification and characterization of the binding site on the TRPA1 receptor may facilitate drug design of organic polysulfides as analgesic and anti-inflammatory drugs.

Support
National Brain Research Program 3.0 (NAP 3.0); RRF-2.3.1-21-2022-00015 National Laboratory for Drug Research and Development; TKP2021-EGA-16 National Research, Development and Innovation Fund of Hungary.

17. MECHANISMS OF NEURO-VASCULAR-IMMUNE INTERACTIONS IN DEEP INFILTRATING RECTOSIGMOID ENDOMETRIOSIS DETERMINED BY TRANSCRIPTOMICS

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Rectosigmoid deep infiltrating endometriosis (DIE) is a severe chronic painful inflammatory disease with an incidence of up to 90% of all intestinal endometriosis cases. DIE is a highly aggressive and progressive disease, which represents a major therapeutic challenge. Its pathophysiological mechanisms are not known, but complex neuro-vascular-immune interactions are likely to be involved. Since its therapy is unsatisfactory, it is considered to be an unmet medical need. Therefore, identification of key mediators and signaling pathways that could lead to potential novel therapies is crucial.

Here, we performed unbiased transcriptomic analysis of rectosigmoid endometriosis lesions (n=9) compared to normal recto-sigmoid bowel wall (n=13) and healthy endometrium (n=9) using next-generation mRNA sequencing. Bioinformatic analysis was performed to determine differentially expressed (DE) genes, KEGG, GO, Reactome databases and the ingenuity pathway analysis (IPA) software were used to identify pathways and networks. Our results were compared to the gene expression microarray data of the Turku Endomet public database.

Altogether 31 DE genes were identified in DIE lesions compared to both healthy endometrium and normal bowel wall samples. Functional annotation identified 8 genes from the extracellular matrix (ECM), 8 genes from growth factors and inflammation-related processes, and 14 genes from different intracellular pathways. The highest fold-changes were found in cartilage oligomeric matrix protein (COMP), immunoglobulin-like and fibronectin III. domain 1 (IGFN1) and cartilage intermediate layer protein 2 (CILP2), the latter 2 have not been reported in endometriosis. IPA analysis revealed that the 31 DE genes are related to cell migration, cell proliferation, collagen trimerization, ECM organization and scar tissue formation. Transcriptomic alterations showed similar patterns in the Turku Endomet database results to our results.

These are the first results providing an integrated view on specific canonical pathways, biological functions and gene networks differentially expressed in DIE. Inflammatory, vascular and ECM organization, as well as cell growth and migration were activated in ectopic endometrial specimens, suggesting their potential roles in disease development and progression. These data may help to elucidate the molecular mechanisms of DIE to identify novel therapeutic targets.

Funding: Hungarian Research Network (Chronic Pain Research Group) , TKP2021-EGA-16, (Phar-maLab, RRF-2.3.1-21-2022-00015), János Bolyai Research Scholarship (BO/00496/21)

18. THE FRACTALKINE RECEPTOR CX3CR1 MEDIATES PAIN AND NEUROINFLAMMATION IN THE PASSIVE TRANSFER-TRAUMA MOUSE MODEL OF COMPLEX REGIONAL PAIN SYNDROME

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Complex Regional Pain Syndrome (CRPS) is a severe chronic primary pain condition that develops after a small injury and is characterized by hyperalgesia, oedema and autonomic dysfunction. Autoimmunity, complex sensory-immune-vascular interactions and neuroinflammation are involved in its pathophysiology. Since its treatment is unsatisfactory, the molecular mechanisms need to be explored to identify novel therapeutic targets. We investigated the role of the inflammatory chemokine fractalkine receptor 1 (CX3CR1) expressed predominantly on microglia and macrophages in the CRPS passive transfer-trauma mouse model.

Female C57Bl/6 mice were treated i.p. daily with purified IgG from CRPS patients or healthy volunteers. Plantar skin-muscle incision was performed to model the microinjury. The role of the CX3CR1 receptor was investigated in gene-deficient mice and wild-type mice treated with the selective receptor antagonist AZD8797 (80 μg/kg i.p/day). The paw mechanonociceptive threshold was measured by dynamic plantar aesthesiometry and paw volume by plethysmometry. Astrocyte and microglia density was detected in pain-related central nervous system regions by glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) immunohistochemistry, respectively.

Plantar incision induced approximately 45-50% mechanical hyperalgesia, which decreased to normal 3 days after the injury in healthy human IgG-treated mice but persisted throughout the 7-day experiment in CRPS IgG-treated animals. Both CX3CR1 deficiency and antagonist treatment significantly reduced CRPS IgG-induced mechanical hyperalgesia-increase. CRPS IgG treatment increased microglia and astrocyte activation in the somatosensory cortex and periaqueductal gray. Microglia immunoreactivity was suppressed by CX3CR1 gene deficiency in the somatosensory cortex, while astrocyte activation was significantly reduced by the antagonist treatment in the periaqueductal gray. Interestingly, CX3CR1 gene deletion increased GFAP immunoreactivity also in healthy IgG-treated mice.

The CX3CR1 receptor may play a role in CRPS-related chronic pain through the regulation of neuroinflammatory mechanisms. Therefore, CX3CR1 antagonists represent a potential novel therapeutic target in this primary chronic pain condition.

Support: National Brain Research Program 3.0, OTKA K-138046; PTE ÁOK János Szolcsányi Research Fund: PTE ÁOK_KA-2020-18, HUN-REN–PTE Chronic Pain Research Group, TKP2021-EGA-16, RRF-2.3.1-21-2022-00015; AG was supported by the Pain Relief Foundation, Liverpool

19. ALTERED METABOLITE PROFILE IN A POTENTIAL MIGRAINE-MIMICKING ANIMAL MODEL

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Migraine is a primary headache affecting 10% of people worldwide. The pathophysiological mechanisms are still not clearly understood, and the therapy is often unsatisfactory. Therefore, disease- and headache-specific mediators and potential novel therapeutic targets need to be identified by hypothesis-free unbiased approaches using patients' plasma samples. Here we tried to mimic migraine via generating neuroinflammation in whisker pad of rats and measuring analyzed the metabolomic changes in the plasma of animals.

The control (saline) and treated (Complete Freud's Adjuvant – CFA) groups had 5-7 Whistar rat parallels. Blood samples were collected on the 3rd day after the treatment based on earlier data. Samples were run using 4 different instrumental setups, with the liquid chromatographic separation of 106 metabolites, and the flow injection analysis of 524 metabolites in targeted way using MxP® Quant 500 kit and also untargeted way. For data analysis, samples with >20 % CV were filtered out To classify the samples on cases and controls orthogonal partial least squares discriminant analysis (OPLS-DA) modelling was used. Multivariate calculations and plots were performed by using SIMCA-P+ 13.0.3.0 (Umetrics, Umea, Sweden). Ingenuity Pathway Analysis (IPA) software by Quiagen for general metabolits and Lipidmaps for mainly lipid analysis was used for gaining further information about the biological meaning of our results.

Data showed Tryptophan metabolism, Alanine, aspartate and glutamate metabolism, Urea cycle, Histidine metabolism, Arginine and proline metabolism, Cysteine and methionine metabolism were affected. Carnosine, Serotonin were significantly upregulated meanwhile Tryptophan, Kynurenine, Tyrosine, Phenyl-alanine, Asparagine, Methionine. IPA showed via Diseases and function Bar chart that Cell Function and maintenance, Amino acid metabolism, Inflammatory response, Small molecule biochemistry, Cell Cycle, Cell Signaling, Immune Cell Traficking, Infectious diseases, Respiratory diseases were found as significant from its database. Our model showed, Glyceroplipids, Glycerophospholipids – confirmed also with untargeted way-, Sphingolipids, Sterol lipids were downregulated and Fatty acids were upregulated in the case of CFA treated rats.

Our data confirm the multiple sides of neuroinflammational progresses in this model. Tryptophan metabolism is affected also in migraine, however further confirmation needs to be done to evaluate this model.

Funding: OTKA FK132587, FK138046, TKP2021-EGA-16, RRF-2.3.1-21-2022-00015 and NAP3.

20. FATTY ACID AMIDE HYDROLASE (FAAH) INHIBITION AGGRAVATES INDOMETHACIN-INDUCED ENTEROPATHY IN MICE

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Non-steroidal anti-inflammatory drugs (NSAIDs) are among the world's most widely used medications to alleviate pain, inflammation and fever. However, chronic use of these drugs has been associated with enteropathy, which refers to damage occuring predominantly in the distal small intestine. Currently, no proven effective treatment or prevention is known for this condition. Although several animal studies have shown that NSAID-induced gastric injury can be prevented by activation of the endocannabinoid system, e.g. by inhibition of fatty acid amid hydrolase (FAAH) and elevation of anandamide (N-arachidonoylethanolamine, AEA) levels, little is known about the effect of endocannabinoids on NSAID enteropathy. Hence, the objective of our study was to assess the effect of FAAH inhibition by URB597 on an animal model of NSAID-induced enteropathy.

NSAID enteropathy was induced by indomethacin (30 mg/kg) in C57BL/6 mice and tissues were harvested

24h later. URB597 (0.5 and 5 mg/kg) or its vehicle (1% hydroxyethyl cellulose) was administered 3 times (2h before and 6h after indomethacin, as well as 2h before termination). Intestinal injury was assessed macroscopically and by measuring the levels of various inflammatory mediators.

Indomethacin treatment resulted in enteropathy, characterized by shortening of the small intestine and increased protein expression of the inflammatory mediators tested (COX2, MPO, PTX3). In addition, a decrease in FAAH and endocannabinoid receptor CB1 mRNA levels was observed in response to NSAID administration. Whereas URB597 treatment alone had no effect on any parameters measured, it aggravated the damaging effect of indomethacin in a dose-dependent manner.

Despite literature data suggest that FAAH inhibition by URB597 can alleviate NSAID-induced gastric inflammation, our results indicate the opposite effect in the small intestine. Whether this is due to off-target effects of URB597 unrelated to AEA elevation, or to effects induced by AEA and mediated by CB receptors remains to be elucidated.

Grants: NKFI FK 138842

21. RATS USE RANDOM STRATEGY FOR SOLVING COMPLICATED COGNITIVE TASKS

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For the treatment of cognitive disorders only a few drugs are available and those are of limited efficacy. Our research aims to study clinically effective potential cognitive enhancer drugs using a rodent cognitive test system. The used tests model different human cognitive functions impaired in psychiatric and neurodegenerative disorders.

We observed that in several cognitive assays rats were rather relying on chance to solve the task instead of learning to give the correct response: e.g. in pairwise visual discrimination (PD) and paired associates visual learning (PAL) in touchscreen box (TSB); episodic memory task in Morris water maze (spatial navigation), working memory 'order test' in a 5-hole operant box (5HOB) as well as in a 'delayed non-matching to sample task' (DNMTS) in 5HOB.

In PD task to get a reward rats should choose one correct image out of two on a screen. The mean correct response of 35 Long-Evans (LE) rats was 80 %. After reversing the images, it decreased to 61 %, showing that rats moved toward the mentally less demanding random strategy.

Another 24 LE rats were trained to do PAL, in that 2 of 3 different images on 3 potential positions were randomly shown on the screen, the third position remained blank. Rats needed to nosepoke the image appeared on its correct position. This resulted in 48 % correct responses in average, also indicating a „guessing" strategy.

In 'episodic memory' test we investigated whether rats could determine at which time of the day they are, so the escape platform was placed onto various locations at different times of the day. Both Lister Hooded and LE rats randomly chose among 3 or 2 possible target locations.

In 'order test' 18 LE rats had to remember in which order the light stimulus moved on in three different holes of the 5HOB. The successful recall was 30,4 %, which was achieved by a better than random choice for the first hole (69 %), and a random choice between the remaining 2 holes (45 %).

In DNMTS task, when the sample and non-matching stimuli could randomly appear at 5 or 4 possible holes, respectively, 16 Wistar rats' average overall correct ratio was only 56 %.

In conclusion, rats could learn the contextual elements of the tasks, but they could not demonstrate memory in the described tasks. A possible explanation is that the 50% probability of being rewarded by the

guessing strategy (chance level) was too high, thus it did not force the rats to invest more cognitive efforts for solving the tasks more efficiently.

In development of novel rodent assays evolutionary built-in behavioural strategies (like a behaviour pattern which is adaptive in a changing environment or cost-benefit calculation of an effort) have to be considered, as these can mask the cognitive trait intended to be measured with the test.

22. TARGETING THE AMPK PATHWAY AS A TREATMENT STRATEGY FOR HUNTINGTON'S DISEASE

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Age-related neurodegenerative diseases are becoming more prevalent as human lifespan increases. Modeling human aging is challenging in the lab. We use transdifferentiation via an all-in-one lentiviral vector to directly reprogram human donor-derived fibroblasts into induced neurons (iN). The advantage of this methodology is the maintaining of age and disease-related signatures of the donor cells as iNs bypass the intermediary cell rejuvenation.

Huntington's disease (HD) is an autosomal dominant age-related neurodegenerative disorder caused by a mutated Huntingtin (HTT) gene containing an expansion of CAG repeats. HD-iNs display a cell type specific alteration in proteins linked to autophagy through the AMPK pathway, only present in the HD-iNs but not in the parental fibroblast. In HD both AMPK and autophagy have neuroprotective roles, downregulation or hyperactivation of these pathways have worsened the disease.

In this project we aim to target the AMPK pathway as a therapeutic strategy in HD-iNs. To quantitatively measure AMPK activity, we will transfect U2OS and RPE-1 cell lines with ExRai AMPKAR, an already published AMPK sensor. We will screen for AMPK affecting drugs using a library of 2401 FDA approved compounds. We will verify the effect of the positive compounds in our HD-iN model and look for disease modifying changes. Top candidates that can efficiently rescue autophagy and elaborate the reduced neuronal complexity of the HD-iNs will be functionally characterized using patch-clamp electrophysiology. Confirmed and carefully validated compounds can potentially be endorsed for drug repurposing and tested further in clinical trials.

23. ACTIONS OF PIEZO-1 CHANNEL ACTIVATION AND INHIBITION ON ASTROCYTE-NEURON COMMUNICATION IN THE MURINE NEOCORTEX

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The Piezo1 mechanosensitive ion channel is abundant on several elements of the central nervous system including astrocytes. It has been already demonstrated that activation of these channels is able to elicit calcium waves on astrocytes which contributes to release of gliotransmitters.

Astrocyte- and N-methyl-D-aspartate (NMDA) receptor dependent slow inward currents (SICs) are hallmarks of astrocyte-neuron communication. These currents are triggered by glutamate released as gliotransmitter, which in turn activates neuronal NMDA receptors responsible for this inward current having slower kinetics than any synaptic events.

In this project, we aimed to investigate whether Piezo1 activation and inhibition is able to alter spontaneous SIC activity of murine neocortical pyramidal neurons. When the Piezo1 opener Yoda1 was applied, the SIC frequency and the charge transfer by these events in a minute time was significantly increased. These changes were prevented by treating the preparations with the NMDA receptor inhibitor D-AP5. Furthermore, Yoda1 did not alter the spontaneous EPSC frequency and amplitude when SICs were absent. The Piezo1 inhibitor Dooku1 effectively reverted the actions of Yoda1 and decreased the rise time of SICs when applied alone.

In conclusion, activation of Piezo1 channels is able to alter astrocyte-neuron communication. Via enhancement of SIC activity, astrocytic Piezo1 channels have the capacity to determine neuronal excitability.

24. IDENTIFYING GENE EXPRESSION PATTERNS LEADING TO SCLC

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Small cell lung cancer (SCLC) constitutes a significant subset of lung malignancies, characterized by its pulmonary neuroendocrine origin and high metastatic propensity. Despite initial responses to platinum-based chemotherapy, the emergence of chemoresistance remains a formidable challenge, contributing to its status as a recalcitrant cancer with limited therapeutic breakthroughs in decades. Recent insights into SCLC subtyping based on neuroendocrine features and transcription factor expression, particularly the NAPY classification (NEUROD1, ASCL1, POU2F3, YAP1), have provided a framework for understanding its molecular heterogeneity. Additionally, the identification of an immune-related subtype, termed SCLC-inflamed, emphasizes the importance of subtype-specific investigations to find the best therapeutic possibilities.

In this study, we conducted a comprehensive analysis of transcriptomic data utilizing both single-cell and bulk RNA-Seq datasets encompassing normal lung tissue, SCLC tumors, and cell lines. By delineating cell-specific gene expression profiles of healthy lung tissue, we aimed to decipher the molecular underpinnings driving SCLC pathogenesis and drug resistance mechanisms to propose alternative therapeutic strategies and improve patient outcomes. Furthermore, comparative analyses with other neuroendocrine and non-neuroendocrine lung tumors offers valuable insights into shared and divergent molecular pathways across lung malignancies.

In summary, our study underscores the importance of transcriptomic profiling in unraveling the complexities of SCLC and other lung tumors, with implications for patient stratification and therapeutic innovation. By integrating multi-omics data and leveraging advances in computational biology, we aim to advance oncopharmacology and enhance the clinical management of SCLC and related malignancies.

25. THE METABOLISM STUDY OF 4-FLUOR-3-METHYL-ALPHA-PVP, A SYNTHETIC CATHINONE TYPE STIMULANT USING UHPLC-QTOF

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4-fluor-3-methyl-alpha-pyrrolidinopentiophenone (MFPVP) is a cathinone type psychostimulant. It was first reported to EMCDDA in Sweden in April 2020.It first appeared in Hungary in November 2020. In 2021, MFPVP was among the most popular illegal cathinones on the European drug market. Forensic toxicology aims to determine drug abuse by analysing biological samples. However, these samples might contain only metabolites of the consumed substance. Therefore, it is essential to study the metabolic pathways and metabolic profile of any newly emerging drug.

In the Department of Forensic Toxicology of HIFS, the metabolic profiling assay of MFPVP was performed on a Waters Xevo G2-XS UPLC-MS-QToF system in MS^e mode by analysing *in vitro* (pHLM and pS9) and *in vivo* (n=3) samples from users. Waters_Connect software was applied. Phase I and Phase II metabolites were investigated and ranked according to their relative abundance. Major metabolic pathways were determined.

In this metabolism study, seven *in vivo* urinary metabolites of MFPVP were tentatively identified. Six Phase I metabolites via reduction of the keto moiety (M01), via oxidation (M08), via the combination of these (M02, M03), via carboxylation (M07) and via ring-opening followed by carboxylation (M10) and one Phase II metabolite via the glucuronidation of the carboxylated metabolite (M06) were detected in three authentic urine samples. According to the metabolite ranking study, four major metabolites (M02, M03, M06 and M08) were more abundant than the parent compound. M01, M07 and M10 were also dominant. M04, M05 and M09 were detected only *in vitro*.

The major metabolic pathways of MFPVP were determined and proved to be highly similar to the metabolism of analogous compounds (alpha-PVP, alpha-PiHP). The metabolite ranking study presented that MFPVP is intensively metabolised in humans. Consequently, monitoring of tentatively identified dominant metabolites might facilitate the sensitive and selective detection of the consumption of the parent compound.

26. SIGNIFICANT IMPROVEMENT OF GUINEA PIG CARDIOVASCULAR TELEMETRY WITH DIRECT LEFT VENTRICULAR PRESSURE MEASUREMENT

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Guinea pig cardiovascular telemetric measurements have been performed by the recording of ECG, blood pressure (aorta), body temperature and activity of animals in our safety pharmacology laboratory for many years. The evaluation of these parameters fulfills the requirements of guidelines and authorities, but in some cases, compounds can have so on-, or off-target effect that necessitate the assessment of cardiac contractility. Although the analysis module of the telemetry system can calculate the QA interval (time elapsed between ECG Q wave and Aortic pulse wave), by which the left ventricular contractility can be evaluated, the length of the QA interval can be influenced by several factors, like changes in blood pressure, which is a common effect of compounds. A much more accurate method to assess contractility is to directly measure left ventricular pressure (LVP) after the cannulation of the ventricle.

After learning and practicing this very challenging surgical technic - when the pressure catheter should be introduced and fixed to the working heart of anaesthetized animals beating at a heart rate of 350-400 beat/minute - we have integrated it to the existing telemetric model. DSI HD-S21 transmitters having two pressure

catheters were implanted under general anesthesia into 8 guinea pigs weighing 400-500 g. The first catheter of the implants was inserted into to left ventricle, and the second one into to abdominal aorta. The negative ECG lead was positioned over the right pectoral muscle, while the positive one approximately 2 cm left from the xyphoid process.

The system validation is in progress by using the following references: the beta blocker atenolol, the beta-adrenergic receptor agonist isoproterenol, and the calcium-channel blocker diltiazem. The latest results will be published at the HUPHAR2024.

27. EFFECTS OF SUMATRIPTAN ON P2X7 PURINERGIC RECEPTOR-MEDIATED SIGNALING IN AN AMPHETAMINE-INDUCED ACUTE MANIA MOUSE MODEL

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

Our research investigated amphetamine-induced hyperactivity in wild-type (WT) and P2X7R gene knockout (P2X7KO) mice using open-field (OF) test, changes in c-fos expression in the striatum using immunohistochemistry and we measured dopamine and serotonin release from the striatum after amphetamine induction.

In the behavioral assay, 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. C-fos expression was increased by amphetamine in both WT and P2X7KO mice. C-fos expression was increased by amphetamine in both WT and P2X7KO mice, which was decreased by sumatriptan in WT but not P2X7KO mice. Dopamine and serotonin release was increased by amphetamine in both WT and P2X7KO mice but was lower in P2X7KO mice. This effect was reduced depending on concentration by adding sumatriptan in WT mice. The 5-$HT_{1B/1D}$ receptor antagonist GR127935 had the opposite effect in the behavioral assay and release experiments than sumatriptan.

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.

28. IN VITRO BREAST CANCER CELL LINE PANEL TO MODEL AND VALIDATE A PRECISION ONCOLOGY PROGRAM

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The complex pathogenesis of tumors can make the selection of optimal cancer treatment challenging. Precision oncology aims to personalize cancer treatment based on the molecular profile. Digital drug delivery (DDA) systems able to select the appropriate tumor therapy by analyzing the molecular profile. These systems has

such complexity, which requires preclinical testing of performance.

Here, we aim to utilize breast cancer cell lines to model a precision oncology program incorporating a DDA system.

We included 8 widely used breast cancer cell lines in our study. Molecular profiles were characterized by gene sequencing and receptor expression measurements. Base on the molecular profile the DDA system scored and ranked oncological agents. From the list of treatment options, 10 agents (afatinib, neratinib, olaparib, thalazoparib, rucaparib, niraparib, crizotinib, palbociclib, tamoxifen, vorinostat) representing different mechanisms of action and DDA scores were selected for further in vitro studies. The inhibitory concentration 50 (IC50) for each cell line was determined for these agents. Finally, the relationship between IC50 values and DDA scores was analyzed.

Molecular profiling identified mutations and copy number variations of 591 genes, as well as expression of hormone receptors. The 8 selected cell lines were classified into three main groups: BRCA-mutant (CAL-85-1, MDA-MB-436), HER-2 overexpressing (HCC-1954, SKBR3, MDA-MB-361) and BRCA-mutant and HER-2 protein overexpressing cell lines (JIMT-1, BT-474, HCC-1569). Subsequently, the molecular profiles of the cell lines were used to calculate the DDA score of the selected drugs and to determine the IC50 values. The correlation of the measured IC50 values with the DDA scores showed a weak correlation for all IC50-DDA score pairs. However, for absolute DDA scores above 500, the correlation was above 0.6, while for absolute DDA scores above 1000, the correlation was above 0.8.

In our work, we present for the first time the in vitro modelling of a DDA-based precision oncology program on a breast cancer cell line panel. The correlation between the scores calculated with DDA and the measured IC50 values emphasizes that a DDA system can support precision oncology decision-making.

29. EXTRACELLULAR MIRNAS INDICATE IN VITRO HEPATOTOXICITY

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Toxicology testing of chemicals (drug candidates, plant protection products and other new active substances) is of paramount importance both for official authorization and for social requirements and economic considerations. One of the major challenges in the field is the early prediction of potential toxicity, especially the hepatotoxic effect of a new chemical entity. The liver is the main organ responsible for the metabolism of drugs and other toxins, and is exposed to xenobiotics at high concentrations. Standard biomarkers currently used for detection of drug-induced liver injury (serum aminotransferases, alkaline phosphatase, bilirubin) are limited in their abilities to accurately monitor toxicity, because they have low organ specificity and do not precisely estimate the current state of the liver. Our main objective is to develop new extracellular microRNA (miRNA)-based biomarkers that are able to identify various adverse effects with great accuracy and specificity than the classical methods. A functionally significant proportion of miRNAs is found in extracellular vesicles, whose quantitative and qualitative characterization and continuous monitoring can provide an opportunity to predict toxic effect even before the classical toxicological endpoints.

We have developed methods for isolation of extracellular vesicles and miRNAs as well as for quantification of extracellular miRNA content (miRNA-specific reverse transcription, 'target' sequence-specific pre-amplification, TaqMan probe quantitative PCR measurement). Quantification of miRNA biomarkers associated with liver-specific toxicity was performed in the Fluidigm Biomark™ HD real-time PCR system based on high-throughput microfluidic "chip" technology, using several internal reference miRNAs (e.g. miR-92, miR-16, miR-23a). Two model compounds were selected for in vitro studies in rat primary hepatocytes: the pain killer paracetamol, which is safe at therapeutic doses, but results in liver-injury at high doses, and the antibiotics amoxicillin/clavulanic acid,

which causes unexplained, late-onset liver damage. Various miRNAs were measured that are potentially related to liver toxicity. The amount of extracellular miR-194, miR-21, miR133a and miR-146a increased as a consequence of paracetamol or amoxicillin/clavulanic acid exposure compared to untreated hepatocytes. The extracellular concentration of miR-122, miR-155 and miR-192 was elevated only in paracetamol treated cells. The established methods can serve as a basis for the measurement of extracellular miRNA concentration in plasma during toxicity testing in rat, and can be a supplement to classical toxicology studies.

30. PREDICTIVE INSIGHTS INTO DRUG RESPONSE: INTEGRATING PROTEOMICS AND GENE EXPRESSION WITH MACHINE LEARNING IN SMALL CELL LUNG CANCER

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Introduction: Small Cell Lung Cancer (SCLC) is characterized by aggressive behavior and limited treatment efficacy. The integration of proteomics and gene expression data offers new avenues for predicting drug responses. This study utilizes dimension reduction and clustering techniques to identify distinct sample clusters within SCLC cell lines, aiming to discover variable drug responses and enhance personalized treatment strategies.

Methods and Results: Proteomic and gene expression data from SCLC cell lines were subjected to dimension reduction techniques (tSNE, UMAP, PCA) and clustering methods (DynamicTreeCut, HDBSCAN, k-means) to form distinct sample clusters. Drug response data, in terms of IC50 values, were obtained from databases such as NCI, GDSC, CCLE, and UTSW. Within each sample cluster, drug responses were analyzed to identify patterns of sensitivity or resistance. Additionally, ssGSEA was employed to derive enrichment scores from gene clusters, which were then correlated with the drug response data to pinpoint biomarkers predictive of treatment outcomes. This dual approach of clustering samples and correlating gene cluster enrichment scores with drug responses enabled the identification of distinct phenotypic profiles with specific drug susceptibilities.

Conclusion: By clustering SCLC cell lines based on proteomic and transcriptomic data and correlating gene expression signatures with drug responses, our study delineates a comprehensive method to discern and exploit therapeutic vulnerabilities in SCLC. This strategy enhances the precision of drug response predictions, paving the way for personalized treatment regimens that could improve patient outcomes in SCLC.

Keywords: Small cell lung cancer, drug response, proteomics, gene expression, machine learning, dimension reduction, clustering, ssGSEA.

31. COMPARISON OF ACCURACY & RELIABILITY OF THE RECEPTORIAL RESPONSIVENESS METHOD (RRM) PERFORMED WITH NON-LOGARITHMIC OR LOGARITHMIC VARIABLE PARAMETERS IN COMPLICATED OR SIMPLIFIED EXPRESSIONS

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RRM is a procedure based on simple (i.e. not multiple) nonlinear regression, the model of which contains two variables (the logarithm of the concentration of an agonist used to generate the curve and the effect evoked) and (at least) one variable parameter, which latter can be expressed in a non-logarithmic form (c_x) as well as in

a logarithmic one ($logc_x$). This variable parameter quantifies a factor that has reduced the responsiveness of the investigated biological system before the generation of the concentration-effect curve. This quantification is made by RRM with the concentration of the agonist used for the curve, which concentration can produce the same decrease in the responsiveness of the system as the original evoking factor. In our present work, the factor to be determined was a known concentration of the agonist also used for RRM, so we could investigate RRM itself. The RRM's model was fitted in the form containing c_x as well as $logc_x$, the former in a more complicated expression as well as in a simplified one, while the latter only in the simplified expression. In addition, for the curve fitting, we used individual (local) regression, one-model global regression and two-model global regression as well. We have found that the more complicated model containing c_x provided the worst results, the simplified model including c_x was significantly better, while the simplified model containing $logc_x$ was the most accurate and reliable. In accordance with our previous experiences, individual fitting and two-model global fitting gave similarly good results, whereas the one-model global fitting was inferior to them. From these findings, three main conclusions can be drawn: 1) the use of $logc_x$ in the RRM's model usually provides more accurate and reliable estimates than that of c_x; 2) the algebraically simpler model of RRM is more useful than the more complicated one; 3) individual and two-model global regression should be preferred to one-model global fitting. Therefore, the simplified model containing the logarithmic variable parameter can be recommended to implement RRM, using individual or two-model global regression.

Acknowledgement: ÚNKP-23-3-II-DE-85, TKP2021-EGA-18

32. INVERTEBRATE MODELS FOR PHARMACOLOGICAL TESTING

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Invertebrate animal models such as the fruit fly (Drosophila melanogaster) and worms (Annelida) (especially redworm (Eisenia fetida)) are the most amenable to high-throughput genetic and chemical screens while they are the least expensive. Moreover, their well-known and conserved gene structure makes them suitable for testing molecular signal transduction pathways, relevant for example in cancer biology.

Furthermore, the fruit fly model is ideal due to its low cost, short life cycle, and lifespan, large number of progenies per adult, easy handling and maintenance, relatively low number of paralogous genes, the epigenetic mechanisms and signaling pathways are highly conserved. Thus, it represents the availability to experimentally modulate gene expression in vivo.

On the other hand, Annelida models are suitable for testing the ecotoxicological effects of pharmaceuticals in soil biota, among others.

Here we summarize the most important literature data on the utilization of the above-mentioned species.

33. TRANSCRIPTOMIC COMPARISON OF MOUSE MODELS OF HEART FAILURE INDUCED BY CHRONIC ANGIOTENSIN-II INFUSION OR TRANSVERSE AORTIC CONSTRICTION

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Heart failure with reduced ejection fraction (HFrEF) is a major public health problem due to its high mortality and prevalence. To achive bench to bedside results, one requires well-established, easy-to-perform *in vivo* preclinical animal models. The currently well-established transverse aortic constriction (TAC) model of HFrEF requires highly experienced personnel and several weeks of follow-up for HFrEF to develop.

We aimed to investigate the feasibility of angiotensin II (Ang II) treatment-induced HFrEF model in Balb/c mice and to compare the model with the well-established TAC model at the molecular level.

In our study, Balb/c mice were treated with Ang II or vehicle with osmotic minipump for 2 weeks, and C57BL/6J mice were treated with TAC or sham surgery. Cardiac function of the mice was followed up by echocardiography. After the animals were sacrificed, cardiac samples were investigated by RNA sequencing in addition to basic pathology. Differential expression profiling was performed by the Hisat2-featureCounts-DESeq2 bioinformatics workflow. Differential expression changes in the two models were compared by correlation analysis with the use of Gene Ontology terms.

Chronic Ang II treatment of Balb/c mice showed systolic cardiac dysfunction, but less ventricular dilatation and hypertrophy compared to the widely used TAC model. Similar transciprotmic changes were observed in general and in most of the investigated functions when comparing the two models.

We showed that chronic Ang II treatment of Balb/c mice is a relevant and reliable preclinical model for the study of HFrEF, which results very similar molecular changes in the heart compared to the TAC model, but it is much easier technically.

34. COMPARISON OF DIFFERENT SAMPLE PREPARATION TECHNIQUES FOR THE DETERMINATION OF CHLORPROMAZINE FROM BIOLOGICAL SAMPLES

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Sample preparation has crucial role in analytical measurements. In the different biological samples most of the analytes of interest can be found at low concentration compared to other components of the sample matrix. The selection of the used technique mainly depends on the characteristics of the analyte(s), and on matrix and volume of the sample. Furthermore, it is important that the selected technique must be adequately fast, accurate, specific and reproducible, as well as, must require low volume of solvents and be cost effective. Microextraction techniques fulfill these requirements. Furthermore, these have many advantages compared "classical" ones including the high enrichment of the analytes, which increases the sensitivity and the clearer extracts after the process.

In our experiments we studied the application of two microextraction techniques (MEPS and SPME) for the determination of chlorpromazine (CPZ). We investigated the effects of different parameters (solvent composition,

cycle time, washing solvents as well as adsorption and desorption time and temperature, agitation speed, etc.) on the extracted amount. After extraction CPZ was measured by GC-MS.

Based on the results the final MEPS procedure was the following:
Plasma samples were diluted 4-5 times with 0.1% HCOOH. MEPS cartridge was conditioned 2x 100µL MeOH + 2x 100 µL H_2O. Sample loading was 4 cycles, 100 µL sample (aspiring-dispensing). Washing was carried out with 100 µL 0.1% HCOOH in 10% MeOH in H_2O. Elution: 5 cycles with 100 µL MeOH (aspiring-dispensing). Total extraction time ~5 min.

The final SPME procedure was the following:
Spiked plasma is incubated for 30 minutes at room temperature, then were diluted 2 times with 0.1% HCOOH prior to the analysis. Conditioning: 10 minutes in MeOH and equilibrate for 10 more minutes in water. Sample loading: 50µL sample for 30 minutes, with agitation speed at 300RPM. Elution: in 50µL of MeOH for 10 minutes, with agitation speed at 700RPM. Total extraction time ~60 min.

The study was supported by NKFI-143360. Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, Financed under the TKP2021-EGA-18 funding scheme.

35. CHLORPROMAZINE INTERRUPTS AUTOPHAGY IN H9C2 CELLS.

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Chlorpromazine (CPZ) is a first-generation antipsychotic drug used in the treatment of schizophrenia; however, its application was shown to be associated with increased risk of cardiovascular diseases including ischemic heart diseases. Autophagy plays a crucial role in the ischemia reperfusion induced injury. Thus, the goal of the present study was to investigate the effect of CPZ on cardiac autophagy. H9c2 cells were treated with different concentrations (0,1, 1, 10 µM) of CPZ in the presence or absence of chloroquine. To measure the cell viability upon CPZ-treatment MTT assay were performed. Western Blott analysis were carried out to quantify the level of autophagy related proteins including (LC3B, Beclin-1, p62). Furthermore, LysoTracker staining was employed to visualize the lysosomes. We have detected a dose dependent decrement in cell viability upon CPZ treatment. Our western blot results indicated an enhanced level of p62 and LC3B, indicating disruption of autophagic process. We have observed similar results in the presence of chloroquine further supporting that CPZ alter the autophagic process. Our immunocytochemistry results indicated fewer number of co-localized lysosome and LC3 puncta. Based on our results CZP disrupt the autophagic process in H9c2 cells. However further studies required to understand the precise underlying mechanisms.

Acknowledgement: NKFI-143360. Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA-18 funding scheme.

36. DUAL RESPONSE OF ACETYLCHOLINE IN THE RIGHT AND LEFT VENTRICULAR MYOCARDIUM OF CANNABIDIOL TREATED ZDF RATS

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Examination of the effects of different drug candidates on the ventricular myocardium *ex vivo* is important to reliably identify potential beneficial and adverse actions. In the present study, we were able to quantify the changes in inotropy following the administration of norepinephrine and acetylcholine by examining the isolated and stimulated right and left ventricular trabeculae of Zucker Diabetic Fatty (ZDF) rats treated *in vivo* with cannabidiol (CBD).

For the present study, metabolically intact (lean) and diseased (obese) types of ZDF rats were used. The lean rats, fed with normal rat chow, formed the negative control group (Lean group), while the obese rats, kept on diabetogenic rat chow, were randomized into two groups receiving 60 mg/kg/day CBD (Obese+CBD group) or vehicle (Obese group), *via* gavage for four weeks (forming the treated group and the positive control group, respectively). After guillotining the animals, right and left ventricular trabeculae were isolated, mounted in organ chambers and paced (3 Hz, 1 ms, 10 mV). On the samples, concentration-effect (E/c) curves were generated with norepinephrine and then (after a wash-out) with acetylcholine, recording the inotropic response.

Norepinephrine was found to induce a positive inotropic effect in all the three groups showing the viability of the ventricular samples. The weakened response of the obese animals in comparison with the lean ones seemed to be slightly improved by the CBD treatment in the samples from both sides. In the Lean and Obese groups, acetylcholine exerted no response or a minimal negative inotropic effect (in the samples from both sides). In the Obese+CBD group, however, two different responses to acetylcholine could be detected (in both the right and left samples): acetylcholine elicited a positive inotropic effect in roughly half of the samples, while no inotropic change occurred in the rest. We assume that the positive inotropic effect of acetylcholine may stem from the known acetylcholinesterase inhibitory effect of CBD. However, it is not clear why this effect does not appear in all CBD-treated rats.

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

37. EFFECTS OF BM-112, A NEW H_2S-RELEASING ASPIRIN DERIVATIVE, ON ISOPROTERENOL-INDUCED CARDIAC HYPERTROPHY

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Cardiac hypertrophy is a compensatory mechanism that occurs in conjunction with cardiovascular diseases. Although hypertrophy of the myocardium provides certain benefits during the early stages of cardiovascular disease, prolonged hypertrophy is potentially harmful to the heart and can result in arrhythmia and heart failure. Development of cardiac hypertrophy involves various pathophysiological signals. Cardioprotective effects of H_2S

and aspirin are being suggested by numerous studies. H_2S plays a role in relaxation of vascular smooth muscle, protects against oxidative stress, myocardial hypertrophy and modulates inflammation. The aim of the project was to investigate the effect of a new H_2S-releasing aspirin derivative (BM-112) on isoproterenol (ISO)-induced cardiac hypertrophy and oxidative stress and study the 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, and biocompatibility was examined using MTT assay. In order to investigate the antihypertrophic effect 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. JC-1 staining was also performed to measure the number of healthy mitochondria. The alteration in autophagic and apoptotic protein expressions were analyzed by Western blot. The effects of BM-112 were compared to a slow releasing H_2S donor, which called GYY4137. 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 lower than 50 μM. 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 pharmacological effects of BM-112 on ISO-induced cardiac hypertrophy.

Acknowledgments: NKFI-143360. Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme.

38. EVALUATION OF THE ANTIOXIDANT EFFECT OF BIXIN CONJUGATED CBD AND CBG

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Background: Cannabidiol (CBD) and Cannabigerol (CBG) have been shown to possess health beneficial effects. We have conjugated CBD and CBG with bixin a known apocarotenoid having antioxidant properties, which may further enhance the beneficial effects of CBD and CBG.

Aims and objectives: In this study our aim was to investigate whether the pretreatment with ILKA 675 and ILKA 676 molecules have a protective effect against hypoxia-reoxygenation (H/R) induced acute injury on cardiac myocytes. Antioxidant effect was compared with the parent molecules Bixin, CBD and CBG.

Methods: In our series of experiments H9c2 rat cardio myoblast cells were treated for 24 and 48 hours and the Ic50 value of the molecules was determined. For comparison of the antioxidant effect of the molecules the concentrations of 30 μM was used to pretreat cardiomyocytes, followed by four/three hours of H/R. Cell viability and cytotoxicity was determined by lactate dehydrogenase (LDH) assay. Western blot analysis was used to examine the expression levels of the proteins involved in apoptosis and autophagy. Antioxidant assays such as TEAC (Trolox Equivalent Antioxidant Capacity), FRAP (Ferric Reducing Antioxidant Power) and ORAC (Oxygen Radical Absorbance Capacity) were carried out to evaluate the Trolox equivalence.

Results: Our findings demonstrate that the pretreatment with the newly synthesised molecules significantly improved the cardiomyocytes viability after H/R, especially the 48 hours pretreatment. ILKA 675 was the least cytotoxic under hypoxic conditions. Furthermore, ILKA 675 showed the highest antioxidant activity according to the results obtained from the antioxidant assays. At the same time, the activities of cytoprotective antioxidant enzymes, heme oxygenase-1 (HO-1), superoxide dismutase (SOD), and catalase (CAT) were also altered.

Conclusions: Overall, ILKA 675 and ILKA 676 may have a beneficial effect in the prevention of H/R

induced cytotoxicity through the reduction of oxidative stress.

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

39. THE ROLE OF DAPAGLIFLOZIN AND EMPAGLIFLOZIN PRETREATMENT ON DOXORUBICIN-INDUCED CARDIOTOXICITY

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Doxorubicin (DOX) is a commonly used anthracycline chemotherapeutic agent. Although it is efficacious in tumor regression, the cardiotoxic side effects limit patients' treatment and overall survival. It is imperative to develop proper management strategies for DOX-receiving patients and decrease the occurrence of impaired cardiac function and adverse outcomes. Dapagliflozin (DAPA) and Empagliflozin (EMPA) are inhibitors of sodium–glucose cotransporter 2 (SGLT2). Recently, it has been shown that SGLT2 mitigate the risk of heart failure or cardiovascular death in patients with a reduced LVEF or type II diabetes. Multiple contributors have been considered to implicate the DOX-induced cardiomyopathy, such as the formation of reactive oxygen species (ROS), induction of apoptosis, or aberrant signaling pathways. However, the detailed molecular mechanisms underlying the protective effects of DAPA and EMPA on DOX-elicited cardiomyopathy remain largely unknown.

The objective of the current study was to examine the protective role of DAPA and EMPA and its effects on DOX-induced cardiotoxicity. Sprague-Dawley rats were randomly divided into four groups. The DOX-treated animals received DOX (2.5 mg/kg 6 times) i.p. The DAPA or EMPA-treated groups received 25 mg/kg/day (EMPA) and 10 mg/kg/day (DAPA) via gavage, respectively, before and during the DOX treatment. Following the treatments, ECG was registered, followed by heart isolation and cardiac function assessment. Cardiac malondialdehyde (MDA) were quantify. Western-blot analysis was conducted for evaluating the expression level of survival pathway, autophagy, and apoptosis-associated proteins.

Based on our results the DOX-exposure induced left ventricular dysfunction. Heart functions were preserved in DAPA+DOX and EMPA+DOX groups. However, further studies needed to determine the precise pharmacological role of these investigated SGLT2-inhibitors against DOX-induced cardiac dysfunction.

Acknowledgement: NKFI-143360. Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, Financed under the TKP2021-EGA-18 funding scheme and supported by the University of Debrecen Program for Scientific Publication

40. PHARMACHOLOGICAL CHARACTERISATION OF CBD DERIVATIVES CONTAINING FLUORINE ATOMS

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In the latest decades different cannabinoids are in the focus of a lot of research. Most of them possess beneficial pharmacological effects such as neuroprotective, antioxidant, antiepileptic properties. The two most

studied cannabinoides are cannabidiol (CBD), and tetrahydrocannabinol (THC) that are naturally founded in the plant Cannabis sativa. However, there are only limited information about synthetically modified cannabinoids. Recently, we have studied the effect of three newly synthesized CDB molecule, one of which was in some aspect superior to the CBD. Many of the new drug contain F atoms and, in many cases, these molecules are more potent than the old ones.

In the current study we aimed to study new synthetic CBD derivatives having substituted on the aromatic ring of CBD and containing fluorine. The major goal was similar to our previous work; however, we paid special attention to the study of the antioxidant properties, which may allow a more precise structure-function relationship to be established.

We have performed an MTT assay to study the cell viability of H9c2 rat heart cells exposed to the new compounds. After that we investigated if the pretreatment with the derivatives can protect the cells from oxidative stress induced by H_2O_2. In order to study the mechanisms of the antioxidant effects of the compound we have employed different antioxidant assays such as FRAP, ORAC and ABTS.

pIC_{50} (the negative log of IC_{50}) values are as follows: PFD14/A/I:3,992; PFD14/B/I:4,386; PFD43/I:4,355; PFD17:4,624; PFD10/A/I:4,445; PFD10/I/I:3,975; CBD:4,976. Our results indicated that the biocompatibility of the new molecules is similar or slightly better than the CBD. Some of the compounds were able to protect the cells against H_2O_2 caused stress. Some of the compounds manly having free OH group have comparable antioxidant activity indicated by the antioxidant assays. However, CBD had the most potent antioxidant ability followed by PFD17 which is the second most potent according to the FRAP, ORAC and ABTS assays. Taken together our ne compound containing F atoms have protective effect and considerable antioxidant properties in some ways they are better than the parent molecule.

Acknowledgement: NKFI-143360, and the University of Debrecen Program for Scientific Publication

41. IMPACT OF INDICATION RESTRICTION AND DIRECT HEALTHCARE PROFESSIONAL COMMUNICATION LETTERS ON PRESCRIBING PATTERNS OF TOLPERISONE IN HUNGARY

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Tolperisone, a centrally acting muscle relaxant used to treat spasms and spasticity, has been linked to life-threatening hypersensitivity reactions since 2003. This safety concern prompted the European Medicines Agency (EMA) to restrict its use to post-stroke spasticity in adults. The Hungarian National Institute of Pharmacy and Nutrition issued direct healthcare professional communication (DHPC) letters in 2013 and 2020 warning healthcare professionals about the indication restriction and risks associated with tolperisone therapy.

This study evaluated the effect of indication restriction DHPC letters for tolperisone on Hungarian trends of the indication of prescription.

We analyzed indication data categorized by ICD-10 codes to track the prescribed use of tolperisone-containing medications before the first DHPC letter, between the two letters, and after the second letter. Indication data were requested from the National Health Insurance Fund of Hungary.

Our analysis of Hungarian prescriptions revealed a concerning finding of off-label use even after the DHPC letters were sent. The 10 most prescribed indications were musculoskeletal disorders after the first letter.

The correct indication still ranked low (9th place) after the second letter.

The DHPC letters had limited impact on prescribing practices for tolperisone. While there was a slight shift towards appropriate use, a significant number of prescriptions continued to fall outside the recommended indications. Routinely evaluating the effectiveness of DHPC letters and monitoring off-label use would contribute to enhancing drug safety.

42. THERAPEUTIC ASPECTS OF PRUNUS CERASUS EXTRACT IN A RABBIT MODEL OF ATHEROSCLEROSIS-ASSOCIATED DIASTOLIC DYSFUNCTION

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This study evaluates the potential therapeutic effects of anthocyanin-rich Prunus cerasus (sour cherry) extract (PCE) on atherosclerosis-associated cardiac dysfunction, described by the impairment of the NO-PKG (nitric oxide-protein kinase G) pathway and the antioxidant capacity. Initially, a rabbit model of atherosclerotic cardiovascular disease was established by administering a cholesterol-rich diet, enabling the examination of the impact of 9 g/kg PCE on the pre-existing compromised cardiovascular condition.

After that, the animals were divided into four groups for 12 weeks: the (1) untreated control group; (2) PCE-administered healthy rabbits; (3) hypercholesterolemic (HC) group kept on an atherogenic diet; and (4) PCE-treated HC group. Dyslipidemia, impaired endothelial function, and signs of diastolic dysfunction were evident in hypercholesterolemic rabbits, accompanied by a reduced cardiac expression of eNOS (endothelial nitric oxide synthase), PKG, and SERCA2a (sarco/endoplasmic reticulum calcium ATPase 2a). Subsequent PCE treatment improved the lipid profile and the cardiac function. Additionally, PCE administration was associated with elevated myocardial levels of eNOS, PKG, and SERCA2a, while no significant changes in the vascular status were observed. Western blot analysis further revealed hypercholesterolemia-induced increase and PCE-associated reduction in heme oxygenase-1 expression.

The observed effects of anthocyanins indicate their potential as a valuable addition to the treatment regimen for atherosclerosis-associated cardiac dysfunction.

43. THERAPEUTIC EFFECTS OF THE DRUG CANDIDATE BGP-15 IN A RAT MODEL OF MONOCROTALINE-INDUCED PULMONARY ARTERIAL HYPERTENSION

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Introduction: Right-sided heart failure is often caused by pulmonary arterial hypertension (PAH) that is characterized by the abnormalities of the pulmonary vessels. This process is followed by an increase in the vascular resistance, which quickly starts depleting compensatory mechanisms in the right ventricle. These impairments eventually lead to irreversible damage of the cardiovascular system and premature death of patients suffering from the disease. The aim of the study was to detect the possible therapeutic effects of the BGP-15 in a rat model of monocrotaline-induced pulmonary arterial hypertension.

Materials and methods: 12-week-old male Sprague Dawley rats were used. At the beginning of the study PAH was induced by subcutaneous monocrotaline (MCT) injection (60 mg/kg). The experimental animals were randomly divided into 3 groups (n = 15 in each): (1) Control group, received only the vehicle (dimethyl sulfoxide (DMSO) buffer); (2) rats that received only MCT and developed PAH (PAH group); (3) and MCT-injected rats that were treated with 100 mg/kg BGP-15 daily via gavage technique. At the end of the study echocardiographic measurements were carried out, followed by a rat adipokine array and the Western blot method.

Results: The outcomes of the cardiac ultrasound showed that the rats from the PAH group suffered from diastolic dysfunction. Both he LA/Ao and the E/e' ratios were significantly elevated due to the MCT administration. Moreover, the E/A ratio and the early diastolic myocardial velocity (e', detected by TDI) were markedly decreased. The BGP-15 treatment improved the diastolic performance, characterized by decreased LA/Ao, E/e' ratios and increased e' and E/A ratio. In the PAH model, the shortening of the pulmonary artery acceleration time (PAAT) and the midsystolic notch were observed. In the BGP-15-treated MCT group these impairments were attenuated. The results of the molecular biological techniques indicate that the BGP-15 treatment significantly restored the molecular abnormalities of the PAH model.

Conclusions: Although further investigations are needed, our results suggest that the drug candidate BGP-15 may have benefits in the treatment of the pulmonary arterial hypertension.

Supported by: TKP2021-EGA-18, ÚNKP-23-3-II-DE-250

44. CHANGES OF ENDOGENOUS PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE (PACAP) LEVELS IN PATIENTS WITH ATRIAL FIBRILLATION UNDERGOING PULMONARY VEIN ISOLATION

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Pituitary adenylate cyclase-activating polypeptide (PACAP) has well known cardioprotective effect. Our research group earlier described that endogenous PACAP level increases in myocardial infarction and decompensated heart failure, on the other hand it decreases in chronic heart failure. The aim of the present study was to examine the changes of PACAP level in patients with atrial fibrillation undergoing pulmonary vein isolation (PVI).

We collected blood samples from patients (n=46) undergoing PVI for atrial fibrillation from the femoral vein at the beginning of the procedure, from the right atrium before septal puncture, from the left atrium before and after the beginning of ablation, from the femoral vein at the end of the procedure and from the cubital vein the next day after the procedure. PACAP levels were determined by ELISA. Patients were divided into intact (n=29) and scarred (n=17) left atrial groups by electroanatomical map of the patients' left atrium. PACAP levels were compared in the total population and in the two groups and correlated with left atrial size and other comorbidity data.

Significantly higher levels of PACAP were detected in atrial blood samples and in post-operative femoral vein samples compared to peripheral blood samples collected at the beginning of the procedure and 1 day after the procedure. We measured higher PACAP levels in the left atrium before ablation and in the femoral vein after the procedure compared to our left atrial samples at the end of the ablation. In patients with scarred left atrium, we found lower PACAP levels in the left atrium after ablation compared to the intact group.

Our study was the first to show a significant difference between PACAP levels in atrial and peripheral blood samples. The elevated PACAP levels measured in atrial samples may be due to myocytes and neurons, whose PACAP production decreases depending on the degree of scarring following ablation-induced injury in the left atrium, but transient systemic elevation of PACAP levels is demonstrated in the periphery, suggesting a potential biomarker role for PACAP in these pathologies.

45. EFFECTS OF H_2S-DONOR ASCORBIC ACID DERIVATIVE AND ISCHEMIA/REPERFUSION-INDUCED INJURY IN ISOLATED RAT HEARTS

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Hydrogen sulfide (H_2S), a gasotransmitter, plays a crucial role in vasorelaxation, anti-inflammatory processes and mitigating myocardial ischemia/reperfusion-induced injury by regulating various signaling processes. We designed a water soluble H_2S-releasing ascorbic acid derivative, BM-164, to combine the beneficial cardiovascular and anti-inflammatory effects of H_2S with the excellent water solubility and antioxidant properties of ascorbic acid. DPPH antioxidant assay revealed that the antioxidant activity of BM-164 in the presence of a myocardial tissue homogenate (extract) increased continuously over the 120 min test interval due to the continuous release of H_2S from BM-164. The cytotoxicity of BM-164 was tested by MTT assay on H9c2 cells, which resulted in no cytotoxic effect at concentrations of 10 to 30 μM. The possible beneficial effects of BM-164 (30 μM) was examined in isolated 'Langendorff' rat hearts. The incidence of ventricular fibrillation (VF) was significantly reduced from its control value of 79% to 31% in the BM-164 treated group, and the infarct size was also diminished from the control value of 28% to 14 % in the BM-164 treated group. However, coronary flow (CF) and heart rate (HR) values in the BM-164 treated group did not show significantly different levels in comparison with the drug-free control, although a non-significant recovery in both CF and HR was observed at each time point. We attempted to reveal the mechanism of action of BM-164, focusing on the processes of autophagy and apoptosis. The expression of key autophagic and apoptotic markers in isolated rat hearts were detected by Western blot analysis. All the examined autophagy-related proteins showed increased expression levels in the BM-164 treated group in comparison to the drug-free control and/or ascorbic acid treated groups, while the changes in the expression of apoptotic markers were not obvious. In conclusion, the designed water soluble H_2S releasing ascorbic acid derivative, BM-164, showed better cardiac protection against ischemia/reperfusion-induced injury compared to the untreated and 46. ascorbic acid treated hearts, respectively.

Keywords: Hydrogen-sulfide, H_2S-donor, ascorbic acid, heart, ischemia/reperfusion, antioxidant activity, apoptotic and autophagic proteins.

46. ESTIMATION OF THE CHANGE IN THE INTERSTITIAL ADENOSINE LEVEL IN THE ATRIAL MYOCARDIUM OF OBESE TYPE ZUCKER DIABETIC FATTY (ZDF) RATS AFTER A 4-WEEK CANNABIDIOL TREATMENT

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Cannabidiol (CBD), the most extensively studied non-intoxicating phytocannabinoid, has been attracting a lot of interest worldwide owing to its numerous beneficial effects. It is investigated as a potential drug in some neurological (e.g. epilepsy), psychiatric (e.g. depression) and endocrine diseases (e.g. type 2 diabetes mellitus: T2DM). Our aim was to indirectly quantify the change of the interstitial adenosine level in the atrial myocardium of male, obese type Zucker Diabetic Fatty (ZDF) rats in response to CBD, a known adenosine transport inhibitor. Any increase in the interstitial adenosine concentration may activate the cell-surface A_1 adenosine receptor (A_1 receptor), an important starting point of several protective pathways in the heart.

Lean (healthy) and obese (suffering from T2DM) type male ZDF rats were put into 3 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). At the end of the *in vivo* treatment period, the animals were guillotined, and the left atria were isolated, mounted at 10 mN resting tension in organ chambers containing Krebs solution oxygenated with 95% O_2 and 5% CO_2 (36 °C; pH = 7.4). Atria were paced during the whole experiment (3 Hz, 1 ms, twice the threshold voltage). 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 by means of the receptorial responsiveness method (RRM).

CBD treatment decreased the response of obese ZDF rat atria to CPA, while it increased their response to adenosine. This result provided functional evidence, on one hand, for the known adenosine transport inhibitory property, and, on the other hand, against the putative direct A_1 receptor agonist property of CBD. 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 the 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: TKP2021-EGA-18 and ÚNKP-23-4-I-DE-469.

47. CHRONIC ROFECOXIB TREATMENT OF RATS CAUSES MAJOR CHANGES IN PROTEIN EXPRESSION AND PHOSPHORYLATION IN THE HEART

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As previously reported, the Cyclooxygenase-2 inhibitor, rofecoxib, increased acute mortality in chronically treated rats exposed to ischemia/reperfusion injury (I/R). This hidden cardiotoxic manifestation was attributed to the proarrhythmic effect of the drug on the ischemic heart. However, the beneficial effects of rofecoxib on ischemic injury manifested as decreased infarct size. In the present study, we aimed to identify molecular changes in the heart caused by chronic rofecoxib treatment.

Rats received a four-week treatment with 5.12 mg/kg rofecoxib or its vehicle. Messenger RNA (mRNA), microRNA (miRNA) deep sequencing data, and proteomic datasets of left ventricular tissue samples were used for an unbiased differential expression analysis followed by *in silico* molecular network analysis and experimental target validation.

Using mass spectrometry and filtering criteria, 26 proteins were identified that exhibited pronounced changes in protein expression or phosphorylation due to chronic rofecoxib treatment. The transcriptomic analysis showed mild alterations in the heart´s mRNA- and miRNA expression. The posttranscriptional regulation of mRNAs by miRNAs did not result in differential protein expression.

This is the first demonstration that the expression and posttranslational modification of several proteins in the heart are affected by chronic rofecoxib treatment. These changes are potentially caused by off-target effects that may account for the hidden cardiotoxic and/or cardioprotective manifestations of rofecoxib. Several modulated proteins are known to be involved in regulating ROS production and mitochondrial homeostasis, which suggests a role of these mechanisms in the hidden cardiotoxic and/or cardioprotective properties of rofecoxib.

48. OSTEOSARCOMA CELLS EXPRESS FUNCTIONAL TRANSIENT RECEPTOR POTENTIAL ANKYRIN 1 AND VANILLOID 1 ION CHANNELS AND SOMATOSTATIN SST4 RECEPTORS

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Osteosarcoma is the most prevalent painful primary bone cancer in adolescents. Although the current treatments improve its prognosis after the operation, it still has high mortality. Therefore, understanding its molecular characteristics is important to identify novel therapeutic options. Transient Receptor Potential (TRP) Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) are non-selective cation channels activated by a range of tissue irritants including methyl-glyoxal, formaldehyde, hydrogen-peroxide, protons, etc. produced by the tumor microenvironment. Somatostatin is synthesized by sensory neurons and immune cells, it acts through 5 Gi-protein coupled receptors, among which subtype 4 (SST4) is a potential target for inflammation and pain without endocrine actions. These receptors have been described in various cancers, they are involved in malignant transformation and apoptosis. Therefore, here we studied TRPA1, TRPV1 and SST4 expression in human and mouse osteosarcoma tissues and the K7M2mouse osteosarcoma cell line.

The mRNAs of all the three receptors were detectable by the highly sensitive RNAscope in situ hybridization

technique in the human and mouse osteosarcoma tissues and the cell line, the receptor proteins were not possible to demonstrate due to the lack of selective antibodies. Ezrin, a cytoskeleton linker protein, which enhanced expression levels are linked with osteosarcoma, was used as a marker to identify cancer cells in mouse and human samples. All three receptor mRNAs were co-expressed with ezrin mRNA demonstrating its localization in the osteosarcoma cells. Both the TRPA1 agonist allyl-isothiocyanate and the TRPV1 agonist capsaicin induced Ca^{2+} influx into the K7M2 cells shown by the radioactive $^{45}Ca2+$ uptake measurements. They significantly reduced cell viability in the ATP-based bioluminescence assay similarly to the non-selective heptapeptide SST4 agonist TT-232. However, the viability decreasing effects of AITC was partially antagonized by the TRPA1 antagonist HC-030031, but the action of capsaicin was not affected by the TRPV1 antagonist capsazepine.

These are the first results demonstrating functional TRPA1 and TRPV1, as well as SST4 receptor expression in osteosarcoma. Further experiments are in process to elucidate the intracellular pathways and consequences of the activation of these receptors.

Funding: RRF-2.3.1-21-2022-00015, TKP2021-EGA-13

49. EFFECT OF ESTRADIOL AND GENISTEIN ON THE TRANSIENT RECEPTOR POTENTIAL VANILLOID-1 AND ANKYRIN-1 RECEPTORS REGULATED PAIN RESPONSES

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Transient Receptor Potential (TRP) ion channels including TRP Vanilloid-1 (TRPV1) and TRP Ankyrin-1 (TRPA1) receptors play a pivotal role in pain sensation through activation of sensory nerves. Sexual steroids influence pain, and clinical studies demonstrate higher sensitivity to chronic pain in women. Classical nuclear- (ERα, ERβ) and membrane-localized G-protein-coupled estrogen receptors (GPER) are expressed in the nervous system, particularly in nociception-relating areas. 17β-estradiol (E2) modulates pain transmission via influencing of TRPV1 activation through sensory neuronal ERα receptors. Female sex hormones notably influence responses to mechanical pain by sensitizing TRPA1 receptors. Genistein (GEN) is a neuroprotective phytoestrogen, which exhibits significant structural similarity to E2.

Here we examined the effect of E2 and GEN on the function of TRPV1 and TRPA1 receptors in mice sensory neurons *in vitro* and *in vivo*. In *in vivo* tests dynamic plantar aesthesiometer has been used to determine the threshold of mechanical pain sensitivity and increasing temperature hot plate has been utilized to determine the thermal pain threshold. We used the TRPV1 agonist resiniferatoxin (RTX)-induced hyperalgesia model to determine changes in mechanical hyperalgesia. In *in vitro* studies, we determined changes in gene expression with qPCR, and receptor activity was determined with fluorescent intracellular (IC) Ca^{2+} imaging and detection of IC Ca^{2+} by fluorescent plate reader.

We demonstrated that mechanonociceptive threshold of C57BL/6J male mice was significantly higher compared to female mice. This marked difference did not disappear in TRPV1 knockout (KO) animals. The same sex difference was observed in TRPA1 wild type (WT) and KO mice too. Both mechano- and thermonociceptive thresholds of TRPV1 WT female mice were significantly lower in proestrus compared to estrus phase. This difference

was absent in TRPV1 KO mice. In TRPA1 WT animals, no differences in pain perception were observed between proestrus and estrus phases. In the RTX-induces hyperalgesia model, we showed that E2 enhanced mechanical hyperalgesia via TRPV1 receptor.

We demonstrated that E2 pretreatment inhibited the capsaicin-induced TRPV1 desensitization in sensory neurons by fluorescent Ca^{2+} imaging, and the tropomyosin-related kinaseA (TrkA) receptor inhibitor abolished this effect of E2. We observed an increase in TRPA1 receptor activation after E2 administration on TRPA1 expressing CHO cells by fluorescent plate reader measurements. Furthermore, we proved that overnight E2 and GEN treatment resulted in increased expression of both receptors.

We provided *in vivo* and *in vitro* evidence for E2 and GEN-induced upregulation of TRPV1 and A1 receptors, and E2-evoked TrkA-mediated sensitization of TRPV1 receptor. We are planning further experiments to elucidate the effects and mechanism of actions of E2 and other estrogen analogs on TRPA1 and TRPV1 receptor activation.

Support: TKP2021-EGA-16, TKP2021-EGA-13, NKFIH-138936, RRF-2.3.1-21-2022-00015, KTIA_NAP_20017-1.2.1-NKP-2017-00002

50. CHEMOSENSOR TRPA1 COVALENT LIGAND MODIFIES T LYMPHOCYTE ACTIVATION IN VITRO

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Transient Receptor Potential Ankyrin 1 (TRPA1) is a non-selective cation channel involved in sensation and sensitization to a plethora of inhaled, touched or orally consumed irritating cysteine-reactive agents and also endogenous mediators of oxidative stress such as nitric oxide, hydrogen peroxide, and inflammatory signals. TRPA1 has been reported to influence neuroinflammation accompanied by feedback mechanisms, macrophage and also lymphocyte function, but its complex role is still controversial in immune cells.

We reported earlier detectable, but orders of magnitude lower level of mRNA in monocytes and lymphocytes than in sensory neurons by qRT-PCR analyses of cells originated from primary and secondary lymhoid organs of mice. Our present goals were to (a) further elucidate the expression of *Trpa1* mRNA in mononuclear cells by single cell RNA scope in situ hybridization and (b) to test the potential role of TRPA1 in peripheral lymphocyte activation.

RNA scope in situ hybridization confirmed that low-copy *Trpa1* transcripts were detectable in $CD14^+$ and $CD4^+$ leukocytes isolated from peritoneal cavity of mice.

The role of endogenous TRPA1 in the activation of lymphocytes was studied in vitro. We analysed the effect of a potent selective small molecule TRPA1 agonist, JT010 on the TcR-mediated activation of T lymphocytes and the IgG-dependent activation of B lymphocytes. JT010 administration stimulated significant changes in intracellular Ca^{2+} level of these cells only at high concentrations. However, a concentration-dependent significant inhibitory effect of JT010 could be observed on TcR-induced Ca^{2+} signal of peritoneal T lymphocytes and $CD4^+$ T lymphocytes, while JT010 neither modified peritoneal B cell activation nor ionophore ionomycin stimulated elevation of intracellular Ca^{2+} level.

Though TRPA1 proved not to be a key regulator of TcR-stimulated calcium signaling in our earlier studies in TRPA1 KO mice, its function negatively modulated T lymphocyte but not B lymphocyte activation. Our results indicate that modulation of TRPA1 receptor/channel by an agonist/agent may lead a more complex impact, a cell type-, localization-, environment-, stage- dependent effect on immune cell activation, then solely influencing elevation of intracellular Ca^{2+} level by opening the Ca^{2+}/cationic channel.

The research was financed by TKP2021-EGA-16, TKP2021 EGA10, Hungarian Brain Research Program (NAP-3) and the NKFIH K_18_128210 Research Grant. Project no. Eötvös Loránd Research Network (Chronic Pain Research Group), Pécs, Hungary. Project no. RRF-2.3.1-21-2022-00015. V.K. was supported by the ÚNKP-23-5-PTE-1991, KA-2022-29 and BO/00750/22/5. The research was performed in collaboration with the Nano-Bio-Imaging core facility at the Szentágothai Research Centre of the University of Pécs.

51. ACUTE INFLAMMATION-INDUCED BARRIER DYSFUNCTION IN TNBS RAT COLITIS: A FOCUS ON THE GUT-BRAIN AXIS

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Inflammatory bowel diseases (IBD) are ailments of the gastrointestinal tract with inflammation and ulceration. It is becoming increasingly clear that IBD can be associated with several neurological comorbidities, such as depression and dementia, thus the role of the gut-brain axis in IBD has been raised.

Our work aimed to investigate the expression of inflammatory markers and tight junction proteins in colon and brain tissue in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced rat colitis.

During our experiments, we used 225-250 g male Wistar Hanover rats. To induce acute colitis, the animals were treated intracolonically (i.c.) once with TNBS (100 mg/kg) dissolved in 50% ethanol after 16 hours of fasting. The animals were euthanized 72 hours after TNBS treatment. The last 8 cm section of the colon from the anus and the brain were dissected and were frozen in liquid nitrogen. Biochemical measurements were performed via western blot.

Our results showed that due to TNBS-induced inflammation, the occludin tight junction protein showed a significant decrease compared to the control group both in the colon and in the brain tissue. Furthermore, an increased expression of the myeloperoxidase (MPO) enzyme was observed in both the intestine and brain tissue compared to the control groups. Among the nitric oxide synthase (NOS) enzymes, we observed a significant decrease in the expression of endothelial nitric oxide synthase (eNOS) in the colon and brain tissue as a result of TNBS treatment compared to the control group.

In conclusion, it can be stated that damage to the barrier functions can be assumed as a result of acute intestinal inflammation caused by TNBS, which can be observed both in the colon and brain tissue. Furthermore, our results suggest that peripheral inflammation in the colon can lead to dysfunction of the blood-brain barrier.

52. MITOCHONDRIAL UNCOUPLING PROTEIN-2 IS INVOLVED IN ACUTE NEUROGENIC MECHANICAL AND THERMAL HYPERALGESIA IN FEMALE RATS

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Background and aims: Uncoupling proteins (UCPs) are located in the mitochondrial inner membrane, which translocate protons (H^+) from the intermembrane space to the matrix, therefore reduce membrane potential and uncouple ATP synthesis from mitochondrial respiration. From the 5 members of this family, UCP-2 has been described in primary sensory neurons. Since there are no data on its involvement in pain sensation, transmission and sensitization, we investigated the role of UCP-2 on heat- and mechanosensitivity changes in acute neurogenic inflammatory and chronic neuropathic pain models.

Methods: Experiments were performed on female and male UCP-2 knockout ($UCP\text{-}2^{-/-}$) and wildtype ($UCP2^{+/+}$) rats (12-15-week-old, 250-300 g). Acute neurogenic inflammation was induced by the capsaicin analog resiniferatoxin (RTX) injected into the right hindpaw (0.3 μg/ml, 50 μl). Chronic traumatic mononeuropathy on the right limb was induced by tight ligation of 1/3 of the sciatic nerve. Heat sensitivity was investigated on the increasing temperature hot plate and touch sensitivity by dynamic plantar aesthesiometry.

Results: There was a sex-, but not genotype-related difference between the baseline nociceptive threshold values: the thermonociceptive threshold was lower in males, while the mechanonociceptive threshold was lower in females. RTX-induced thermal hyperalgesia and mechanical allodynia were significantly enhanced and prolonged in female $UCP2^{-/-}$ rats 20 min and 4-24 h after the RTX administration, respectively, compared $UCP2^{+/+}$ ones. In contrast, no differences were observed in males between the genotypes. Sciatic nerve ligation induced mechanical allodynia was not affected by UCP-2 deletion in either sex. However, females showed prolonged allodynia than males 14, but not 7 days after nerve ligation.

Conclusions: UCP-2 contributes in acute neurogenic inflammatory heat hyperalgesia and mechanical allodynia in female, but not in male rats. In contrast, UCP-2 does not play a substantial role in chronic neuropathic allodynia. Sex-dependent nociceptive differences in pain-related parameters were supported by these findings.

Funding: RRF-2.3.1-21-2022-00015, Eotvos Lorand Research Network, National Brain Research Program-3, TKP2021-EGA-13 and TKP2021-EGA-16

53. NEW, BIOACTIVE DERIVATIVES OF (-)-CANNABIDIOL AND (-)-CANNABIGEROL SYNTHESIZED BY MANNICH TYPE REACTION

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(-)-Cannabidiol (CBD) and (-)-cannabigerol (CBG) are among the most studied non-psychotropic phytocannabinoids present in *Cannabis sativa* L. Many different beneficial pharmacological effects are attributed to them: they have antioxidant, anti-inflammatory, analgesic, neuroprotective, cardioprotective properties, moreover, antibacterial, antifungal, and antiviral activities as well. However, due to their low water solubility and prominent first-pass metabolism, their oral bioavailability is moderate. Therefore, there is a great need for appropriate chemical modifications to improve their physicochemical and biological properties.

Our research group aimed to modify CBD and CBG synthetically and study the possible biological effects of the derivatives. Mannich reaction was used for the introduction of amino groups with different side chains to CBD and CBG and the biological properties of the new derivatives were investigated.[1] Some of the derivatives have very good antioxidant and protective effect on H9c2 cells, the modified CBD derivatives showed remarkable antiviral activity against SARS-CoV-2 with reduced cytotoxic effect, while synthetic modifications on CBG resulted in a significant increase in antiproliferative activity in some cases compared to the parent compound.

This research was funded by the National Research, Development and Innovation Office of Hungary (FK 142315)

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54. THE NOVEL MULTITARGET SEMICARBAZIDE-SENSITIVE AMINE OXIDASE INHIBITOR SZV-1287 DECREASES THE VIABILITY OF DIFFERENT CANCER CELL LINES

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Introduction: Osteosarcoma, melanoma and breast cancer are highly malignant tumors with bad prognosis, but different vasularization and immonogenity profiles. Cell viability is a crucial indicator of their proliferation dynamics and an important tool for testing potential novel anti-cancer compounds. The s**emicarbazide-sensitive amine oxidase (**SSAO) is expressed by cancer cells and metabolizes primary amines to several irritants like methylglyoxal, formaldehyde and hydrogen peroxide. Many studies have found higher levels of SSAO activity in different types of cancers, however the role of SSAO in carcinogenesis is still unclear. Here we tested here the effect of our patented multi-target SSAO inhibitor SzV- 1287 with additional direct antagonistic actions on Transient Receptor Potential Ankyrin 1 and Vanilloid 1 receptors on 3 cancer cell lines.

Materials and methods: Viability of mouse K7M2 osteosarcoma, 4T1 breast cancer and B16 melanoma cell lines was measured by the CellTiter-Glo® Luminescent Cell Viability Assay. Cells were seedd in 96-well tissue culture plates at a density of 5000 cells/ well in 100 µl media and cultured for 24 hours. Incubation was performed with SzV-1287 (10 µM, 50 µM, 100 µM, 250 µM, 500 µM) and the vehicle DMSO for 24 h. The ATP-based luminescent signal from the metabolically active proliferating cells was determined by EnSpire® Multimode Plate Reader, concentration-response curves were generated and EC_{50} values were calculated.

Results: SzV-1287 concentration-dependently decreased the luminescent signal corresponding to ATP synthesis and viability in all the 3 cell lines. Melanoma cells were the most sensitive to its inhibitory action between the 50-500 uM concentration range with 84 mM EC_{50} value. Significant viability decrease on the osteosarcoma cells was detected by 100-500 uM SzV-1287 providing an EC_{50} of 95.5 mM. Breast cancer cells were the least sensitive with the EC_{50} of 183 mM. 10 μM SzV-1287 did not affect cell viability, but 500 μM induced elimination of living cells in all cases.

Conclusion: Our novel multi-target SSAO significantly reduces the viability of mouse cancer cell lines with moderately different potencies. Therefore, investigating its effects on tumour growth in *in vivo* models provides interesting perspectives.

Acknowledgement: TKP2021-EGA-16; TKP2021-EGA-13; RRF-2.3.1-21-2022-00015; ELKH-PTE, Hungarian Research Network (Chronic Pain Research Group), Pécs, Hungary, National Brain Research Program 3.0.

55. RGH-397 INHIBITS THE FUNCTION OF RECOMBINANT AND NATIVE α5 $GABA_A$ RECEPTORS AND SHOWS EFFICACY IN ANIMAL MODELS OF COGNITIVE IMPAIRMENT

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Excessive activity of α5 subunit containing $GABA_A$ receptors in the hippocampus might play an important role in cognitive impairments and memory deficits. Selective reduction of the function of α5 $GABA_A$R could restore the normal neuronal activity and alleviate the symptoms. Chemical optimization of novel naphthyridine derivatives resulted in 1-(2-{[3-(4-fluorophenyl)-5-methyl-1,2-oxazol-4-yl]-methoxy}-5,6,7,8-tetrahydro-1,6-naphthyridin-6-yl)-2-methanesulfonylethan-1-one (RGH-397) a subtype-selective negative allosteric modulator (NAM) of α5 $GABA_A$Rs [1]. The biological characterisation of this molecule is presented here.

Displacement assays on human α5β3γ2, α1β3γ2 and α2β3γ2 $GABA_A$Rs were performed using the allosteric radioligand [^{3}H]Ro15-1788. Receptor function was investigated using automated and manual whole-cell patch clamp in human α1β3γa2, α2β3γ2 and α5β3γ2 $GABA_A$R-expressing HEK-293 cell lines. Additionally, conventional voltage clamp experiments were carried out to measure the inhibition of tonic $GABA_A$ currents in rat hippocampal brain slices. The compound was tested at concentrations ranging from 10 nM to 10 μM. Finally, RGH-397 (1, 3 and 10 mg/kg) was tested in two models of cognitive impairment in schizophrenia, the phencyclidine (PCP) (2 mg/kg, i. p. or 10 mg/kg s.c.) induced impairment of novel object recognition (NOR) and social recognition (SI) in rats.

RGH-397 had high affinity to human α5 $GABA_A$Rs and moderate affinity to α1 and α2 $GABA_A$Rs (K_i values of 4.1, 221 and 161 nM (all n = 3), respectively. The compound inhibited α5 $GABA_A$R-mediated current with an IC_{50} of 330 nM and an efficacy of 41% with relatively fast on-kinetics and slow off-kinetics when compared to the reference α5 NAM compound basmisanil. RGH-397 showed no significant inhibition of α1 and α2 $GABA_A$R currents up to 30 μM. In hippocampal interneurons RGH-397 partially blocked the α5 $GABA_A$R-mediated tonic currents in rat brain slices at the concentration of 100 nM and above (all n = 83). In the cognitive models RGH-397 reversed the PCP-induced cognitive deficit at the effective doses of 3 mg/kg (SI) and 10 mg/kg (NOR).

RGH-397 is a potent and selective NAM of α5 $GABA_A$Rs. The compound inhibited tonic current at the minimal effective concentration of 100 nM which is in accordance with its inhibitory potency on human recombinant α5β3γ2 $GABA_A$Rs. Altogether, the efficacy of RGH-397 was proven on human recombinant receptors, in rat brain tissue and in animal models of cognitive impairment as well.

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56. SYNTHESIS OF MORPHOLINE-RINGED AND THIO-SUBSTITUTED NUCLEOSIDE ANALOGS AND EVALUATION OF THEIR CYTOTOXIC, ANTIVIRAL AND ANTIMALARIAL EFFECTS

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Nucleoside analogs, chemically modified derivatives of natural nucleosides, are key drugs in the treatment of cancer and viral diseases, and are also used as antiprotozoal, antifungal, and antibacterial agents.[1] Nucleosides used in therapy are typically modified at the ribose sugar or the nucleobase. Important representatives of sugar-modified analogues are morpholine ring nucleosides, called morpholinos, which contain a morpholine heterocycle instead of the native ribofuranose ring.

We have developed efficient synthetic methods for the preparation of new types of morpholine ring nucleoside derivatives that contain either an N-fluoroalkylated morpholine ring or different bi- and tricyclic ring systems instead of the ribofuranose unit. The synthetic strategy was based on oxidative ring cleavage of the vicinal diol unit in ribofuranose of nucleosides, followed by cyclization of dialdehyde intermediates with various functionalized amines, including fluorinated primary amines.[2]

To modify ribofuranose, we also used the photoinitiated thiol-alkene coupling reaction, thus obtaining configurationally altered nucleoside derivatives, which carry sulfanylmethyl-linked substituents in different positions of the furanose ring.[3,4]

Among the above-mentioned nucleoside derivatives, we identified some promising anti-tumor, anti-SARS-CoV-2 and anti-malarial compounds.

This research was funded by the National Research, Development and Innovation Office of Hungary (K 132870).

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57. THE ROLE OF HEMOKININ-1 IN MOTOR COORDINATION AND MUSCLE STRENGTH OF YOUNG AND AGED MICE

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Musculoskeletal problems and consequent fractures significantly reduce the quality of life in old age. A group of neuropeptides, tachykinins, have regulatory functions in the central and peripheral nervous system, and this group includes hemokinin-1 (HK-1). It is present in high concentrations in the cerebellum and reproductive organs and can be detected in bone and muscle. We investigated its role in locomotor coordination and muscle function and we were also looking for possible sex differences in 3-4, 12 and 18 month old C57BL/6 wild type and HK-1 deficient (Tac4 KO) male and female mice.

In the static rod test, which is used to investigate locomotor coordination, mice are placed on the ends of rods of different thicknesses and the time it takes them to turn and reach the end of the rod is measured. In the grid test, the mice have to cling upside down on a metal grid. In the horizontal bar test, the animals have to grip the bars with their forelegs and climb out to the edge of the bar. These last two tests measure muscle strength.

No difference was found between the wild and gene-deficient groups in the young animals. In the ageing animals (12 and 18 months), both males and females, a significant deterioration in locomotor coordination was observed in the static bar test, which was significantly more severe in 12-month-old male Tac4 gene-deficient animals compared to respective wild types, but the opposite effect of gene deficiency was observed in 18-month-old females. A significant decline in muscle strength was also detected in older wild-type animals in both tests. In the grid test, the loss of muscle strength was significantly smaller in females compared to males, a phenomenon also observed in the horizontal bar test.

Our results suggest that HK-1 may play a complex regulatory role in motor coordination in old age, where important sex differences can be observed. However, HK-1 minimally affects muscle strength. Therefore, elucidating the mechanism of action of HK-1 and its interactions with sex hormones may be important for drug developmental purposes.

Funding: Hungarian 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, TKP2021-EGA-13, János Bolyai Research Scholarship of the Hungarian Academy of Sciences (BO/00592/19/5), Project no. RRF-2.3.1-21-2022-00015 has been implemented with the support provided by the European Union.

58. INVESTIGATING THE RELATIONSHIP BETWEEN ANTIMICROBIAL PEPTIDES AND GUT MICROBIOTA IN NSAID ENTEROPATHY

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Introduction: The impact of nonsteroidal anti-inflammatory drugs (NSAIDs) on the gut microbial ecosystem is a growing area of concern, with evidence suggesting that these medications not only cause significant harm to the small intestine but also lead to shifts in gut microbiota, known as dysbiosis. Although NSAID-induced mucosal damage and inflammation are likely to be the major causes of dysbiosis, some evidence suggests that other factors may also contribute to it. Our research builds on previous findings that NSAID enteropathy is linked with changes in the intestinal expression of AMPs, suggesting these alterations may be key drivers behind NSAID-induced microbial imbalance.

Aim: Our objective was to investigate whether changes in AMPs correlate with bacterial alterations in a rat model of NSAID-induced acute enteropathy.

Methods: Wistar rats received a single dose (20 mg/kg) of indomethacin by gavage and were euthanized after 6, 12, 24, 48, and 72 h. A sixth group, treated with vehicle (1% hydroxyethylcellulose), was euthanized at 72 h. Tissue levels of some inflammatory proteins and AMPs were assessed, as well as the composition of small intestinal microbiota by 16S rRNA sequencing. Associations between inflammatory proteins, AMPs, and bacterial abundances were tested by calculating Spearman's rank correlation coefficients.

Results: Indomethacin-induced gut inflammation was associated with a modest elevation of the antimicrobial peptides cathelicidin (CAMP), α-defensin 5, and β-defensin 2. Indomethacin also caused gut dysbiosis, mainly characterized by expansion of Gram-negative bacteria and loss of some Gram-positives. Correlation analysis has revealed that several bacteria, in particular Gram-negative, showed positive associations with inflammation and CAMP expression, whereas some Gram-positives correlated negatively with them. We observed only weak associations between defensins and microbes or inflammation.

Conclusion: Our study revealed substantial correlations between the levels of CAMP and the abundance of certain gut bacteria. However, these relationships did not mirror the established antimicrobial actions of CAMP, suggesting that the observed correlations might be attributed to modifications in both CAMP levels and bacterial composition triggered by inflammatory processes, rather than the direct antimicrobial effects of CAMP. Additionally, defensins showed only weak correlations with bacteria. These results suggest that AMPs do not have a major role in the development of dysbiosis associated with acute enteropathy.

Grant: NKFI FK 138842.

59. IN VITRO STUDY TO ELUCIDATE THE ROLE OF TRPA1 RECEPTOR IN THE PATHOMECHANISM OF ALLERGIC CONTACT DERMATITIS.

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Background: Allergic contact dermatitis (ACD) is an increasing health problem with limited treatment options. Formaldehyde, DNCB (dinitrochlorobenzene) and DNFB (dinitrofluorobenzene) are known contact sensitizers and a potent cause of ACD. These allergens have been used to create an animal model in the field of ACD research. DNCB, DNFB, formaldehyde have been reported to activate Transient Receptor Potential Ankyrin 1 (TRPA1) cation channel in other studies. Here, we aimed to investigate the role of TRPA1 in ACD using these allergens, and TRPA1 as a drug target to prevent/treat ACD.

Methodology: In vitro studies were used to assess the activity and the selectivity of those allergens on TRPA1 using $TRPA1^{+/-}$ CHO cell lines as well as primary keratinocytes from wild type (WT) and TRPA1 knock out (KO) mouse strains. Using Flu-o-4 intracellular calcium measurements, agonistic and antagonistic activity were

investigated for each allergen. ATP assays and trypan blue staining were done for testing the cytotoxicity. A pre-treatment of a TRPA1 antagonist (HC030031) was used to further prove the role of TRPA1 in cellular activity.

Results: DNCB, DNFB, formaldehyde have a strong TRPA1 agonist action.

These allergens at certain concentrations (>300μm, >3μm, >300μM respectively) are cytotoxic on the TRPA1$^+$ CHO cells and primary WT keratinocyte but not on the TRPA1$^-$CHO cells and primary KO keratinocyte.

Pre-treatment of the cells with TRPA1 antagonist increased viability significantly in case of TRPA1$^+$ CHO cells and the primary WT keratinocyte.

Conclusion: The tested allergens activate TRPA1 channels and induce cytotoxicity via this receptor as TRPA1 antagonist HC030031 was able to prevent this effect, therefore TRPA1 may play a regulatory role in the pathomechanism of ACD. In our ongoing project we aim to further investigate the underlying mechanism of TRPA1 mediated inflammatory response in ACD.

60. CASK SILENCING PROMOTES GEFITINIB RESISTANCE IN PC9 CELLS VIA INDUCING PROTECTIVE AUTOPHAGY FUNCTION

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Purpose: Tyrosine kinase inhibitors (TKIs) are the first-line drugs for non-small cell lung cancer (NSCLC); however, the problem of drug resistance is inevitable. Calcium/calmodulin-dependent serine protein kinase (CASK) functions as a scaffold protein and the interest of its role in tumor progression has been raised. In this study, we like to understand the role of CASK in TKI drug resistance in NSCLC and underlying mechanisms.

Materials and Methods: CASK expression in PC9 and H1299 cells were silenced by lentivirus transfection. Annexin V/PI and immunofluorescence staining, co-immunoprecipitation, immunoblotting and Q-PCR were conducted.

Results: EGFR-exon 19 deletion mutation PC9 cells are more sensitive to gefitinib than EGFR wild type H1299 cells with IC_{50} of 22.4 and 70.7 μM, respectively. As to afatinib and osimertinib, we found their cytotoxicity potencies are similar in PC9 and H1299 cells. Interestingly, we found CASK silencing increases gefitinib resistance in PC9 but not in H1299 cells, and has no effects on the death caused by afatinib and osimertinib. To define the protective effect of CASK silencing in PC9 cells, we treated autophagy inhibitor 3MA, and found the effect of CASK silencing in PC9 cells was inhibited by 3MA. In supporting the role of autophagy in cell protection, we detected higher LC3II/I and LC3 punctate in gefitinib-treated CASK silencing PC9 cells. In exploring the signaling mechanism, we found gefitinib-induced inhibition of Akt/mTOR was enhanced, while inhibition of ERK was reduced in CASK silencing PC9 cells, but both effects were not observed in H1299 cells. According to previous studies, complex beclin 1/vimentin/14-3-3 has the inhibitory effect on autophagy function. Interestingly, our results revealed that vimentin expression is transiently up-regulated by EGFR TKIs and this effect was reduced by CASK silencing. Moreover, 14-3-3σ mRNA and protein expressions were declined upon CASK silencing. Not only the reduction of protein level, knockdown of CASK also decreased the complex beclin 1/vimentin/14-3-3σ formation.

Conclusion: CASK is involved in gefitinib-induced cell death in PC9 cells by reducing the protective autophagy function. CASK can reduce LC3 punctate formation but promote Akt- and ERK-dependent complex beclin 1/vimentin/14-3-3σ formation.

61. NARINGIN-INDUCED PROTECTION IN AN EXPERIMENTAL NAFLD RAT MODEL

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Non-alcoholic fatty liver disease (NAFLD) is a chronic liver ailment, which prevalence has increased significantly in recent decades. NAFLD is associated with high lipid accumulation in hepatocytes and has been associated with obesity. Naringin (NAR), a natural bioflavonoid found in citrus fruits, such as oranges and grapefruit, has anti-hyperglycaemic and antioxidant effects and may reduce hepatic lipid accumulation. Thus, NAR may be a potential treatment for NAFLD.

The present study aimed to investigate the effect of NAR in a 45% fat diet (HFD)-induced NAFLD rat model.

A total of 47 male Wistar-Hannover rats were divided into 5 groups: 1) control (CTRL), 2) high-fat diet (HFD 45%), 3) carboxymethyl-cellulose (CMC), 4) naringin 40 mg/kg (NAR1 + HFD), 5) naringin 80 mg/kg (NAR2 + HFD). NAR was prepared with a CMC vehicle in the form of suspension and was administered orally by gavage needle daily for 4 weeks.

Our histopathological results showed that after 12 weeks of HFD, the number of fat droplets increased, liver scaffolds collapsed and wider sinuses were observed. Furthermore, white blood cell count and inflammatory marker levels were also increased in the HFD group compared to the CTRL group, however, 4 weeks of NAR treatment resulted in a decrease at both doses. Our histopathological and biochemical results demonstrated that 4 weeks of NAR treatment had a dose-dependent effect on inflammatory changes.

Our results demonstrate that 4 weeks of oral administration of NAR is protective against HFD-induced liver damage and thus may be effective in attenuating the progression of NAFLD.

62. EFFECTS OF BLUNTING ANNEXIN A1 EXPRESION IN SMALL CELL LUNG CANCER CELL LINES

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Small cell lung cancer (SCLC) is one of the malignancies with the worst prognosis, for which there have been no major breakthroughs in treatment for a long time. The majority of patients is diagnosed at extensive-stage, where the only option is chemotherapy, and even the addition of immune-checkpoint inhibitors results in modest benefits. Therefore, characterization of any molecular mechanism behind therapy resistance has relevance in finding novel therapeutic approaches. Previous studies showed a possibility of annexin A1 (ANXA1) involvement in an immunosuppressive tumor microenvironment in SCLC, and there are studies showing direct effects of ANXA1 modulation on cancer cell proliferation. We aimed to investigate the effects of silencing ANXA1 expression in an

SCLC cell line. We hypothesized that silencing ANXA1 expression in cell culture influences malignant phenotype *in vitro*, such as growth rate and epithelio-mesenchymal transition, which could highlight roles of ANXA1 in SCLC therapy outcome.

An SCLC cell line with high ANXA1 expression (H1048) 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. Success 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 120-hour-long trypan blue exclusion assay. Expression of mesenchymal markers, vimentin and ß-catenin was evaluated with western blot. Statistical analysis was done with two-way ANOVA, followed by Tukey's post hoc test for the trypan blue assay, and one-way ANOVA, followed by Tukey's post hoc test for the western blots. The n number of the groups was 7-8 (n=7-8) 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 unexpectedly higher than the native and control shRNA transfected SCLC cells. ANXA1-silenced cells showed less mesenchymal morphology in culture, which observation was supported by decreased mesenchymal marker expression on western blots.

Modulation of ANXA1 expression resulted in mixed effects on the small cell lung cancer line used. An inhibitory effect on growth rate was not detected in H1048 cell line, which suggests that annexin A1 is probably not an optimal therapeutic target. However, decreased mesenchymal phenotype of ANXA1-silenced cells is a feature that supports complex mechanisms in aggressiveness of this cancer, demanding further research.

63. EXAMINATION OF THE ANTIDEPRESSANT AND ANXIOLYTIC ACTIONS OF DIMETHYL TRISULFIDE MEDIATED BY THE TRPA1 ION CHANNEL

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Dimethyl trisulfide (DMTS) is a polysulfide naturally occurring in garlic and is used as a food additive. DMTS inhibited spontaneous motor activity and respiration in mice in our previous experiments. The depressant effect was mediated by the TRPA1 ion channel. We now intend to investigate the effect of DMTS on anxiety and depression-like behavior induced by chronic unpredictable mild stress (CUMS). We used gene knockout mice to test the involvement of TRPA1.

The three-week CUMS paradigm was composed of 4 types of daytime and 3 types of overnight stressors. Male 8-10-week-old TRPA1 wild-type (WT) and knock-out (KO) mice on C57B1/6 background were used. Both WT and KO animals were divided stressed and non-stressed main groups, with untreated, vehicle-treated and DMTS-treated subgroups. Five behavioral tests were used to confirm depression-like behavior and to explore the effects of DMTS: the open field test (OFT), marble burying test (MBT), sucrose preference test (SPT), tail suspension test (TST) and forced swimming test (FST). FosB immunohistochemical staining was applied to specific brain areas relevant to stress processes.

Stress exposure significantly reduced the time spent in open areas in every group of TRPA1 WT animals in the OFT. It increased the number of marbles hidden in the MBT. Inactive duration in the FST and TST was longer in untreated stressed animals. Sucrose preference was reduced in stressed animals. Relative adrenal weight was larger, and thymus weight was reduced after exposure to chronic stress. Stress exposure did not lead to depression-like behavior and anxiety in KO mice. DMTS treatment significantly reduced the time spent in the open field and

increased the number of marbles hidden in non-stressed animals in WT mice. The treatment reduced immobility time and anhedonia in stressed animals. DMTS administration increased the relative weight of the adrenal gland and the relative weight of the thymus in stressed mice. Stress-induced FosB activation was detected in the Edinger-Westphal nucleus, dorsal raphe nucleus, lateral septum, periaqueductal gray, paraventricular nucleus of the hypothalamus, paraventricular nucleus of the thalamus. FosB activation was decreased in the stressed DMTS-treated TRPA1 WT group. Stress and DMTS treatment had no effect on FosB activation in TRPA1 KO animals. Except in the paraventricular nucleus of the hypothalamus, where stress increased FosB activation.

DMTS treatment relieved depression-like behavior in stress-exposed WT mice. DMTS administration increased anxiety in non-stressed WT animals, but reduced anxiety in chronic stress-exposed animals. According to our results, DMTS might be an ideal candidate for further study as a dietary supplement for the complementary treatment of depression.

64. SYNTHESIS AND CYTOTOXICITY STUDIES OF CO(III) COMPLEXES AS HYPOXIA-ACTIVATED ANTICANCER DRUGS

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1. Introduction: Selective targeting is crucial in cancer therapy. Currently used Pt(II) complexes have serious side effects due to their lack of selectivity. Hence, other metal complexes are being investigated. Co(III) complexes can act as hypoxia-activated prodrugs. By incorporating Co(III) into a cytotoxic ligand, the ligand can be rendered inactive under normoxic conditions of healthy cells. Under hypoxic conditions of tumors, Co(III) is reduced to unstable Co(II) complexes, releasing the cytotoxic ligand. Ru(II) complexes are also currently investigated as potential anticancer agents with less toxicity and resistance than Pt(II) complexes.

2. Aim of the study: In this study, an ambidentate ligand (**L_5**) was used to synthesize two Co(III) complexes and two (Ru(II), Co(III)) bimetallic complexes. Subsequently, cytotoxicity assays of the four complexes and the ligand under normoxic and hypoxic conditions were performed to study their hypoxia activation.

3. Material and methods: Starting materials and (**L_5**) were synthesized according to literature procedures. [Co(tren)L]$(PF_6)_2$ (**1**), [Co(tpa)L]$(PF_6)_2$ (**2**), [Co(tren)L(η^6-p-cym)RuCl]$(PF_6)_3$ (**3**) and [Co(tpa)L(η^6-p-cym)RuCl]$(PF_6)_3$ (**4**) were synthesized from the starting materials, and the purity was verified. Redox properties were investigated by cyclic voltammetry. Both for normoxic (approx. 20% O_2) and hypoxic (1% O_2) conditions, MCF-7 human breast cancer cells were seeded, treated at different concentrations of the substances after 24 hours, and incubated for 72 hours. MTT protocol was then performed to determine cell viability. RNA was isolated from MCF-7 cells after 24-hour treatment with (**L_5**) at different concentrations and used to synthesize cDNA. *TfR1, Ndrg1* and *p21* genes were quantified by qPCR.

4. Results and discussions: Cyclic voltammetry study showed that all four complexes can be irreversibly reduced, with $[Co(tpa)]^{3+}$ complexes ((**2**), (**4**)) being more easily reduced than $[Co(tren)]^{3+}$ complexes ((**1**), (**3**)). MTT assays confirmed that (**2**) and (**4**) have significant hypoxia activation with a low-moderate potency. (**L_5**) was shown to be moderately active under normoxia; however, less active under hypoxia, which is undesirable. Incorporation of Co(III) rendered the ligand less toxic under normoxia, while activated under hypoxia. This study presented the possibility of Co(III) complexes as bioreductive carriers of cytotoxins.

Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme. It was also funded by the National Research, Development and Innovation Office of Hungary (NKFI-143360, NKFI-146656)

65. A SELECTIVE CDK12 INHIBITION IN COMBINATION WITH PARP INHIBITION SENSITIZE PARP INHIBITOR RESISTANT BREAST CANCER CELL LINES

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DNA instability plays a major role in the development of tumours. DNA damage can be repaired by homologous recombination mechanisms. Poly (ADP-ribose) polymerases (PARPs) and BRCA1/2 genes are both key components of homologous recombination repair pathways. BRCA1/2 loss of function mutation and PARP inhibition together induce high level of genome instability that leads to cell death. PARPi resistance is a major challenge in their application. PARPi resistance can be induced by increased cyclin dependent kinase 12 (CDK12) expression, a transcription promoter of several DNA repair genes. We aim to investigate the role of CDK12 overexpression in PARP resistance.

We tested two breast tumour cell lines harbouring BRCA1/2 mutations. We determined the copy number of the CDK12 gene specific to the cell lines by DNA sequencing and then measured the expression of CDK12 protein by Western blot. We then investigated the response of the cells to PARP inhibitors in a luminescent cell survival in vitro model, characterizing the inhibitory effect by the IC50 value and the area under the inhibition curve (AUC) size.

According to our results, we found a normal (n=2) CDK12 gene copy number in the MDA-MB-436 cell line by DNA sequencing, while BT-474 had an elevated (n=27) copy number. Similarly, Western blot CDK12 overexpression was observed in BT-474 (MDA-MB-436: 1.00±0.13 a.u. vs BT-474: 1.71±0.08* a.u.; t-test; *$p<0.05$). Furthermore, the BT-474 line showed reduced sensitivity to the PARPi effect of rucaparib compared to the MDA-MB-436 line (IC50 MDA-MB-436: 52.04 mM vs. IC50 BT-474: 210.00 mM; AUC MDA-MB-436: 269 a.u. vs. AUC BT-474: 328 a.u.).

In conclusion, the BT-474 cell line with higher CDK12 gene copy number and protein level were less sensitive to rucaparib PARPi. CDK12 overexpression may be responsible for the reduced PARPi efficacy. In our further experiments, we aim to demonstrate the effect of the CDK12 inhibition on PARPi sensitivity by combined treatment.

66. CARDIOPROTECTIVE EFFECT OF ISCHEMIC PRECONDITIONING IN THE PRESENCE OF ULCERATIVE COLITIS

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Background: The prevalence of cardiovascular diseases is higher in several inflammatory conditions such as in inflammatory bowel disease. Clinically, both remote intestinal damage after myocardial infarction (MI) as well as an increased severity of myocardial infarction in the presence of ulcerative colitis (UC) have already been shown. However, there is currently no data regarding whether endogenous myocardial protection by ischemic preconditioning (IPC) remains in this co-morbid state. Therefore, in the present study, we set up a novel comorbid

mouse model of UC and acute MI to investigate the sustainability of cardioprotection by IPC in the presence of UC.

Methods: Ulcerative colitis was induced in male C57Bl/6 mice by administering 2,5 % dextran sulfate sodium (DSS, 2.5%) for 7 days into drinking water. The development of UC was shown by measuring inflammatory biomarkers from colon samples as well as by their histological analysis. Cardiac ischemia/reperfusion injury was induced at the 7th days of DSS treatment by occlusion of the left anterior descending coronary artery for 45 minutes followed by 120 minutes of reperfusion in the presence or absence of IPC (3 cycles of 5 min ischemia/5 min reperfusion). For controls, acute MI without UC as well as sham-operated animals were used from both healthy and UC groups. Infarct size was assessed by Evans blue and triphenyltetrazolium chloride double-staining.

Results: The histology of the colon, in line with elevated levels of myeloperoxidase, cathelicidin antimicrobial peptide, and pentraxin-3, indicated the development of UC at a moderate level. Interestingly, this was alleviated in the IPC group. Infarct size was approximately doubled in comorbid acute MI group as compared to acute MI without UC (43.4%±8.0% vs. 19.8%±1.1%, respectively). This severe increment in infarct size in the presence of UC was also reduced by IPC (19.0%±6.7%).

Conclusion: This is the first demonstration that IPC can protect the heart even in the presence of inflammatory bowel disease by decreasing myocardial infarct size *in vivo* in an UC-acute MI comorbid mouse model.

Keywords: acute myocardial infarction, ulcerative colitis, myocardial infarct size, diseaseome, ischemic preconditioning

Funding: Hungarian National Scientific Research Fund (OTKA-138223), János Bolyai Research Scholarships of the Hungarian Academy of Sciences (bo_481_21), New National Excellence Program of the Ministry of Human Capacities (ÚNKP-23-5-SZTE-704), Cooperative Doctoral Programme (KDP-2020) of the Ministry for Innovation and Technology

67. EFFECTS OF A NEW CANNABIGEROL DERIVATIVE, LE-127/2 ON HUMAN CUTAN MELANOMA CELLS

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Melanoma is responsible for the majority of skin cancer-related deaths having a high propensity to metastasize. Despite the targeted- and immunotherapies used in a last few years, survival rate among patients with metastatic melanoma remains low. Efforts directed to the development of natural anti-tumor agents against melanoma have been increased. Among such a plant-derived agents, cannabigerol (CBG), one of the non-psychoactive cannabinoids of *Cannabis sativa*, has been shown to exhibit cell growth inhibition in some types of tumors. These findings encouraged us to study the effect of a recently synthesized by Mannich-type reaction, LE-127/2, a CBG derivative on melanoma cells, with particular regard to its effect on cell proliferation and on the underlying processes causing cell death, such as autophagy.
Three different human melanoma cell lines, namely WM35, A2058 and WM3000 were utilized for our studies.

Cell proliferation activates of cells upon treatment with LE-127/2, its parent compound CBG or vemurafenib were determined by Cell Titer Blue Assay (Promega). To compare the effect of the above mentioned different drugs, the cells were treated with a 1.25-80 μM of compounds, and as a result of the treatments, it was found that at 20 μM concentration all drugs showed comparable effective inhibition on cell growth of melanoma cell lines, however,

vemurafenib and CBG proved to be more effective than LE-127/2.

In addition, we performed clonogenic cell survival assays to examine the inhibitory effect of LE-127/2 on the clone formation ability of melanoma cell lines. All tree cell lines treated with 20 μM of LE-127/2 for 14 days showed about a 50% decrease in colony formation ability. However, LE-127/2 exerted most intensive inhibition on colonies in A2058 cells.

Furthermore, to investigate the cytotoxic effect of LE-127/2 on non-malignant human keratinocytes; HaCaT cells were treated with LE-127/2 and its parent compound CBG, and the cytotoxic effect of the drugs was detected by LDH cytotoxicity assay. Interestingly, LE-127/2 proved to be cytotoxic on HaCaT cells only at higher concentration, such as 80 μM while CBG was cytotoxic at concentration as low as 5 μM, suggesting that this drug candidate may be applied in human pharmacotherapy without causing a substantial damage in intact cells.

We also studied the influence of LE-127/2 on the expression of basic proteins involved in autophagic signal transduction processes (LC-3, Belcin-1 and p62) in three different melanoma cell lines. Expression of these proteins was elevated upon the treatment with 20 μM of LE-127/2. Based on the results obtained, it can be concluded that LE-127/2-induced autophagy could lead to the inhibition of cell proliferation and death in melanoma cells.

Acknowledgement: The project was supported by the HUN-REN-DE-TKI Pharmamodul Research Group, University of Debrecen.

68. ANTIOXIDANT EFFECTS OF A HYDROGEN SULFIDE DONOR IN EXPERIMENTAL COLITIS

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Inflammatory bowel diseases (IBD) are chronic, relapsing disorders, which affect the gastrointestinal tract with inflammation and ulceration. It is well known that oxidative stress contributes to the development of IBD. It is increasingly clear that peroxiredoxins (Prdxs) seem to play an essential role in inflammation and redox balance with other antioxidant components such as glutathione (GSH) and superoxide dismutase (SOD). Several studies demonstrated that hydrogen sulfide (H_2S) as a gasotransmitter, has a wide range of regulatory functions in many physiological and pathological processes. In pathological conditions, H_2S reduces inflammation among others by upregulating antioxidant enzymes.

Our research aimed to investigate the effect of H_2S donor treatment on the antioxidant system, with particular attention to the Prdx enzymes, in experimental colitis. To model IBD 2,4,6-trinitrobenzene sulfonic acid (TNBS) was administered intracolonically (i.c.) to Wistar–Hannover male rats. Then animals were treated orally with the H_2S donor Lawesson's reagent twice a day (18.75 μM/kg/day), and after 72 hours the distal 8 cm section of the colons was dissected. GSH level, SOD activity, peroxiredoxin (Prdx) 1,-2,-4, -6 and nitrotyrosine (3-NT) levels were measured by ELISA method.

Our results showed that H_2S treatment significantly decreased the extent of the colonic lesions and the level of oxidative stress marker 3-NT compared to the model group. Furthermore, H_2S significantly increased the level of the antioxidant GSH, SOD activity, and among the four measured Prdx isoforms the levels of Prdx1 and Prdx6 compared to the TNBS group.

In summary, our results suggest that H_2S treatment can be a potential therapeutic method against IBD through the activation of GSH, SOD, and Prdx1 and Prdx6 antioxidant enzymes.

69. SEMMELWEIS FACS AND VIRAL VECTOR CORE FACILITY

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Universities and research centers worldwide adopt the practice of establishing Core Facilities to provide researchers access to high-value devices and services requiring specialized expertise. This approach facilitates accessibility to instruments beyond the capacity of individual research groups. Semmelweis University also followed this approach with the creation of unified Core Facilities (https://semmelweis.hu/corefacility/).
In 2021 we established a Viral Vector Core – a BSL-2 certified laboratory within the Institute of Translational Medicine. This facility, operating on a non-profit basis, uniquely produces lentiviral vectors in Hungary for university and external academic customers. Our comprehensive services encompass design, cloning, virus production, and titration, enabling researchers to seamlessly integrate this technique into their work. Lentiviral vector transduction is invaluable for difficult-to-transfect cells and non-dividing cells. The introduced DNA is efficiently integrated into the genome and stably transcribed. Notably, the widely adopted CRISPR/Cas9 gene editing system is preferentially delivered into cells using lentiviruses, both in vitro and in vivo.
We also acquired a Beckman Coulter Cytoflex SRT cell sorter to address the absence of suitable live cell sorting devices in the Basic Medical Science Center. Subsequently, we aimed to extend access to the entire university, resulting in the establishment of a FACS Core Facility. Supplemented with a Sony LE-SH800SFP sorter supervised by Dr. Edit Buzás and Dr. Zsolt Komlósi, located in the NET building, we created a FACS Core Facility, which serves the whole university. Moreover, upon request, we can accommodate external academic users. Our facility includes a state-of-the-art device equipped with four lasers capable of detecting up to 15 channels. This enables simultaneous separation up to four cell populations into 5- or 15-ml tubes, cell culture plates (up to 384 wells), or microscopic slides. The collection of individual cells facilitates downstream genomic and/or transcriptomic studies.

70. INVESTIGATION OF THE PROTECTIVE EFFECT OF A BIOACTIVE POLYPHENOL IN TNBS RAT MODEL

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Inflammatory bowel diseases (IBD) are a group of chronic, incurable diseases of the digestive tract. Chronic inflammation underlies the aetiology of IBD and is closely associated with oxidative/nitrosative stress and a vast generation of reactive oxygen/nitrogen species. Several substances with antioxidant and anti-inflammatory properties are now intensively researched as possible adjunctive or independent treatment options in IBD. Among them, resveratrol (RES), a natural polyphenol is increasingly studied for its possible protective properties against IBD.

In the present study, we aimed to investigate the anti-inflammatory effects of RES in three different doses. For this reason, RES supplementation was carried out for 28 days per os. A total of 65 male Wistar-Hannover rats were randomly divided into 7 groups: CTRL, EtOH, TNBS, RES: 5, 10, 20 mg/kg, SASP. On the 25th day of the experiment, animals were challenged by intracolonic injection of 2,4,6-trinitrobenzene sulfonic acid (TNBS) to model IBD.

Animals were sacrificed on the 29th day of the experiment. The potential anti-inflammatory effect was investigated by enzyme-linked immunosorbent assay (ELISA) and western blot.

The histological features of the gut wall, especially the tunica mucosa layer showed clearly visible differences in the investigated groups. Based on our histological and planimetric analysis 10 mg/kg dose of RES is considered to be effective and significantly attenuated ulceration of the colon compared to the TNBS group. Furthermore, RES-induced protection at a concentration of 10mg/kg/day was mediated by the modulation of inflammatory parameter, such as myeloperoxidase (MPO). RES supplementation also caused a decrease in inflammation by reducing the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α). In addition, our immunohistochemical findings showed that 10 mg/kg/day of RES attenuated the intensity of TNF-α receptors, TNFR1 and TNFR2 in the colon compared to TNBS. In conclusion, our results indicate the protective effects of RES in acute low-grade inflammation in TNBS rats and suggest that RES may be a promising therapeutic alternative in the treatment of IBD.

71. MAO-B INHIBITION BY SELEGILINE BLUNTS CARDIAC FUNCTIONS IMPROVED BY HIGH-FAT DIET: ROLE OF INFLAMMATION, APOPTOSIS, AND CALCIUM-HANDLING.

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Obesity is a major risk factor for the development of cardiovascular diseases, which is associated with oxidative stress and chronic inflammation. On the other hand, epidemiological studies show, that moderate obesity reduces the risk of cardiovascular disease. We have shown previously that MAO-B selective inhibitor selegiline reduced visceral adiposity in obesity. Therefore, we aimed to assess if selegiline can influence the beneficial or harmful cardiac effects of obesity. We investigated the effects of selegiline on cardiac redox homeostasis and cellular damage in a high-fat high-sucrose diet (HFD)-induced moderate obesity model in rats by western blot. We demonstrate that selegiline reduces cardiac mitochondrial reactive oxygen species (ROS) production in healthy, but not in obese rats. HFD did not deteriorate major cardiac parameters and improved contractility parameters, which were blunted by selegiline. HFD-increased contractility might be mediated by altered Ca2+ handling as evidenced by increased expression of sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2a) and pentamerization of phospholamban (PLN), whereas selegiline reduced SERCA2a expression in HFD. HFD induced other cardiac molecular changes as well, such

as increased expression of inflammatory mediators Tumor necrosis factor (TNF) and Nuclear factor-kappa B (NF-kB) which was not affected by selegiline. HFD decreased sequestosome-1 level and B-cell lymphoma 2-associated X protein/B-cell lymphoma 2 (Bax/Bcl-2) ratio, which was restored by selegiline. Autophagy, mitophagy, necroptosis, and pyroptosis were not affected by HFD or selegiline. Moderate obesity improves cardiac function through Ca2+ homeostasis and inflammatory processes and MAO-B inhibition reverses this effect.

72. EFFECT OF HYPERCHOLESTEROLEMIA ON CIRCULATING AND CARDIOMYOCYTE-DERIVED EXTRACELLULAR VESICLES

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Hypercholesterolemia (HC) induces, propagates and exacerbates cardiovascular diseases via various mechanisms that are yet not properly understood. Extracellular vesicles (EVs) are involved in the pathomechanism of these diseases. To understand how circulating or cardiac-derived EVs could affect myocardial functions, we analyzed the metabolomic profile of circulating EVs, and we performed an in-depth analysis of cardiomyocyte (CM)-derived EVs in HC. Circulating EVs were isolated with Vezics technology from male Wistar rats fed with high-cholesterol or control chow. AC16 human CMs were treated with Remembrane® HC supplement and EVs were isolated from cell culture supernatant. The biophysical properties and the protein composition of CM EVs were analyzed. THP1-ASC-GFP cells were treated with CM EVs and monocyte activation was measured. HC diet reduced the amount of certain phosphatidylcholines in circulating EVs, independently of their plasma level. HC treatment significantly increased EV secretion of CMs and greatly modified CM EV proteome, enriching several proteins involved in tissue remodelling. Regardless of the treatment, CM EVs did not induce the activation of THP1 monocytes. In conclusion, HC strongly affects the metabolome of circulating EVs and dysregulates CM EVs, which might contribute to HC-induced cardiac derangements.
KEYWORDS: exosome, obesity, dyslipidemia, proteomics, metabolomics, inflammation
FUNDING: VEKOP-2.3.3-15-2017-00016, NVKP-16-1-2016-0017, TKP2021-EGA-23, 2020-4.1.1.-TKP2020, RRF-2.3.1-21-2022-00003, Marie Skłodowska-Curie grant agreement No 101007931; ZG was supported by the National Research, Development and Innovation Office (NKFIH) of Hungary (K139105); K.W. was supported by the SFI Comprehensive Molecular Analytical Platform (CMAP) (18/RI/5702).

73. EFFECTS OF TOFACITINIB ON CARDIOMETABOLIC SYNDROME IN EXPERIMENTAL ARTHRITIS

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Rheumatoid arthritis (RA) is a systemic autoimmune inflammatory disorder accompanied by several comorbidities. Studies have shown that the prevalence of metabolic syndrome (MetS) in RA patients can range from 10% to 56%. Research has delved into the association between obesity, MetS, disease progression, and treatment responses in individuals with RA. The objective of this study was to assess the impact of the Janus kinase inhibitor tofacitinib on cardiac homeostasis in an experimental model of RA and MetS comorbidity.

In this experimental model, ZSF1 rats were inoculated with complete Freund's adjuvant. *In vivo* Transthoracic echocardiography (TTE), Fluorodeoxyglucose (FDG)-positron emission tomography (PET), and *ex vivo* endothelial function test were conducted to assess cardiovascular function. Histological, molecular analysis, as well as blood sample measurements including Masson's trichrome staining, cytokine array, western blot analysis and measurements of serum parameters related to cardiometabolic syndrome, were conducted to gain further insights.

TTE revealed moderate diastolic dysfunction among all obese groups. Lowe FDG uptake in the hearts of obese animals was noted, indicating a link to the progression of the autoimmune disease and insulin resistance associated with MetS. However, a notable improvement was observed in animals treated with tofacitinib, as evidenced by the endothelial function test and blood analyses. Haematoxylin eosin staining demonstrated an increase in cardiomyocyte diameter in the diseased ZSF1 group. Fibrosis was observed in the cardiac tissues of lean groups treated with tofacitinib, which was confirmed by elevated cytokine levels. Conversely, ZSF1 animals treated with tofacitinib did not exhibit any fibrotic alterations.

Our findings align with previous studies suggesting that disease outcomes and response to therapy may differ in patients with both rheumatoid arthritis (RA) and MetS.

Project no. TKP2021-EGA-18 has been implemented with the support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-EGA funding scheme, by the GINOP -2.3.4-15-2016-00002 and the NKFIH-1150-6/2019 projects co-financed by the European Union and the European Social Fund.

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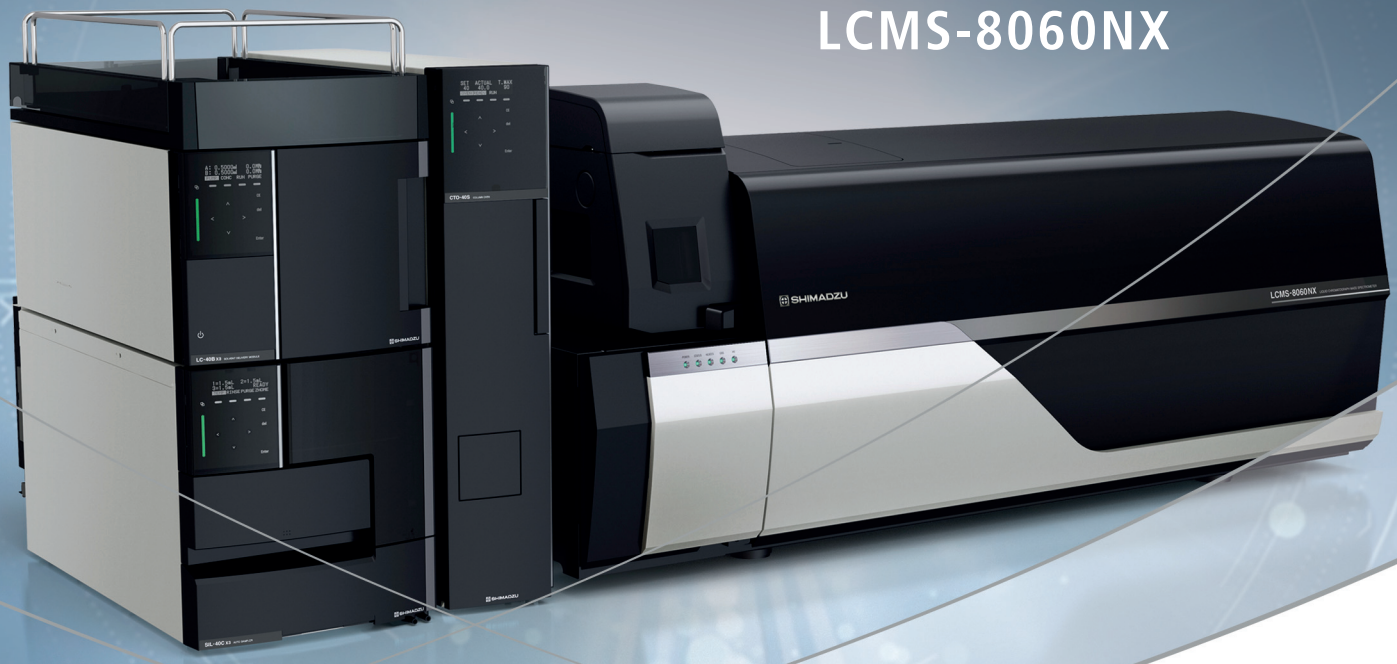

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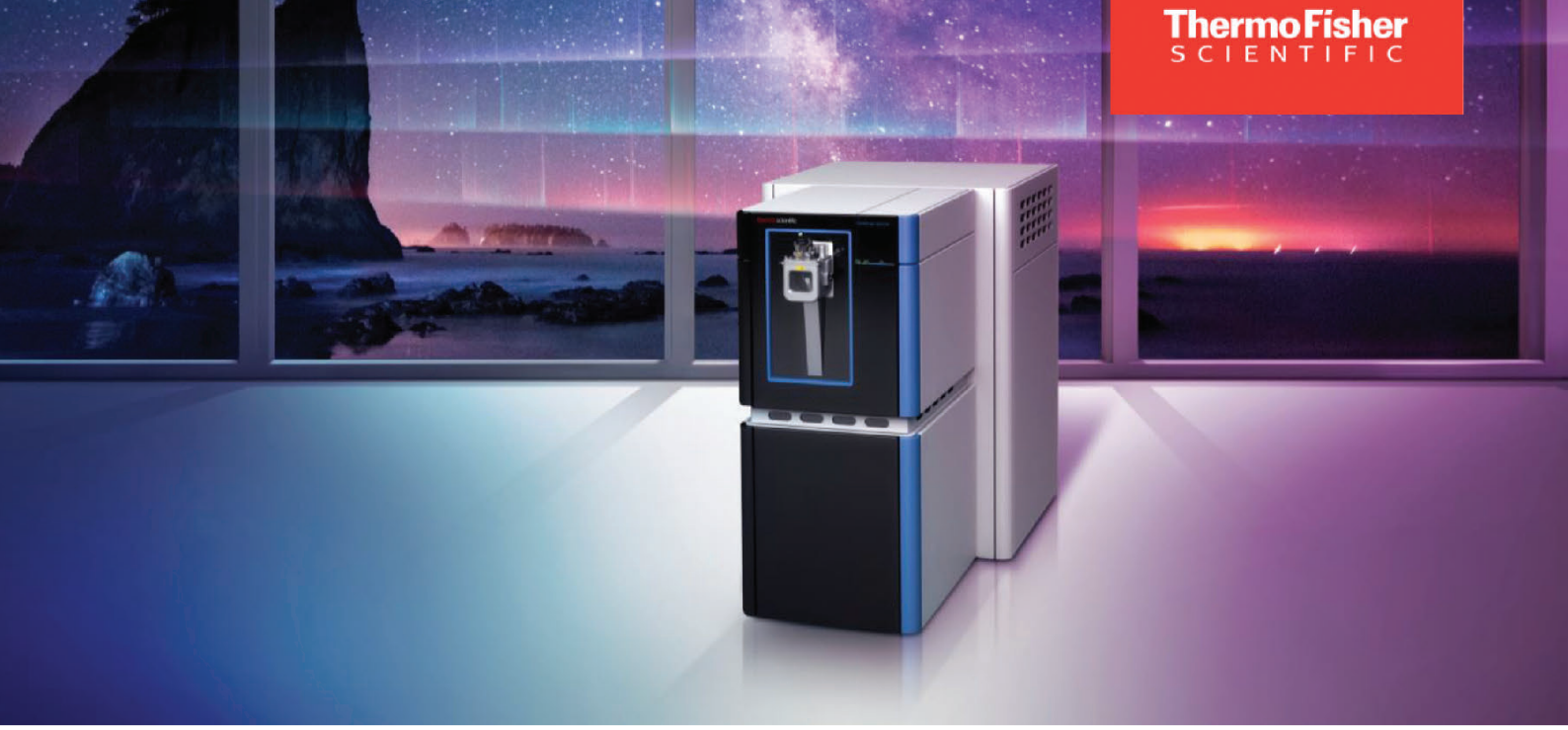

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