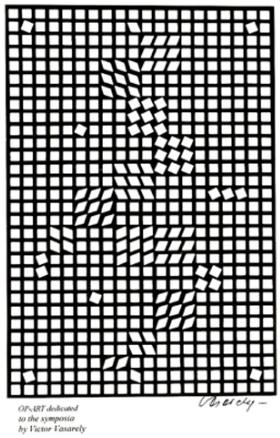
11th ISCTICO - HUPHAR-IUPHAR CONFERENCE

OCTOBER 27-30, 2021
PÉCS, HUNGARY



OP-ART dedicated to the symposia by Victor Vasarely

11th INTERNATIONAL SYMPOSIUM ON CELL/TISSUE INJURY AND CYTOPROTECTION/ ORGANOPROTECTION (ISCTICO),

JOINT MEETING OF THE HUNGARIAN SOCIETY FOR EXPERIMENTAL AND CLINICAL PHARMACOLOGY (HUPHAR) AND THE INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY (IUPHAR)

ABSTRACT BOOK

IUPHAR INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY

HUPHAR Hungarian Society for Experimental and Clinical Pharmacology

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UNIVERSITY OF PÉCS SZENTÁGOTHAI RESEARCH CENTRE

The underappreciated but essential role of glycosphingolipids in ageing, motorneurone disease, Parkinson's disease – and viral infections. New therapies

Michael Spedding

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The complexity of sugars and lipids in the glycocalyx surrounding cells has discouraged research, particularly as molecular biology is blind to the changes, except for mutations is the enzymes of synthesis and degradation – where powerful phenotypes are are revealed for rare diseases. We used 3000 lipid metabolomics and a network of research teams around the world to uncover a new approach to ALS and other neuromuscular diseases involving glycosphingolipids (GSLs). Glucosylceramidase (GCase, GBA1 and GBA2) mutations are the principle genetic cause of Parkinson's Disease (linked to synuclein). We showed in metabolomic studies that major changes in GSLs, associated with denervation, occur in ALS, with a specific increase of GBA2 in spinal cord, associated with a reduction of the GSL neurotrophin GM1 in neuromuscular junctions. We have progressed a safe generic drug to phase II trials. Furthermore, GSLs are the targets of many viruses: influenza viruses and some coronaviruses target the sialic acid in GM1, the neurotrophin which is also involved in viral Guillain-Barré syndrome, and ALS. Thus the glycocalyx, and GSLs, are at last revealing their critical roles in disease pathology.

Dimethyl trisulfide as a promising therapeutic organosulfure molecule in experimental acute pancreatitis

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Acute pancreatitis (AP) is an inflammatory disease of the pancreas which doesn't have any adequate therapy. Several members of organic sulfides or hydrogen sulfide donors have anti-inflammatory effects. Dimethyl trisulfide (DMTS) belongs to the organosulfur molecule family, it shows biological activity, decreases paw inflammation and has analgesic effect through TRPA1 and SST4 receptors.

Our aims were to investigate the in vivo effect of DMTS on AP. Furthermore, we wanted to reveal how DMTS affects cellular viability, the production of reactive oxygen species (ROS) and intracellular calcium concentrations ($[Ca^{2+}]_i$).

AP was induced in FVB/n mice with 10x50µg/kg intraperitoneal (ip) hourly injections of cerulein. DMTS was administered subcutaneously (sc.) 3-hourly in different doses. The first DMTS and cerulein injections were given simultaneously. AP severity was evaluated with histological scoring of pancreatic oedema, leukocyte infiltration, necrosis. Abdominal pain was measured by von Frey filament test. Isolated mouse pancreatic acinar cells were used for in vitro assays. Cellular viability was determined by MTT and propidium iodide. For ROS and $[Ca^{2+}]_i$ measurements, carboxy-H_2DCFDA and FURA-2 dyes were used.

Administration of 2x75, 2x100, 4x50, 4x75mg/kg DMTS significantly ameliorated AP severity by reducing pancreatic necrosis, oedema or leukocyte infiltration in mice. The 4x75mg/kg DMTS significantly reduced the abdominal pain. 3-60µg/ml DMTS concentrations increased the acinar metabolic activity and <100µg/ml DMTS showed no effect on viability. DMTS in itself did not induce ROS production, but reduced the ROS signal in case of menadione and H_2O_2 treatments. DMTS treatment slightly increased the $[Ca^{2+}]_i$ compared to the control treatment.

High doses of DMTS significantly alleviate experimental AP in mice. DMTS did not affect cell viability and ROS production, whereas it increases $[Ca^{2+}]_i$. Our results suggest that DMTS has anti-inflammatory effects and is worth further investigation in AP.

Study was supported by NKFIH-PD-129114, NKFIH-K-119938, GINOP-2.3.2-15-2016-00034, GINOP-2.3.2-15-2016-00048,

and EFOP-3.6.2-16-2017-00006.

The transient receptor potential ankyrin-1 channel mediates the thermal effects of hydrogen sulphide

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Hydrogen sulphide (H_2S) is produced endogenously by different enzymes (e.g., cystathionine β-synthase) and serves as a gasotransmitter. In earlier studies, it was shown that H_2S causes hypothermia and hypometabolism in young mice. In other studies, it was also found that H_2S can act on the transient receptor potential ankyrin-1 (TRPA1) channel, but it has remained unclear if the interaction between H_2S and TRPA1 contributes to the thermal effects of H_2S. Hence, we aimed to investigate whether the thermal effects of H_2S are mediated, at least in part, by TRPA1 channels. In adult, male TRPA1 knock out (KO) and wild type (WT) mice, we studied the effects of H_2S on the thermoregulatory parameters of the animals. To circumvent any drug-specific effect, we tested both fast and slow H_2S-releasing donors. Neither of the compounds showed any thermal effect when administered peripherally to TRPA1 WT mice, whereas a dose-dependent drop in core temperature (0.5-1.0°C) and oxygen consumption (~15 ml/kg/min) developed when the compounds were delivered into the central nervous system ($P < 0.05$ for both). In contrast with our findings in TRPA1 WT mice, the hypometabolic and hypothermic effects of the H_2S-donors were markedly attenuated in TRPA1 KO mice after intracerebroventricular administration. We conclude that H_2S causes hypothermia in mice via an action triggered from the central nervous system. The thermal effects of H_2S are mediated, at least in part, by TRPA1 channels, presumably located on thermoregulatory neurons in the brain.

Dimethyl trisulfide reduces duration of immobility and increases activity frequency in the mouse forced swim test via TRPA1 ion channel

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Present-day antidepressants have a delayed onset of effect and anxiolytics are highly addictive. The search for rapid-acting antidepressants and non-addictive anxiolytics identified only few potential drug candidates. Sulfide was possesses antidepressant and stress-relieving effects. Dimethyl trisulfide (DMTS) is a stable organic polysulfide with impressive pharmacokinetic properties. Our previous work identified central nervous system depressant effect of DMTS. The effect of DMTS relied on TRPA1 ion channel activation. The aim of the present study was to investigate the activity of DMTS in the forced swim test, as well as the involvement of TRPA1 ion channels.

Male TRPA1 gene-knockout (KO) and wild-type (WT) mice were used. Animals either received no treatment, vehicle (1.5% m/v polysorbate 80 in physiological saline) or DMTS (50 mg/kg i.p.). Animals were placed into 24 °C water for 6 min 30 min after drug application and were filmed. The last 4 min of the recordings were evaluated with Noldus EthoVision XT 15 software. Highly active duration and activity frequency parameters were utilized. Duration of inactivity was calculated by subtracting highly active duration from the duration of observation.

DMTS treatment reduced inactive duration, increased highly active duration and activity frequency in TRPA1 WT animals compared to either naïve mice or vehicle. TRPA1 KO animals exhibited overall elevated highly active duration, activity frequency and smaller inactive duration in comparison with the respected WT groups. However, differences between TRPA1 WT and KO mice were statistically not significant. DMTS did not alter parameters in TRPA1 KO animals relative to naïve mice or vehicle treatment.

DMTS reduced depression-like behavior in the forced swim test. The effect was mediated by TRPA1 ion channels. DMTS might offer an alternative for the complementary treatment of depression.

The study was financed by the grant OTKA FK 132454 from the National Research, Development and Innovation Office.

Computational binding studies of sulfide-containing irreversible agonists of ion channel TRPA1

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Transient receptor potential ankyrin 1 (TRPA1) is a transmembrane protein functioning as a calcium ion channel. TRPA1 is a polymodal nocisensor, that can be activated by thermal, mechanical stimuli and a wide range of chemically damaging molecules including small volatile environmental toxicants and endogenous algogenic lipids. After such compounds activate the channel, it opens up by the widening of its central pore to allow calcium influx, that consequentially induces signal transduction pathways in the cytosol.
Recent experimental determination of structures of apo and holo forms of TRPA1 opened the ways towards computational binding studies of electrophile agonists in order to unravel their binding mechanisms. The binding mechanism of agonists is investigated applying molecular docking and dynamics methods, suitable for covalent and prerequisite binding studies.
In the apo form, prerequisite binding modes were detected, and their contribution to the activation of the binding site was shown on the nanosecond level scale. Furthermore, prerequisite binding properties were identified, that can forecast a strong covalent bond forming agonist. Together, these findings might contribute to the overall understanding of the activation of the TRPA1 receptor, as well as to the design of new agonists.
Funding: This work was funded by the Hungarian National Research, Development and Innovation Office (K123836, K134214), 2017-1.2.1 NKP-2017-00002 (NAP-2; Chronic Pain Research Group), EFOP-3.6.1.-16-2016-0004 and GINOP 2.3.2-15-2016-00050 “PEPSYS”. This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.
Acknowledgments: We acknowledge the support from the Governmental Information Technology Development Agency, Hungary. The work was supported by the ÚNKP-21-5 and ÚNKP-21-3-II. New National Excellence Program of the Ministry for Innovation and Technology. Supported by the PTE ÁOK-KA No:2019/KA-2019-31. The project has been supported by the European Union, co-financed by the European Social Fund. Project name and code: Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, EFOP-3.6.1-16-2016-00004.

Hydrogen-sulfide (H2S)-releasing pharmacological tools in the Prevention of Drugs-Induced Gastrointestinal Mucosal Injury.

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Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) display a range of therapeutic effects, but they also exert serious gastrointestinal adverse effects including hemorrhage and ulceration. It has been discovered that anti-inflammatory and anti-oxidative H_2S supports the maintenance of gastric mucosal integrity. H_2S-releasing chemical donors have been developed and reported to protect gastric mucosa against damage induced by NSAIDs, ethanol or stress.
Aim: We aimed to evaluate the molecular mechanisms of increased GI safety of novel H_2S-releasing ketoprofen and the impact of H_2S released from AP39 on mitochondrial activity altered by NSAIDs.
Methods: Rats were administered intragastrically with i) vehicle, ii) ketoprofen (10 mg/kg/day), iii) its H_2S-releasing derivative, ATB-352 (14mg/kg/day), iv) AP39 alone (0.02 mg/kg)
or v) combined with aspirin (125 mg/kg). Gastric and intestinal damage score was assessed macro- and microscopically. H_2S production in GI mucosa was analyzed using the modified methylene blue assay. GI mucosal mRNA expression for HIF-1⟨, HMOX-1 and SOCS3 was evaluated
by real-time PCR. Mitochondrial complex IV activity in gastric mucosa was evaluated by biochemical assay.
Results: Ketoprofen induced gastrointestinal injury and decreased H_2S production level in GI mucosa, while the toxicity of ATB-352 was significantly reduced due to the release of H_2S. Ketoprofen, but not ATB-352, elevated intestinal mRNA expression of HIF-1⟨ and SOCS3. ATB-352, in contrast to ketoprofen, upregulated the mRNA expression of HMOX-1 in intestinal mucosa. AP-39 prevented gastric mucosa against aspirin-induced injury due to modulation
of complex IV activity.
Conclusion: H_2S-releasing NSAIDs exert increased GI safety profile based on mechanisms involving compensation of decreased H_2S production, upregulation of protective HMOX-1 and lower impact on mRNA expression of inflammatory markers within GI mucosa. Gastroprotective effect of H2S against NSAIDs-gastropathy may be due to the maintenance of mitochondrial activity affected by these drugs. [Founding source: The National Centre for Research and Development, Poland (LIDER/9/0055/L8/16/NCBR/2017)].

The novel multi-target drug SzV-1287 is a promising candidate for neuropathic pain: summary of the preclinical results

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SzV-1287 (3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime) is a novel multi-target drug, which inhibits amine oxidase copper-containing 3 (also known as semicarbazide-sensitive amine oxidase) metabolizing primary amines to irritants like methyglyoxal and formaldehyde activating the transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) receptors. Furthermore, it also directly antagonizes these non-selective cation channels located on primary sensory neurons, immune cells and several central nervous system regions.

We provided proof-of-concept for the ability of i.p. administered SzV-1287 (20 mg/kg) to significantly reduce acute and chronic pain in mice via inhibiting TRPA1 and TRPV1 activation. It significantly inhibited the TRPV1 agonist resiniferatoxin- and the TRPA1 agonist formalin-evoked acute chemonociception and hyperalgesia. Repeated SzV-1287 injections attenuated chronic arthritis (edema, myeloperoxidase activity, histopathological changes) and related hyperalgesia, L4-L6 spinal dorsal horn microgliosis in wildtype, but not in TRPV1- and TRPA1-deficient mice. Acute SzV-1287 administration resulted in approximately 50% significant reduction of neuropathic hyperalgesia 7 days after sciatic nerve ligation, which was not observed in mice lacking either TRPA1 or TRPV1 receptors. Since mainly under acidic conditions, SZV 1287 is transformed to the cyclooxygenase inhibitor oxaprozin, which is ineffective for neuropathy, an enterosolvent capsule is suggested for oral formulation and preclinical drug development. SzV-1287 (20, 50, and 200 mg/kg, p.o.) significantly, but not dose-dependently, reduced neuropathic hyperalgesia by 50%. It was quickly absorbed; plasma

concentration was stable for 2 h, and it entered into the brain. Although SzV-1287 significantly decreased the proton-induced TRPV1-mediated calcium-influx potentially leading to hyperthermia, it did not alter deep body temperature. It did not show any considerable toxicity either in rodents or in dogs.

Oral SzV-1287 inhibits neuropathic hyperalgesia, it is safe and - despite its TRPV1 antagonistic action and brain penetration - does not influence thermoregulation, which makes it a promising analgesic candidate.

Acknowledgements: GINOP-2.2.1-15-2016-00020, EFOP-3.6.1-16-2016-00004, National Brain Research Program 20017-1.2.1-NKP-2017-00002

Calculation of complex structures of somatostatin receptor subtype 4

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Somatostatin is an endogenous cyclic neuropeptide, released from the capsaicin-sensitive sensory nerves and mediates analgesic and anti-inflammatory effects via the somatostatin sst_4 receptor without endocrine actions. Therefore, sst_4 is considered to be a novel target for drug development in pain including chronic neuropathy, which is an emerging unmet medical need. The therapeutic use of the somatostatin is limited due to its diverse biological effects and rapid degradation. Despite the development of numerous somatostatin analogues, the molecular structure of sst_4 receptor and the exact binding site or modes of its ligands have not been determined experimentally. However, such information could be essential for the design of potent, receptor-selective drugs.
The aim of our group is the modelling of the structure of sst_4, identification of the binding sites of somatostatin and analogues and the examination of binding mechanisms using target-ligand complexes.
Due to the size of the receptors and the complexes, molecular mechanics methodologies are involved besides homology modelling and molecular docking. To answer the challenge of blind docking prediction of the binding mode of somatostatin, our recent techniques Wrap 'n' Shake and fragment blind docking were also adopted for the work.
Acknowledgments: This work was supported by the Hungarian National Research, Development and Innovation Office (K123836), 2017-1.2.1-NKP-2017-00002 (NAP-2; Chronic Pain Research Group), GINOP 2.3.2-15-2016-00050 "PEPSYS", the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, the ÚNKP-20-5 New National Excellence Program of the Ministry for Innovation and Technology and the University of Pécs (PTE ÁOK-KA No:2019/KA-2019-31).

Protective effects of the novel amine oxidase inhibitor multi-target drug SZV 1287 on pancreatic beta cell damage and neuropathic cold hypersensitivity in the streptozotocin-induced rat diabetes model

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Background: Diabetes is a common metabolic disease leading to hyperglycemia due to insufficient pancreatic insulin production or effect. Amine oxidase copper containing 3 (AOC3) belongs to the semicarbazide-sensitive amine oxidase family, which may be a novel therapeutic target to treat diabetic complications.
Aim: We aimed to explore the effects of AOC3 inhibition and test the actions of our novel AOC3 inhibitor multi-target drug candidate, SZV 1287, compared to a selective reference compound, LJP 1207, in an 8-week long insulin-controlled streptozotocin (STZ)-induced (60 mg/kg i.p.) rat diabetes model.
Methods and results: Both AOC3 inhibitors (daily 20 mg/kg s.c.) were protective against STZ-induced pancreatic beta cell damage determined by insulin immunohistochemistry and radioimmunoassay, and neuropathic cold hypersensitivity measured by paw withdrawal latency decrease from 0°C water. SZV 1287 showed greater effects. Functional retinal damage was significantly attenuated by both compounds, but SZV 1287 was more beneficial on the morphological alterations. Mechanical hypersensitivity measured by esthesiometry, cardiac dysfunction and nitrosative stress determined by echocardiography and immunohistochemistry/Western blot, respectively, serum Na^+, K^+, fructosamine, and urine microalbumin, creatinine, total protein/creatinine ratio did not change in response to diabetes. None of these parameters were influenced by the treatments except for SZV 1287 reducing serum fructosamine and LJP 1207 increasing urine creatinine.

Conclusion: We provide the first evidence for protective effects of AOC3 inhibition on STZ-induced pancreatic beta cell damage and neuropathic cold hypersensitivity, and demonstrate its beneficial actions against retinopathy. Our novel multi-target analgesic candidate, SZV 1287, is chronically safe and effective under diabetic conditions.
Supports: This work was supported by **GINOP 2.2.1-15-2016-00020, 20765-3/2018/FEKUTSTRAT.**

Comparative evaluation of two hyaluronic acid gel products for the treatment of interdental papillary defects

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Background: The successful restoration of interdental papilla defects is a major challenge for dental clinicians. A non-surgical method introduced recently is the injection of hyaluronic acid fillers into the deficient papillae.
Aim: Our aim was to investigate the efficacy of single injections of two different hyaluronic acid products, Flex Barrier and Revident, in reducing the size of black triangles for the treatment of Nordland-Tarnow class I and II type recessions.
Methods: Forty adult patients were recruited with at least two upper and two lower interdental papilla defects in the front region between canine teeth. According to the Nordland-Tarnow classification of papillary defects, both class I and class II recessions were included in the investigation. Patients were randomly assigned to experimental groups to receive single injections of two different hyaluronic acid products, either Flex Barrier or Revident. The untreated sites served as controls. Photographs were taken before and immediately after treatment, and again after one week and one month. To determine the size of the black triangles, ImageJ software was used. For statistical analysis, a mixed-design ANOVA was applied.
Results: Both Flex Barrier and Revident significantly decreased the size of the treated defects immediately after treatment and also one week later ($p<0.001$). The beneficial effect of Revident lasted longer than Flex Barrier as it remained significant even after one month in Revident-treated patients but not in the Flex Barrier-treated group. Furthermore, Nordland-Tarnow Class I lesions generally showed a greater improvement than Class II lesions.
Conclusion: In this proof-of-concept, randomized clinical trial we have demonstrated the clinical applicability of both Flex Barrier and Revident, although Revident gave longer-lasting improvements than Flex Barrier. Further trials are needed to optimize multiple-application protocols for treating gingival black triangles.
Acknowledgements: This work was supported by the Hungarian Human Resources Development Operational Program (EFOP-3.6.2-16-2017-00006).

The pharmacological effect of thyme (*Thymus vulgaris* L.) essential oil in an endotoxin-induced acute airway inflammation mouse model

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Background: Inflammatory lung diseases affect a large population worldwide. Essential oils can easily reach the respiratory tract via inhalation. The anti-inflammatory effect of essential oils is poorly studied *in vivo*. Transient Receptor Potential Vanilloid1 (TRPV1) and Ankyrin1 (TRPA1) ion channels are expressed on the sensory neurons and epithelial cells of the airways and play a role in sensory-immune interactions.
Aim: Therefore, we aimed to examine the chemical composition and effects of thyme oil (TO) inhalation in the model of lipopolysaccharide (LPS)-induced airway inflammation and the potential role of TRPA1/V1 ion channels in mediating TO effect. The essential oil was selected on the basis of its potent antibacterial activity.
Methods: The chemical composition of TO was determined by GC-MS. Lung inflammation was evoked by the intratracheal administration of 60 µL LPS (*E. coli* 083: LPS) in female TRPA1/V1$^{+/+}$ (WT) and TRPA1/V1$^{-/-}$ (KO) mice. TO or the control oil was inhaled 3 times for 30 min during the 24-h period of the experiments. Airway function was measured in awake, spontaneously breathing animals by unrestrained whole body plethysmography. Lung myeloperoxidase (MPO) activity was determined by spectrophotometry. The histopathological alterations were evaluated from hematoxylin-eosin stained lung sections by semiquantitative scoring.
Results: Thymol (46.3%) was the main component of TO. TO inhalation significantly alleviated airway hyperreactivity in WT, but aggravated it in KO mice. Histological parameters were not affected significantly by TO inhalation in either WT or KO mice. LPS treatment induced a remarkably increased MPO activity, which was significantly reduced by TO inhalation in WT, but not in KO mice.
Conclusion: Therefore, thyme oil can be considered as a potential treatment in airway inflammation, and its protective effect is potentially mediated by TRPA1/V1 ion channels.

Emerging role of exercise and intestinal alkaline phosphatase in amelioration of inflammatory bowel disease (IBD) in experimental animals and humans. Importance of adipokines, myokines and intestinal microbiota

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Over the past few years, the role of intestinal alkaline phosphatase (IAP) as a crucial mucosal defense factor essential for maintaining gut homeostasis has been established. IAP is an important apical brush border enzyme expressed throughout the gastrointestinal tract and secreted both into the intestinal lumen and into the bloodstream. IAP exerts its effects through dephosphorylation of pro-inflammatory molecules including lipopolysaccharide (LPS), flagellin and adenosine triphosphate (ATP) released from cells during stressful events. Therefore, the aim of our present study was to determine whether voluntary exercise could affect experimental colitis induced by intrarectal administration of TNBS in mice fed standard diet (SD) or high-fat diet (HFD), possibly by affecting muscle-fat crosstalk. We assessed the underlying mechanisms involved in the beneficial action of voluntary exercise, namely the alterations in disease activity index (DAI), colonic blood flow (CBF), irisin release as well as at the molecular level, the colonic expression of proinflammatory biomarkers and adipokines. We have also evaluated release of myokines and appetite hormones in exercising young volunteers. We found that that exogenous treatment with IAP exerted a significant protective effect on the intestinal inflammation and potentiated the beneficial ameliorating effect of exercise on experimental colitis. Combined administration of IAP and wheel running resulted in a substantial decrease in DAI index along with mRNA expression for proinflammatory biomarkers TNF-α, IL-1β, IL-6 in colonic mucosa and such an effect was accompanied by a significant elevation in CBF, known to play an important role in the intestinal mucosa defense mechanisms. In human study, acute bout of exercise resulted in enhanced release of irisin and appetite hormones such as ghrelin and pancreatic polypeptide (PP). Our data have shown that exogenous IAP can ameliorate gut inflammation, enhances effect of exercise and favors healing of colitis due to enhancement in CBF, the downregulation of intestinal proinflammatory factors and upregulation of anti-inflammatory factors released from adipose tissue and working skeletal muscle. The relevance of these experimental findings in IBD patients is warranted and should be conducted in properly designed clinical trials.

Microbial and pharmacological implications of high-fat diet-induced depression

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Background: The global prevalence of diabesity is rising, and the clinical, social and economic health burden arising from this epidemic is aggravated by comorbid neuropsychiatric diseases, particularly depression. There is evidence that the interoceptive input to the brain may in part arise from the gastrointestinal tract.
Aim: To advance the understanding of the relationship between metabolic dysregulation and affective disturbances we set out to analyse potential links between diet-related obesity and depression-like behaviour.
Methods: Two major tasks were pursued. Firstly, we examined the impact of high fat diet (HFD) on intestinal microbiome, cerebral metabolome and emotional-affective behaviour in mice. Secondly, we sought to provide direct evidence that the gut microbiota and obesity-related hormones are relevant to HFD-evoked depression-like behaviour.
Results: Male mice were fed a HFD (60 kJ% from fat) or control diet (12 kJ% from fat) for 4 or 8 weeks, which led to a depression-like phenotype as revealed by reduced sociability, anhedonia and lethargy. In the caecum, HFD diminished the relative abundance of Bacteroidetes and increased the relative abundance of Firmicutes and Cyanobacteria. In the brain, HFD modified the metabolome of prefrontal cortex and striatum, changing the relative concentrations of molecules involved in energy metabolism and neuronal signalling. While HFD-induced anhedonia remained unaltered by antidepressant treatment, antibiotic-induced depletion of the gut microbiota blunted the HFD-induced obesity, the HFD-induced increase in plasma leptin and the HFD-induced depression-like behaviour. The anhedonic effect of HFD was absent in leptin-deficient *ob/ob* mice.
Conclusion: Depression-like behaviour induced by prolonged HFD in mice is associated with distinct alterations of intestinal microbiome, leptin signalling and brain metabolome Both gut microbiota and leptin play essential roles in HFD-induced depression-like behaviour.
Supported by EU grant 613979 (MyNewGut) and the Austrian Science Fund (P25912-B23 and W1241-B18).

The Physiological and Pharmacological role of Microbial Biofilms at the Gut Mucosa Surface.

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Microorganisms are colonizing various ecological niches in the human habitat, as they do in nature. Predominant forms of multicellular communities called biofilms colonize human tissue surfaces. The gastrointestinal tract is home to profusion of microbes living in intertwined, but not synonymous, lifestyles: isolated planktonic cells, biofilms and biofilm-dispersed. It is therefore of major importance for our comprehension of homeostatic and altered host-microbe interactions processes to consider not only planktonic lifestyle, but also biofilms and biofilm-dispersed forms.
In this presentation, we will elaborate on the natural organization of microorganisms at gut surfaces, the stratification of microbiota taxonomy, biogeographical localization, and transkingdom interactions occurring within this habitat. The contribution of the host/mucosal biofilm relationship to gut homeostasis and to diseases will be questioned. How host factors can shape the organization, structure and composition of mucosal biofilms, and how biofilms themselves are implicated in a variety of gut biological homeostatic and pathological processes will be discussed. The pharmacological role of Biofilms in drug metabolism will also be discussed. Future studies characterizing biofilm nature, physical properties, composition and intrinsic communication will shed new light on gut physiology, and potential novel therapeutic options for intestinal diseases.

The effect of nonsteroidal anti-inflammatory drugs on the small intestinal microbiota and bile acids in rats

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Background: The pathogenesis of nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal injury is still poorly understood. The enterohepatic circulation of NSAIDs, increased toxicity of bile and intestinal bacteria all have been implicated in the development of enteropathy. NSAIDs induce intestinal dysbiosis, and there is some evidence that they also alter the composition of bile acids, but little is known about the time-course of these changes, and about the correlation between dysbiosis, inflammation and bile acid alterations.
Aim: To determine the effect of indomethacin on the microbiota and bile acids at different time points, and to analyze their correlation with each other and with intestinal damage.
Methods: Rats were treated once with 20 mg/kg indomethacin by gavage and were euthanized 24, 48 or 72 h later. A fourth group was treated with vehicle and euthanized at 72 h. Intestinal injury was assessed macroscopically and by measuring the level of various cytokines. The composition of jejunal microbiota was determined by deep sequencing of 16S rRNA, whereas the amount of bile acids by LC-MS/MS.
Results: Although the tissue levels of cytokines peaked already at 24 h, the severity of macroscopic damage increased with time. Indomethacin induced time-dependent alterations in the intestinal bile acid profile of animals, which was, for example, characterized by a reduction of primary unconjugated-, and elevation of conjugated bile acids. The abundance of several Gram positive genera decreased with time, whereas the proportion of Gram negative bacteria increased, however, the changes of some bacteria showed a different pattern. The proportion of some hydrophilic bile acids and Gram positive bacteria correlated negatively with inflammation, and some Gram positive genera showed positive correlation with unconjugated bile acids.
Conclusion: Our time-course and correlation analysis helps to understand the complex interaction between NSAID-induced intestinal inflammation, dysbiosis and bile acid alterations. Grants: NKFI FK 124878, STIA-KF-17.

Sulforaphane normalizes human bowel habits by increasing Nrf2-dependent antioxidant system and butyrate-producing gut microbiota

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Background & Aims: Chronic functional constipation is caused by enhanced mental stress and imbalance of intestinal microbiota. Since chronic oxidative stress disturbs regular defecation,we assume that dietary approach with anti-oxidant foods may improve defecation during stressful dailylives. Sulforaphane (SFN), rich in broccoli sprouts (BS), enhances anti-oxidant systems, therebyprotects cells from oxidative stress. We have previously shown that SFN upregulates anti-oxidant enzymes, and inhibits activity of Helicobacter pylori, and intestinal anaerobic bacteria (Curr Pharm Des 2017, 23, 4066-75). The present study was conducted to determine if daily intake of SFN-rich BS mitigates chronic constipation.
Methods: This study was approved by the research ethics committee in Hitachi General Hospital, and was registered to U-Min Clinical Trial Registration System in Japan. Study 1: Effects of Sprouts Intake on Defecation: This study was designed as a placebo-controlled blinded intervention trial. Forty-eight healthy subjects, recruited from the hospital staff, who showed more than 2 points by a constipation scoring system (CSS) (Dis Colon Rectum 1996 ; 39 : 681-685) were divided into either broccoli sprouts
(BS) group (n=24), or alfalfa sprouts (AS) group (n=24). The subjects assigned to BS/AS group were requested to eat either raw BS/AS 20 g every day for 4 weeks, respectively. BS contains 4.4 mg/g sulforaphane glucosinolates (SGS), a precursor of SFN, while AS contains no SGS. There was no significant difference in other components between BS and AS. The CSS-based questionnaires and stool samples were collected at 4 weeks intervals during the trial period. Study 2: Effects of Sprouts Intake on Intestinal Microbiota. This study was conducted by a crossover trial using 24 subjects with chronic constipation recruited from the participants in Study 1. Each subjects were assigned to eat BS (or AS), 20 mg/day, for 4-wks period, with a subsequent 4-wks washout phase, followed by intake of AS (or BS) for the ensuing 4-wks period. Stool samples were collected twice at the end of the each 4 weeks periods. Percentages of the Bifidobacterium, Lactobacillus, Bacteroides, Prevotella, Clostridiums [cluster IV, IX, XI, XIVa, XVIII], were evaluated by T-RFLP flora analysis.
Results: 1. Intake of BS, but not AS, showed a significant decrease in the total constipation score, characterized by an increase in the defecation frequency, and a decrease in length of time to attempt.
2. Intake of BS, but not AS, up-regulated HO-1, and increased percentages of Bifidobacterium and Clostridium XVIa, which has been shown to normalize defecation by enhancing butyrate production.
Conclusion: Daily intake of BS improves bowel habits in human subjects. These beneficial effects of BS appear to be mediated by enhancement of antioxidant system and by normalizing intestinal microbiota.

The stressed gut: influence of microbiom on susceptibility for posttraumatic stress disorder in rats

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Posttraumatic stress disorder (PTSD) is a highly prevalent, devastating psychiatric disorder. However, only vulnerable subject develop symptoms after a traumatic experience. Identifying markers of resilience maybe helpful for prevention. Our goal was to explore the possible link between the gut microbiota and PTSD in a rat model with the hope to identify a suitable probiotic cocktail, which will increase resiliency.

Male Long Evans rats were traumatized using electric footshock and - based on z-score calculated from the behavioral parameters (social interest, freezing in trauma context, extiction of freezing etc.) – vulnerable and resilient subgroups were determined. After sacrifice ileal morphology and caecal microbiota content were studied.

The results of the histological examinations confirmed that the thickness of the intestinal villi was significantly smaller in the vulnerable subjects compared to both resilient and control animals. The most prominent difference in the intestinal microbiota composition determined by next-generation sequencing was in the relative abundance of the bacterium *A. muciniphila*, which was nearly ten times higher in the susceptible group compared to the control and resistance groups.

Our findings show that trauma may contribute to the development of PTSD symptoms in susceptible individuals by altering microbial composition and intestinal anatomy.

Clinical pharmacology of modern vaccine development

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During the Covid-19 pandemics new DNA or mRNA related technologies emerged for producing convincingly effective vaccines. In the lecture the development of recombinant spike protein, replication-incompetent vector and mRNA based vaccines will be briefly presented. For producing recombinant spike protein vaccine, insect cells are transfected with DNA to produce antigenic spike proteins, which are stud on synthetic particles to obtain a virus like structure. The addition of saponine adjuvant is necessary for achieving high immunogenicity. Replication-incompetent virus vectors transmit the DNA code of the spike protein inserted into the virus DNA. The virus causes an infection and the body develops antibodies against both the virus and the spike protein. In the newest approach the mRNA coding for the spike antigen is enclosed into lipid nanoparticles (LNP) for i.m. administration. The mRNA vaccine represents a new drug class as well as a new production technology used the first time during the Covid-19 pandemics. I shall shortly cover the modifications of the mRNA molecule needed for increasing the stability and translation efficiency of the nucleic acid medicinal product and also the development of suitable biopharmaceutical formulation for the safe delivery of mRNA to the cells.

P2X7 receptors amplify CNS damage in neurodegenerative diseases

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ATP is a (co)transmitter and signaling molecule in the CNS. It acts at a multitude of ligand-gated cationic channels termed P2X to induce rapid depolarization of the cell membrane. Within this receptor-family, the P2X7 receptor (R) allows the transmembrane fluxes of Na^+, Ca^{2+}, and K^+ in the channel-mode, but long-lasting activation by ATP drives it into the pore-mode allowing the passage of molecules previously not permeating the channel. This is supposed to cause necrosis by excessive Ca^{2+} influx, depletion of intracellular ions and metabolites. Cell death may also occur by apoptosis due to the activation of the caspase enzymatic cascade. Because P2X7Rs are localized in the CNS preferentially on microglia, but also at a lower density on neuroglia (astrocytes, oligodendrocytes) the stimulation of this receptor leads to the release of neurodegeneration-inducing bioactive molecules such as pro-inflammatory cytokines, chemokines, proteases, reactive oxygen and nitrogen molecules, and the excitotoxic glutamate/ATP. Various neurodegenerative reactions of the brain/spinal cord following acute harmful events (mechanical CNS damage, ischemia, status epilepticus) or chronic neurodegenerative diseases (neuropathic pain, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis) lead to a massive release of ATP via the leaky plasma membrane of neural tissue. This causes cellular damage superimposed on the original consequences of neurodegeneration. Hence, blood-brain-barrier permeable pharmacological antagonists of P2X7Rs with excellent bioavailability are possible therapeutic agents for these diseases. The aim of this review article is to summarize our present state of knowledge on the involvement of P2X7R-mediated events in neurodegenerative illnesses endangering especially the life quality and duration of the aged human population.

Central inhibition of P2Y12R differentially regulates survival and neuronal loss in MPTP-induced Parkinsonism in mice

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Background: Parkinson's disease (PD) is a chronic, progressive neurodegenerative condition. Accompanying the relatively selective degeneration of the nigrostriatal dopaminergic pathway, neuroinflammation has been identified as one of the fundamental features of the disorder. During PD progression, microglia, the resident immune cells in the central nervous system, are activated and may contribute to maintaining Parkinson's development. The purinergic $P2Y_{12}$-receptor is the molecular target of widely used antithrombotic drugs and has also been shown to have pivotal role in microglial activity and responses.
Aim: Our aim was to identify the function of the $P2Y_{12}$-receptor during PD progression.
Methods: We have used the MPTP-induced experimental PD model in C57BL/6J or P2Y12R-KO mice.
Results: Here we show that MPTP-induced PD symptoms in mice are associated with marked neuroinflammatory changes and $P2Y_{12}$-receptors contribute to the activation of microglia and progression of the disease. Surprisingly, while pharmacological or genetic targeting of the $P2Y_{12}$-receptor augments acute mortality in MPTP-treated mice, these interventions protect against the neurodegenerative cell loss and the development of neuroinflammation *in vivo*. Additionally, we show that pharmacological blockade of $P2Y_{12}$-receptors during disease development reverses the symptoms of PD and halts disease progression. We found that $P2Y_{12}$-receptors regulate ROCK and p38 MAPK activity, consequently promoting transcription and increasing pro- and anti-inflammatory cytokine production. Furthermore, we have found prominent $P2Y_{12}$R expression on activated microglia in human patients with neurodegenerative diseases.
Conclusion: The presence of functional $P2Y_{12}$R is essential to prevent acute neurotoxicity and associated mortality, while prolonged activation of $P2Y_{12}$R may alter pro- and anti-inflammatory cytokine production and maintain neuroinflammation during experimental PD. Chronic inhibition of P2Y12-receptors during disease progression mitigates inflammation, protects against dopaminergic neuronal cell loss and alleviates motor impairments. Understanding protective and detrimental $P2Y_{12}$ receptor-mediated actions in the CNS may reveal novel approaches to control neuroinflammation and modify disease progression in PD.

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE, A POTENTIAL NEW BIOMARKER IN PARKINSON'S DISEASE

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Background: Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with antiapoptotic, antioxidant, regulatory and antiinflammatory effects proved by numerous *in vitro* and *in vivo* studies. In the last few years several studies examined changes of PACAP levels in human samples to show alterations in various physiological and pathological conditions.
Plasma PACAP concentrations were significantly increased after acute spontaneous basal ganglia hemorrhage and reduced in the cerebrospinal fluid (CSF) of Alzheimer's disease patients. We showed correlation between patient mortality and PACAP levels in severe traumatic brain injury. Earlier experiments did not show changes of PACAP levels in the CSF of Parkinson's disease (PD) patients.
Aim: Our aim was to examine the PACAP in blood samples of patients with PD (n=107, control=39).
Methods: We measured the plasma PACAP38 level of PD patients with sandwich-type ELISA and searched for correlations with clinical parameters such as gender, age, stage and subtype of disease, type of treatment and specified scores for PD [Hoehn-Yahr, Movement Disorder Society - Unified Parkinson's Disease Rating Scale (MDS-UPDRS)].
Results: We showed significantly decreased PACAP38 levels in PD patients over 50 compared to younger group and in Hoehn-Yahr scale stage 3 and 4 compared to stage 2 group. Elevated levels were found after deep brain stimulation. We didn't find significant correlations between plasma PACAP38 levels and the MDS-UPDRS or the type of pharmacological treatment.
Conclusion: Earlier experiments did not find significant changes in CSF PACAP levels of PD patients, however, our experiments showed decreased level in plasma samples. The reduced levels of PACAP found in PD patients and the significant increase after DBS treatment confirm the hypothesis that the neuroprotective effect of PACAP could have a role in PD. Based on our results we assume the possibility of using PACAP as a biomarker to monitor the course of the disease.

RainsfordGI-Safer Salicylates – the case for Salsalate (Diplosal)

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Non-steroidal anti-inflammatory (NSAIDs) and non-narcotic analgesics have been the mainstay over the past century for the treatment of arthritic and other painful conditions. They originated from the isolated extracts from salicylate-containing plants (e.g. willow bark), later followed in the 19th Century by the chemical synthesis of salicylic acid and then to acetyl – salicylic acid (ASA, or aspirin) in the early 20th Century (Rainsford, 2004, *Aspirin and Related Drugs.* London: Taylor & Francis). Shortly afterwards, salicyl-O-salicylic acid (SSA, *aka* Diplosal®, Disalcid®, salysal, salsalate) a non-acetylated salicylate was introduced. It is now recognised that the acetyl group of aspirin while responsible for the unique pharmacological activity of aspirin in acetylating the active site of cyclo-oxygenases (COX-1 and COX-2) that underlies the antiplatelet (COX-1) and prostaglandin synthesizing (COX-2) effects of this drug, also accounts for the gastro-intestinal (GI) and other adverse effects of this drug. SSA is totally different, being without the acetyl group of aspirin, and it has markedly lower GI ulcerogenic activity. SSA is comprised of 2 molecules of salicylic acid to form an ester. It was patented in 1909 (Lorenz Ach & Theodor Sutter, US Patent No 922,995, also German patents) and developed by CF Boehringer & Soehne GmbH of Mannheim-Waldorf, Germany as Diplosal®. During the 1950s, Dart Industries Inc, Riker Laboratories, Northridge, California, USA, marketed salsalate as Disalcid® first in Canada in 1950, then USA and in the UK in 1969. Later in 1969 the 3M Company (Minnesota) bought Riker Labs and proceeded with the development of Disalcid® with considerable emphasis on developing newer high strength tablet and capsule formulations (500mg, 750mg), extensive pharmacokinetic and pharmacodynamic studies in humans, and clinical trials in rheumatic and painful conditions. In long-term studies (e.g. ARAMIS), showed that the drug was amongst the lowest of NSAIDs for gastro-intestinal adverse events. This was supported by upper GI endoscopic investigations and studies showing the drug had little if any effects on gastric mucosal prostaglandin production.

Recently, there has been a major interest in the clinical and experimental actions of SSA (Anderson et al., Am Health Drug Benefits 2014; 7:231-5). Extensive investigations has shown that salsalate has new indications among these treatment for type II diabetes, pre-diabetes, and obesity which relate to the effects of salicylate as a metabolite of SSA on inhibiting IκB-kinase, consequent inhibition of the nuclear factor-κB cascade, reduction in inflammatory cytokines, insulin resistance, levels of glucose, triglycerides and free fatty acids (Salastekar et al., *Diabetes Obes Metab*, 2017; 19:1458-62). Some of the effects on glucose and fatty acid metabolism may relate to the well-established enzymic effects of salicylate on intermediary and lipid metabolism. Thus, in addition to controlling joint pain in arthritic diseases SSA reduces the weight-bearing problems in obese patients. There are also indications that SSA may have beneficial effects in cancer, including enhancing responses in prostate cancer radiotherapy, and in Alzheimer's and other dementias (Panza et al. Immunotherapy 2016:8:1119-34).

Novel transgenic rabbit models for drug-induced arrhythmia prediction

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Drug-induced proarrhythmia represents a potentially lethal adverse effect of various drugs. This proarrhythmic side effect is often linked to the drug's potential to modulate repolarizing cardiac ion channels causing a prolongation of the QT interval on the ECG. Despite sophisticated screening approaches during drug development, reliable prediction of proarrhythmia remains an unmet need. Drug-induced proarrhythmic events are often favored by pathological conditions that impair the patient's repolarization reserve, however, most cellular, tissue, and whole animal model systems used for current preclinical drug safety screening are based on normal, healthy tissues and animals. Several transgenic rabbit models for different types of long QT syndromes (LQTS) with differences in the extent of impairment in repolarization reserve have been generated recently. The potential use of these models for screening/prediction of drug induced arrhythmia is discussed in this talk. Also, summarize the electrophysiological characteristics of the available transgenic LQTS rabbit models are summarized along with proof-of-principle studies these models – identifying advantages and disadvantages of rabbit LQTS models.
Supported by Ministry of Human Capacities Hungary (EFOP-3.6.2-16-2017-00006).

Is inhibition of matrix metalloproteinase-2 cardioprotective against acute myocardial infarction in normo- and also in hypercholesterolemia?

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Background: In our previous study, novel thiazole and imidazole carboxylic acid type matrix metalloproteinase-2 (MMP-2) inhibitors were developed and the most potent compounds were selected for cardioprotection after testing in vitro and in vivo. The **Aim** of our present study was to achieve cardioprotection by using two previously efficacious inhibitors in an in vivo rat model of acute myocardial infarction (AMI) combined with hypercholesterolemia.
Methods: A group of male Wistar rats were fed with cholesterol-enriched diet (2% cholesterol, 0.25% cholic acid) and age-matched controls with normal rat chow for 12 weeks. At the end of feeding, animals were subjected to 30 minutes coronary occlusion followed by 120 minutes of reperfusion. Ischemic preconditioning (IPC) induced by 3 cycles of 3 min ischemia/5 min reperfusion, was used as positive, vehicle of the inhibitors as negative controls. MMPI-1154 at 1 µmol/kg and MMPI-1260 at 3 µmol/kg were administered intravenously at the 25th minutes of ischemia as a single bolus. Infarct size was determined by Evans blue and TTC staining, while microvascular obstruction (MVO) by thioflavine-S staining.
Results: The development of hypercholesterolemia was confirmed by increased serum total cholesterol levels (6.6±0.3 vs 0.5±0.04 mmol/L). In the presence of hypercholesterolemia, IPC (from 45.6±4.8% to 36.8±6.6%) and both inhibitors (44.8±6.1% and 44.0±2.4%) failed to reduce infarct size, whereas a significant decrease was found in the normocholesterolemic rats in all the 3 treated groups as compared to vehicle (IPC: 26.2±6.2 %, MMPI-1154: 40.6±3.4 %, MMPI-1260: 36.8±4.6 %, respectively vs. vehicle: 55.6±3.4 %). Both in the normo- and hypercholesterolemic groups, IPC significantly reduced, the MMP inhibitors did not change MVO as compared to the vehicle.
Conclusion: MMPI-1154 and MMPI-1260 reduced infarct size in normocholesterolemic rat AMI model. Although, hypercholesterolemia abolished their infarct size-limiting effect, the lack of cardioprotective potential of our novel MMP inhibitors cannot completely be excluded.
Grant support: NKFIH, TÉT_15_IN-1-2016-0068; GINOP-2.3.2-15-2016-00040.

Identification of protectomiRs in a porcine model of acute myocardial infarction

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Introduction: Changes in microRNAs expression profile contributes to cardioprotective signaling. We have previously shown a workflow to identify cardioprotective miRNAs (protectomiRs) with a comparison of miRNA expressions induced by myocardial infarction with or without ischemic conditioning (Varga ZV et al, JMCC, 2014). Therapeutic use of protectomiRs could be important in the future therapeutic interventions.

Aim: We aimed to identify protectomiR microRNAs in the pig heart.

Methods: Here we used cardiac tissues samples generated in our previous extensive study in closed chest domestic pigs (Baranyai et al, J Transl Med. 2017). Pigs were subjected to sham operation (Sham), ischemia/reperfusion (Isch) or preconditioning (IPreC), postconditioning (IPostC) and remote perconditioning (RIPerC) by inflations and deflations of a balloon catheter. In the RIPerC group, ischemic conditioning of the hind limb was performed during LAD occlusion. Tissue samples were collected after 3 hours of reperfusion from the infarcted regions of the left ventricles. MicroRNA profile was detected with high-throughput qPCR. Based on the "ProtectomiR" concept, those microRNAs were selected, which were significantly up- or downregulated in Isch vs. Sham and were counter-regulated by ischemic conditioning vs. Isch. To validate the functional role of selected microRNAs in cardioprotection, isolated cardiomyocytes were transfected with specific microRNA mimic or antagomiR and cell viability was measured after simulated ischemia/reperfusion injury.

Results: Expression of 224 microRNA was assessed. Expression of 19 microRNAs were increased and 18 were decreased in Isch vs. Sham (min. $1\times\log_2$ expression change, $-\log_{10}p>1$ vs. Sham). Expression of 43 microRNAs were changed in the IPreC, 34 in the IPostC and 39 in the RIPerC group vs. Isch (min. $1{,}5\times\log_2$ fold-change, $-\log_{10}p>1{,}31$ vs. Isch). Expression of 8 microRNAs changed significantly due to all three conditionings vs. Isch (3 microRNA was downregulated and 5 upregulated). 7 of the selected microRNAs were modulated (3 with microRNA mimics and 4 with antagomiRs) in isolated cardiomyocytes. Modulation of 2 of these microRNAs (both with mimic antagomiR) improved cell viability after ischemia/reperfusion injury (due to ongoing patenting, we do not disclose these potent protectomiRs).

Discussion: Here we identified 2 microRNAs in a clinically relevant pig model of myocardial infarction which modulation with microRNA mimics can serve as potential therapeutic approach in ischemic heart diseases.

Improving the quality and the time complexity of network visualization by hierarchical relative entropy optimization

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Background: We previously demonstrated, that so-called 'hairball' visualizations of complex biological networks achievable by currently available network layout softwares can be improved by utilizing the EntOptLayout plugin for the Cytoscape network analysis framework. Although the underlying relative entropy optimization algorithm and raising the adjacency matrix to the second power enable the visual discrimination of functional modules, the software is still limited by suboptimal running times and inadequate placing of the large-scale components of networks.
Aim: We aimed to improve the macroscopic layout and to reduce the running time of the relative entropy optimization algorithm by implementing a hierarchical version of the original methodology.
Methods: Multiple clustering algorithms were tested for the iterative merging of nodes and edges to generate a reduced network hierarchy with an increasing level of detail. The relative entropy optimization algorithm implemented in C++ programming language, was applied on each level of the hierarchy until convergence. The hierarchical algorithm utilizing different clustering methods and the original EntOptLayout software were compared based on the running times and the quality of the resulting layouts expressed in terms of normalized information loss values.
Results: Complete-linkage clustering was selected as the best-performing clustering algorithm. By applying the hierarchical version of the algorithm significant reduction of the normalized information loss values ($p < 0.001$ for each tested network) were achieved accompanied by up to 4 times reduction of the running times ($p < 0.001$ in most of the cases) when compared to the original algorithm.
Conclusion: Our hierarchical approach as a faster alternative to the original algorithm could improve the quality of network layouts therefore facilitating the visual identification of key modules in biological networks.
Acknowledgements: This study was supported by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017 National Heart Program).

Running as a stressor: the effects on the gastrointestinal tract

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Background: Physical activity is a natural stressor activating the hypothalamic-pituitary-adrenocortical (HPA) axis. Previously we have shown that preconditioning stress protects the gastric mucosa against ulcerogenic action through glucocorticoid involvement.
Aim: The aim of this work was to study the preconditioning effects of forced treadmill and voluntary wheel running on the HPA axis activity and a vulnerability of the gastric mucosa to ulcerogenic stimuli in rats.
Methods: Treadmill running was used as forced exercise paradigm. Two protocols of treadmill running were used: moderate (9 m/min for 15 min) and intensive (15 m/min for 30 min). Moderate treadmill running was applied in single as well as repeated for 5 days (15 min/day) mode. Intensive treadmill running was used only once. Wheel running was used as voluntary exercise paradigm. Rats were given access to running wheels once (2 h/ day) or repeatedly (for 5 days) modes. Three ulcerogenic stimuli were used: 1) gastric ischemia/reperfusion (I/R, 30 min occlusion of celiac artery followed by 3 h of reperfusion); 2) indomethacin (IM, 35 mg/kg, sc); 3) cold-restraint stressor (10°C).
Results: Both forced and voluntary running by itself resulted in an elevation of corticosterone levels suggesting the HPA axis activation. A single intensive forced treadmill running as well as voluntary wheel running in 5 days exerted gastroprotective effect against IM-induced injury but proulcerogenic action on cold-restraint-induced injury. At the same time moderate treadmill running in 5 days before ulcerogenic stimulus attenuated both IM- or stress-induced gastric erosions. Both a single and regular voluntary wheel running attenuated I/R-induced gastric erosions.
Conclusion: Running might have both beneficial and harmful effects on the vulnerability of gastric mucosa to ulcerogenic stimuli depending on the nature of ulcerogenic stimulus as well as the intensity of running and its duration.
Acknowledgements: The study was supported by grants from Russian Science Foundation №19-15-00430.

Brain-gut interactions: implications to understand and treat postoperative ileus (POI)

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Background: Abdominal surgery (AS) induced POI characterized by the suppression of propulsion of gut content including gastric emptying (GE). Experimental models showed that the underpinning mechanisms encompass a first neurogenic phase with the recruitment of capsaicin sensitive afferents activating hypothalamic corticotropin-releasing factor (CRF) signaling pathways suppressing the vagal cholinergic outflow to gastric myenteric neurons. The second inflammatory phase starts few hours later with an influx of leucocytes into the intestinal muscularis externa. Electrical vagal stimulation induces anti-inflammatory action through activation of cholinergic α7 nicotinic receptors.
Aims: To assess whether 1) AS induce a gastric inflammatory response and 2) central vagal activation by TRH curtails this response and the delayed GE.
Methods: The stable agonist, TRH agonist, RX77368 (50 ng/rat) was injected into the cisterna magna "intracisternally (ic)" 1 h prior AS and gastric inflammation and GE was monitored 6 h later in conscious rats.
Results: AS 1) increases the number of M1-like macrophage immunoreactive for MHCII (M1 marker) but not for CD206 (M2 marker) (MHCII+/CD206-) in the whole mount preparation of gastric myenteric plexus; 2) upregulates mRNA expression of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in the gastric submucosa plus muscle layers and 3) induces the occurrence of neutrophils infiltration labeled by myeloperoxidase in the muscularis externa. RX77368 injected ic inhibited AS-related delayed GE and gastric inflammatory changes while not altering these parameters in sham group. There was a significant negative correlation between GE and IL-1β (r=-0.46), TNF-α (r=-0.44), M1 macrophage (r=-0.82) and neutrophils (r=-0.91). Similar results were obtained with the gut-brain peptide, ghrelin using a long acting, orally active, brain penetrant agonist, HM01 that activates the dorsal motor nucleus vagal neurons. **Conclusion:** Brain-gut compounds stimulating vagal activity are able to abrogate AS-induced gastric M1 macrophages, proinflammatory cytokines expression, opening a new venue to alleviate.

Somatostatin receptor subtype 4 alleviates indomethacin-induced gastrointestinal mucosal injury in mice

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Gastrointestinal ulceration is still associated with high mortality despite the recent advancements in acid secretion inhibiting agents and endoscopic management. Therefore, understanding mucosal protective mechanisms is of high importance. Somatostatin (SOM) released from endocrine cells, sensory nerves, and its analogues reduce mucosal blood flow, pepsin and gastric acid secretion. SOM also mediates anti-inflammatory and analgesic actions via its receptor subtype 4 (sst_4), as discovered by our group. Therefore, we investigated the role of sst_4 in the indomethacin (IM)-induced gastrointestinal injury mouse model.

Sst_4 gene-deleted ($sst_{4-/-}$) and wildtype mice were treated s.c. with 35 mg/kg IM or vehicle. Gastric and small intestinal lesions were evaluated after 4 and 48 h macroscopically as well as microscopically. Plasma protein extravasation (at 30 and 60 min) and myeloperoxidase (MPO) activity (at 4, 24 and 48 h) were assessed by *ex vivo* imaging, TNFα, IL-1, COX2, sst_4 and somatostatin mRNA levels by qPCR. RNAscope was performed to determine the localization of both sst4 and SOM.

Gastric (4h) and small intestinal macroscopic (24 h) lesions, MPO activity (24, 48 h), and plasma extravasation (30 min) were significantly greater in IDM-treated $sst_{4-/-}$ mice, while there were no strain differences in histopathological scores and small intestinal length. TNFα, IL-1, COX2 significantly upregulated after 4h in the stomach of $sst_{4-/-}$ mice, and the same pattern was observable in wildtype mice at later timepoint (48 h). SOM is abundantly expressed in the glandular epithelial cells, while sst_4 was observed mainly in the myenteric plexus of gastric and small intestinal mucosa.

These results provide evidence for the protective role of the sst_4 receptor against IDM-induced gastrointestinal mucosal injury. This suggests the safety of sst_4 agonist drug candidates in the gastrointestinal tract, and even their potential use for gastroprotective indication.

Supported by: GINOP-2.3.2-15-2016-00050 – PEPSYS, GINOP-2.3.2 STAY ALIVE, EFOP-3.6.2-16-2017-00008

The Role of Associted Adherent-Invasive Eschericchia Coli in Crohn's disease

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Background and Aim: There is increasing evidence that Escherichia Coli (*E. coli*) organisms are important in Crohn's disease (CD) pathogenesis. In CD tissue they are found within macrophages, and the adherent-invasive CD ileal *E. coli* isolate LF82 can replicate inside macrophage phagolysosomes. The sequential etiological steps of the disease induced by adherent-invasive E. coli (AIEC) are: (1) abnormal colonization *via* binding to the CEACAM6 receptor, which is overexpressed in the ilel-colonic mucosa of CD patients; (2) ability to adhere to and to invade intestinal epithelial cells, which allows bacteria to cross the mucosal barrier; (3) survival and replication within infected macrophage in the lamina propria; and (4) induction of tumor necrosis factor secretion and grauloma formation. We investigated the pathogenic role of two AIEC strains, LF82 and O83:H1, in CD patients.
Methods: Organ cultures of colonic biopsies from CD patients were set up to assess the effects of LF82 and O83:H1 on the expression of CEACAM6, LAMP1, HLA-DR, ICAM1 by immunohistochemistry and of IL-8, IFN-γ, and TNF-α genes by RT-PCR.
Results: Epithelial and lamina propria mononuclear cells (LPMNC) expression of CEACAM6 and LAMP1 were higher in biopsies cultured in the presence of both O83:H1 and LF82 than in biopsies cultured with non-pathogenic E. coli. Both AIEC strains induced increased expression of ICAM-1 on blood vessels and HLA-DR on LPMNC. We observed higher levels of TNF-α, IFN-γ, and IL-8 transcripts in biopsies cultured with both AIEC strains than in those cultured with NP.
Conclusions: Our data suggest that LF82 and 083:H1 strains of *E. coli* are able to increase in CD colonic biopsies the expression of all the pro-inflammatory cytokines and all the mucosal immune markers investigated. Clinical trials are indicated to asses the efficacy of a combination antibiotic therapy targeting intramacrophage *E. coli.*

The Role of Mechanotransduction in the Development of Ileus

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Background: Ileus often develops in trauma patients due to hemostatic changes, fluid administration, and/or injury-related inflammation. Both intestinal edema and the development of ileus cause mechanical stretching of smooth muscle in the small intestine. While the physiological effects of mechanical strain in the gut are well-known, the effects of pathological mechanical changes are poorly understood and underestimated.
Aim: We aimed to determine the effects of stretch on smooth muscle cell function.
Methods: A primary human intestinal smooth muscle cell model and an ileus mouse model were used.
Results: Increased cyclical stretch induced decreased myosin light chain phosphorylation and, consequently, decreased smooth muscle contractility. P21-activated kinase (PAK1) increased in stretched primary human intestinal smooth muscle cells (hISMCs) and in smooth muscle tissue from an ileus model compared with unstretched cells/control tissue. The development of ileus was attenuated in PAK1 knockout mice or by PAK1 inhibition in hISMC. Mast cell and neutrophil activation and the development of intestinal edema occurred in both wildtype and PAK1 knockout mice, indicating that the deleterious effects of PAK1 on intestinal contractility were not mediated by increased inflammation. CXCL1 was induced in the intestinal smooth muscle of wildtype but not PAK1 knockout mice after the induction of ileus. CXCL1 was also induced in hISMC in response to increased cyclical stretch. CXCL1 inhibited agonist-induced contractile activity in a CXCR2-dependent manner. Pretreatment of hISMC with a PAK1 inhibitor before stretching did not prevent the upregulation of CXCL1.
Conclusion: We conclude that stretch induces increased PAK1 activity in intestinal smooth muscle leading to the induction of CXCL1, which inhibits intestinal contractile activity. However, PAK1 does not mediate increased CXCL1 in hISMC. In future experiments, we will examine the effects of PAK1 on CXCL1 expression in enteric neuronal cells and macrophages.
Funding: National Research, Development and Innovation Office (K120669)

Vascular endothelial injury: Common element in the cellular & molecular pathogenesis of GI ulceration & COVID-19

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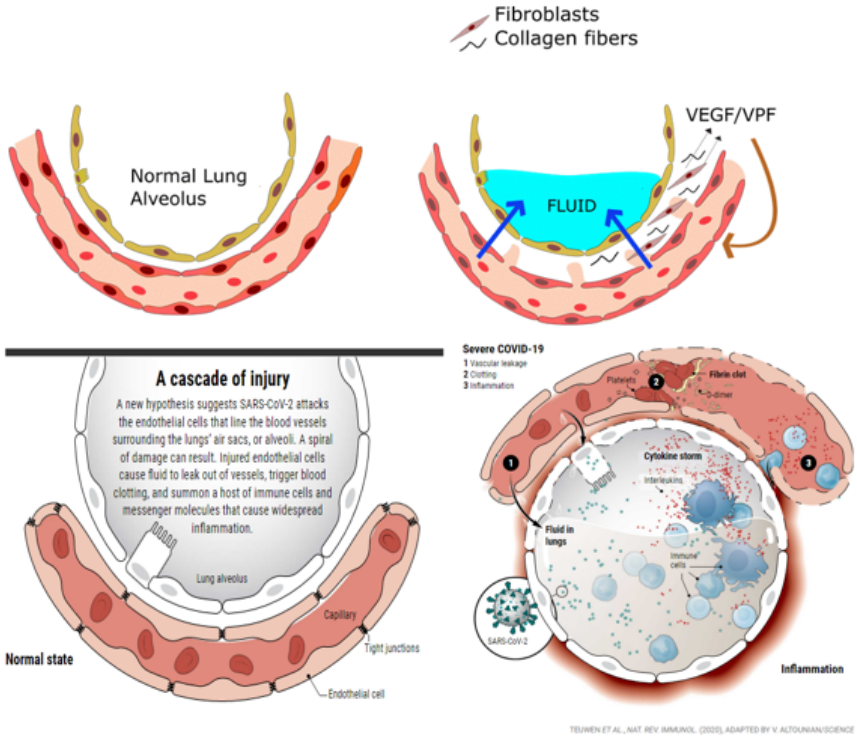
The initial tissue injury induced by chemicals & microbes (e.g., bacteria, viruses) is often investigated only in epithelial cells, since no functional & morphologic methods were available to look for damage in vascular endothelial cells. In the rapidly progressing investigations during the initial stages of "gastric cryoprotection" research in the 1980s, our laboratory was the first to use Evan's blue extravasation & monastral blue labeling of damaged endothelial cell in gastric subepithelial capillaries, arterioles & venules. We demonstrated increased vascular permeability & morphologic damage in vascular endothelial cells occurring before the appearance of gastric hemorrhagic erosions that could be prevented by pretreatment with small doses of prostaglandins or antioxidant sulfhydryls (Szabo et al., *Science*, 1981; *Gastroenterology*, 1985; Pihan et al., *Gastroenterology*, 1986; Trier et al., *Gastroenterology*. 1987).



Based on these initial & our subsequent studies on the role of VEGF/VPF (Vascular Endothelial Growth Factor, which was first called Vascular Permeability Factor, since it increases vascular permeability) in the pathogenesis & healing of gastroduodenal ulcers & ulcerative colitis (UC), we predicted (figures on left) that VEGF/VPF may play a role in the development of pulmonary edema in severe COVID-19 patients. Indeed, subsequent, independent clinical studies found elevated levels of VEGF/VPF in blood of these patients & administration of neutralizing anti-

VEGF improved clinical conditions. New results demonstrated that endothelial cells also have ACE-2 receptors that bind the spike proteins of SARS-CoV-2 that initiate the cascade of 'cytokine storm' which causes extensive damage to vascular endothelium not only in the lungs, but also in brain & myocardium, often leading to microinfarcts & stroke (figures on right, modified from *Science*, 2010).

Conclusion: Our initial experiments in animal models of GI ulceration & new clinical studies demonstrate a critical role of early vascular injury not only in the pathogenesis of gastroduodenal ulcers & UC, but also in the development of multiorgan lesions in COVID-19.

Functional Polarization and Bicarbonate Transport of HAT-7 Ameloblasts

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Background: During enamel maturation, ameloblasts have crucial role in mineral transport and bicarbonate secretion to neutralize excess protons liberated during hydroxyapatite formation.
Aim: We aimed to develop and optimize a novel in vitro model using the HAT-7 rat ameloblast cells to functionally study epithelial bicarbonate transport during amelogenesis.
Methods: To obtain monolayers, HAT-7 cells were cultured in various culture media for 1-7 days on Transwell membranes. The expression of ion transporters and tight junction proteins was investigated by quantitative RT-PCR. We monitored transepithelial resistance as an indicator of tight junction formation and polarization. We also evaluated intracellular pH changes by microfluorometry using BCECF fluorochrome. The activities of specific bicarbonate and chloride transporters, anion exchangers were tested using selective inhibitors such as H_2DIDS, DIDS and bumetanide.
Results: HAT-7 cells formed polarized epithelia on permeable supports in all tested culture media tested, but the DMEM-F12 medium containing 2.1 mM calcium and 10-8 M dexamethasone yielded the best reproducible results. HAT-7 cells expressed the RNA of claudin-1, -4 and -8 tight junction proteins and the ion transporters previously found in ameloblasts. We detected the basolateral activity sodium-proton exchange NHE transporter, using amiloride. Basolateral but not apical anion exchanger and NKCC activities were also demonstrated applying DIDS and bumetanide. High apical membrane CO_2 permeability and substantial basolateral bicarbonate uptake (which was sensitive to Na^+ withdrawal) were inhibited by the carbonic anhydrase inhibitor acetazolamide and by H_2DIDS inhibition. Measurements of transepithelial bicarbonate transport showed a marked increase in response to ATP and forskolin.
Conclusion: We succeeded to optimize the culture and functional test conditions to evaluate formation of polarized monolayers transepithelial resistance and the high the activity of important ion transporters affecting the pH regulation and vectorial bicarbonate transport of HAT-7 cells.
Supported by NKFIH K-125161 and by NIH NIDCR 1R01DE027971.

The colitis-induced supraspinal neuronal alterations contributing to intestinal hyperalgesia

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Background: Gut inflammation triggers intestinal hypersensitivity and hyperalgesia, which can persist after the pathology resolves thereby promoting recurrent abdominal pain. Current evidence suggest that this condition can develop due to the peripheral injury-induced neuroplastic changes along the gut–brain axis that may affect central processing of gastrointestinal sensory signals. However, the precise neuronal alterations responsible for enhanced gut sensation, especially occurring at the supraspinal level, remain unclear, hampering the development of efficacious visceral analgesic strategies.
Aim: In this work we evaluated the colitis-induced changes in neuronal properties of viscerosensory and pain-related brain structures in order to identify those which can underlie the development of intestinal hyperalgesia.
Methods: We used c-fos immunohistochemistry and extracellular microelectrode recording in anesthetized male Wistar rats for assessing background and noxious colorectal distension (CRD)-evoked neuronal activity within studied brain regions in norm and under intracolonic Trinitrobenzenesulfonic acid-induced colitis.
Results: Intestinal inflammation (6-8 days after the induction) caused a rise in both basal and CRD-evoked c-fos labeling in the caudal ventrolateral medulla, nucleus of the solitary tract, and lateral parabrachial nuclei. The nociceptive c-fos activation within the midbrain periaqueductal gray and midline thalamus was reduced, whereas neurons of the dorsal raphe, paraventricular hypothalamic and central amygdalar nuclei were indifferent to noxious CRD under colitis. The microelectrode recording in inflamed rats revealed an augmentation of CRD-induced neuronal firing within medullary viscerosensory areas and reduced neuronal reactivity to CRD in the periaqueductal gray. Colitis was also associated with enhanced excitatory and decreased inhibitory effects of the periaqueductal gray electrostimulation on the medullary visceral nociceptive neurons.
Conclusion: The colitis-induced neuronal sensitization of the supraspinal viscerosensory pathways and associated impairment in functioning of brain areas involved in endogenous antinociception can be important factors contributing to intestinal hyperalgesia.
Acknowledgements: This work was supported by the Russian Foundation for Basic Research (grant 18-015-00055).

Combined CFTR modulators reduce human epididymis protein 4 (HE4) expression in cystic fibrosis airway epithelial cells

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Background: CFTR plays a role in epithelial differentiation/function. In cystic fibrosis (CF), CFTR dysfunction results in impaired ion transport and thick mucus secretion in the lung that leads to chronic inflammation, while expression of several epithelial proteins is altered. We described elevated serum human epididymis protein 4 (HE4) in CF correlating with lung disorder severity, and HE4 level was higher in cystic fibrosis bronchial epithelial (CFBE) cells expressing *p.Phe508del-CFTR* compared to cells with wt-CFTR.
Aim: We here investigated whether HE4 expression could be affected via pharmacological modulation of CFTR function using CFTR specific drugs in CFBE cells *in vitro*.
Methods: HE4 protein levels were measured in the supernatants of CFBE 41o- cells expressing F508del-CFTR or wild-type CFTR (wt-CFTR) after administration of *lumacaftor/ivacaftor* or *tezacaftor/ivacaftor*, while HE4 expression in CFBE 41o- cells were also analyzed following application of adenylate cyclase activators Forskolin/IBMX or $CFTR_{inh172}$. The effect of all of these compounds on CFTR function was monitored by the whole-cell patch-clamp technique. Induced HE4 expression was studied with interleukin-6 (IL-6) in F508del-CFTR CFBE 41o- cells under TNF-α stimulation for 1 hour up to 1 week in duration. In parallel, plasma HE4 was determined in CF subjects homozygous for *p.Phe508del-CFTR* mutation receiving *lumacaftor/ivacaftor* (Orkambi®) therapy. NF-κB-mediated signaling was observed via the nuclear translocation of p65 subunit by fluorescence microscopy together with the analysis of IL-6 expression by an immunoassay. In addition, HE4 expression was examined after NF-κB pathway inhibitor BAY 11-7082 treatment with or without CFTR modulators.
Results: CFTR modulators partially restored the activity of F508del-CFTR and reduced HE4 concentration was found in F508del-CFTR CFBE 41o- cells that was close to what we observed in CFBE 41o- cells with wt-CFTR. These data were in agreement with decreased plasma HE4 concentrations in CF patients treated with Orkambi®. Furthermore, CFTR inhibitor induced elevated HE4 levels, while CFTR activator Forskolin/IBMX downregulated HE4 in the cell cultures and these effects were more pronounced in the presence of CFTR modulators. Higher activation level of baseline and TNF-α stimulated NF-κB pathway was detected in F508del-CFTR vs. wt-CFTR CFBE 41o- cells that was substantially reduced by CFTR modulators based

on lower p65 nuclear positivity and IL-6 levels. Finally, HE4 expression was upregulated by TNF-α with elevated IL-6, and both protein levels were suppressed by combined administration of NF-κB pathway inhibitor and CFTR modulators in CFBE 41o- cells.
Conclusion: CFTR dysfunction contributes to abnormal HE4 expression via NF-κB in CF.

Investigation of esophageal ion transport mechanism using mice esophageal 3D organoid cultures

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Introduction: Esophageal epithelial cells (EECs) protect the lower layers during esophageal reflux. One of the major components of the epithelial defensive mechanisms is the ion transport processes, however their role under pyhsiological and pathophysiological conditions is not completely clear.
Aims: Therefore, our aim was to generate esophageal organoid cultures (OeOs) from epithelial tissue of two different mice strains and to investigate the presence of ion transporters on them.
Methods: EECs were isolated from 8-20 weeks old male mice (C75BL/6; CD-1). Cells were then suspended in Matrigel® and cultured for 8-12 days. Changes in intracellular pH (pH_i) and Cl^-level was measured using microfluorometry and the pH-sensitive fluorescence dye, BCECF-AM, or MQAE. For determining the resting pH_i, the high K^+/nigericin technique was used whereas buffering capacity was measured by the ammonium prepulse technique. For immunostaining, for OeOs were fixed in 4% PFA and dehydrated in 30% saccharose for 3 days before embedding in Cryomatrix™, whereas PCR was used to study mRNA expression of the transporters.
Results: In each mice, organoids had a 3D spheroidal structure. The average size of OeOs were between 50-250 micrometer. We have detected Lgr5 expressions in all cells of EOCs; whereas cytokeratin-14 staining showed granular arrangement. The resting pH_i of C57BL/6 mice was 7.61±0.03, whereas in the case of CD1 mice it was 7.58 ±0.03. Microfluorometric measurement showed the presence of functionally active NHE1, NHE2, AE, NBC and CFTR. In addition, mRNA expression of the major epithelial ion transporters have been shown by PCR and the presence of these channels was also confirmed by immunostaining.
Conclusion: We have successfully set up the culturing of mice OeOs and our results showed that OeOs express both alkalizing and acidifying transporters. We strongly belive that OeOs are a relevant and suitable, experimental model to study esophageal epithelial function *in vitro*.
This study was supported by the National Research, Development and Innovation Office (FK123982) and the Economic Development and Innovation Operative Programme Grants (GINOP-2.3.2-15-2016-00015) and by the National Research, Development and Innovation Office, by the Ministry of Human Capacities (EFOP 3.6.2-16-2017-00006).

Saliva Specimens for detecting SARS-CoV-2: A Meta-Analysis

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Background: COVID-19 caused by the SARS-CoV-2 virus is a severe and possibly lethal infection. Early and quick diagnosis of the disease may play a crucial role in preventing further escalation of the outbreak. At the moment, the nasopharyngeal swab specimens are the gold standard for sampling. However, the latest literature suggested that saliva sampling could be a promising alternative to accelerate and simplify the testing of SARS-CoV-2 infected individuals.
Aim: To systematically assess and analyze the available literature on the reliability and consistency of SARS-CoV-2 viral RNA detection in saliva samples.
Methods: We have conducted our meta-analysis following the Cochrane Handbook. Six major databases (Cochrane Library, Embase, Pubmed, Scopus, Web of Science and clinical trial registries) were searched between 1 January and 25 April 2020 for eligible records. As raw data, we collected and extracted the number of positive tests and the total number of tests conducted. We assessed the ratio of positive test in the pooled data by score confidence-interval estimation using the Freeman-Tukey transformation. The I^2 measure and the χ^2 test was used to evaluate heterogeneity.
Results: Our systematic literature search discovered 96 records without duplicates. We included 26 publications for qualitative analysis and 5 records for quantitative synthesis. Our analysis revealed that the sensitivity for saliva tests is 91% (CI 80-99%), while sensitivity for nasopharyngeal swab (NPS) tests is 98% (CI 89-100%) in previously confirmed COVID-19 patients. We found moderate heterogeneity among studies. Furthermore, 18 registered, ongoing clinical trials were identified, which investigate the applicability of saliva-based testing for virus detection.
Conclusion: Saliva-based specimens provide a promising alternative to NPS test for diagnosing COVID-19. However, further diagnostic accuracy studies are needed to enhance their sensitivity and specificity.
Acknowledgements: This work was supported by the Hungarian Human Resources Development Operational Program (EFOP-3.6.2-16-2017-00006).

Gut region-specific expression of tumor necrosis factor alpha and its receptor in the myenteric neurons of streptozotocin-induced diabetic rats

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Background: Our previous studies reveal that the myenteric neurons which located in different gut segments display different susceptibilities to diabetic injuries. Moreover, the capillaries in the gut wall, the intestinal microbiota, the endogenous antioxidant defence and also the oxidative markers exhibit gut segment-dependent diabetic insults. These evidence indicate the crucial importance of the neuronal microenvironment in the pathogenesis of intestinal region-specific enteric neuropathy. The tumor necrosis factor alpha (TNFα), as a pro-inflammatory cytokine, is involved in diabetes-related neuronal damage. However, it may be capable of exerting neuroprotective or neurodegenerative effects via different TNF receptors (TNFR1 or TNFR2) and signalling pathways.
Aim: Therefore, we aim to elucidate the effects of type 1 diabetes and immediate insulin treatment on the expression of TNFα and its receptor TNFR2 in the myenteric neurons in different intestinal segments.
Methods: Ten weeks after the onset of hyperglycemia, tissue samples from duodenum, ileum and colon of control, streptozotocin-induced diabetic and insulin-treated diabetic rats were processed for post-embedding electron microscopy and enzyme-linked immunosorbent assay (ELISA).
Results: In the diabetic rats, the number of TNFα labeling gold particles was significantly increased in the duodenal, decreased in the colonic myenteric neurons, while did not show any significant differences in the ileal ganglia. The number of TNFR2 labeling gold particles was decreased in the diabetic duodenum.
In the diabetic rats, the tissue level of TNFα was markedly increased, while the level of TNFR2 was decreased in the tissue homogenates of duodenum.
Conclusion: Based on these findings we presume that expressional alterations in the TNFα and TNFR2 system may contribute to region-specific diabetic enteric neuropathy. Further investigation of the TNFR1/TNFR2 distribution along the gastrointestinal tract may help to understand the underlying molecular mechanisms.

Pituitary Adenylate Cyclase-Activating Polypeptide alleviates intestinal, extra-intestinal and systemic inflammatory responses during acute *Campylobacter jejuni* induced enterocolitis in mice

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Background: Human *Campylobacter jejuni*-infections are progressively rising and constitute a significant health burden worldwide. The ubiquitously expressed pituitary adenylate cyclase-activating Polypeptide (PACAP) is well-known for its cell-protective and immunomodulatory effects.
Aim: In our actual clinical intervention study applying an acute campylobacteriosis model we assessed potential disease-alleviating effects of exogenous PACAP.
Methods: Therefore, secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* and treated with synthetic PACAP38 intraperitoneally from day 2 until day 5 post-infection.
Results: Whereas PACAP did not interfere with the gastrointestinal colonization properties of the pathogen, mice from the verum group displayed less severe clinical signs of *C. jejuni*-induced disease as compared to mock controls which was accompanied by alleviated apoptotic, but enhanced cell proliferative responses in colonic epithelia on day 6 post-infection. Furthermore, PACAP dampened the accumulation of macrophages and monocytes but enhanced regulatory T-cell responses in the colon which was paralleled by less IFN-γ secretion in intestinal compartments in PACAP versus mock treated mice. Remarkably, the inflammation-dampening properties of PACAP could also be observed in extra-intestinal and strikingly, even systemic tissue sites on day 6 post-infection.
Conclusion: For the first time we here provide evidence, that synthetic PACAP might be a promising candidate to combat acute campylobacteriosis and post-infectious sequelae.

Murine fecal microbiota transplantation alleviates intestinal and systemic immune responses in *Campylobacter jejuni* infected mice harboring a human gut microbiota

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Background: Human campylobacteriosis constitutes a zoonotic food-borne disease and a progressively rising health burden of significant socioeconomic impact. We have recently shown that conventional mice are protected from *Campylobacter jejuni* infection, which was not the case for human microbiota associated (hma) mice indicating that the host-specific gut microbiota composition primarily determines susceptibity towards or resistance against *C. jejuni* infection.
Aim: In our present preclinical intervention study we addressed whether gut microbiota changes in stably *C. jejuni* infected hma mice following murine fecal microbiota transplantation (mFMT) could alleviate pathogen-induced immune responses.
Methods: To accomplish this, secondary abiotic C57BL/6 mice were generated by broad-spectrum antibiotic treatment, perorally reassociated with a complex human gut microbiota and challenged with *C. jejuni* by gavage. Seven days later *C. jejuni* infected hma mice were subjected to peroral mFMT on three consecutive days.
Results: Within a week post mFMT fecal pathogenic burdens had decreased by two orders of magnitude, whereas distinct changes in the gut microbiota composition with elevated numbers of lactobacilli and bifidobacteria could be assessed. In addition, mFMT resulted in less *C. jejuni* induced apoptotic responses in colonic epithelia, reduced numbers of macrophages and monocytes as well as of T lymphocytes in the large intestinal mucosa and lamina propria and in less distinct intestinal pro-inflammatory cytokine secretion as compared to mock challenge. Strikingly, inflammation dampening effects of mFMT were not restricted to the intestinal tract but could also be observed systemically as indicated by elevated serum concentrations of pro-inflammatory cytokines such as TNF-α, IL-12p70 and IL-6 in *C. jejuni* infected hma mice of the mock, but not the mFMT cohort.
Conclusion: In conclusion, our preclinical mFMT intervention study provides evidence that changes in the gut microbiota composition which might be achieved by pre- or probiotic formulations may effectively lower intestinal *C. jejuni* loads, dampen both, pathogen-induced intestinal and systemic inflammatory sequelae and may represent a useful tool to treat continuous shedding of *C. jejuni* by asymptomatic carriers which is critical in the context of food production, hospitalization and immunosuppression.

Extracellular vesicles: lessons *in vitro*, *ex vivo* and *in vivo*

Edit Buzas

Semmelweis University, HCEMM-SU and ELKH-SE

During the past 25 years, we carried out several studies mostly focusing on the separation and characterizations of extracellular vesicles. In these studies, we used *in vitro* systems, primary cell line-derived extracellular vesicles. These *in vitro* studies led us to the recognition that LDL particles may associate with the surface of extracellular vesicles and that a sustained exposure to the antibiotic Ciprofloxacin results in a genotoxic stress and subsequent release of mitochondrial DNA associated with the surface of small extracellular vesicles. Both *in vitro* analysis of nascent extracellular vesicles incubated in extracellular vesicle-depleted blood plasma and *ex vivo* analysis blood plasma-derived extracellular vesicles resulted in the recognition that a protein corona is formed spontaneously around extracellular vesicles in blood plasma. *Ex vivo*, confocal microscopic analysis of immunostained formalin fixed, paraffin embedded sections of colorectal cancer patients revealed the presence of a novel type of extracellular vesicles: *en bloc* released MVB-like structures. Finally, *in vivo* analysis using a simple implantable device enabled us to follow the *in vivo* spread of the pro-inflammatory response within a local network of mast cells. Lessons from such *in vitro*, *ex vivo* and *in vivo* systems may help us to take one step further towards diagnostic and/or therapeutic application of extracellular vesicles.

Quantitative and qualitative characterization of extracellular vesicles by IR spectroscopy

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Background: Extracellular vesicles (EVs) are one of the most actual research areas in biology and nanomedicine with growing interest in clinical applications. EVs are lipid bilayer enclosed nanostructured bodies released by cells into the extracellular space and they participate in cell-to-cell communication by cargoing proteins, lipids, DNAs and RNAs. Despite intense academic investigation, however, there is still a need for EVs structural characterization.

Aim: Infrared spectroscopy (IR) is a label-free method that can detect changes of functional groups in molecules from biological samples. IR spectroscopy, completed with standardized measurement conditions and data processing procedures, was introduced only in the last few years to characterize EVs. Since the IR spectra provide information about proteins, lipids and other EV components simultaneously, a single assay protocol for EV screening might be feasible.

Results: By detailed spectral analysis we identified changes in protein structures and the relative lipid content for different EV subpopulations isolated form Jurkat T-cell line. We proposed a 'spectroscopic protein-to-lipid ratio' (P/L), which provides a useful index for EV characterisation. Using proper calibrations, IR spectroscopy allows a label-free quantification of EVs in terms of their total protein and/or lipid content. Selected IR markers such as spectroscopic P/L, α-helix/β-sheet ratio of protein secondary structure were used to monitor EVs formed and accumulated *in vitro* during the storage of red blood cells (RBCs). These IR markers alter in function of storage time and medium and might be indicative for biochemical and morphological changes occurring in RBC concentrates. Furthermore, additional information about biomolecular composition of EVs (ATP, lactose, oxidized haemoglobin, e.g.) was be obtained, providing a biochemical fingerprint.

Conclusion: IR spectroscopy is a rapid, label-free method for EV characterization, even to be used complementary to traditional omics approaches. However, to get direct information on size/morphology of EVs, combination with other techniques is required.

This study was funded by the NRDIO, Hungary under grants K131594 and K131657. ZV is supported by the János Bolyai Research Scholarship of the HAS.

PERITONEAL DIALYSIS EFFLUENT DERIVED EXTRACELLULAR VESICLES REDUCE RISK OF DEVELOPMENT OF PERITONEAL DIALYSIS ASSOCIATED PERITONEAL FIBROSIS

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Introduction: Extracellular vesicles (EVs) have significant therapeutic potential. They can protect against myocardial infarction, acute kidney injury or liver fibrosis, as well. However, the impact of EVs derived from dialysis effluents (PDE) of PD patients on the development of peritoneal fibrosis during PD remained unclear. Our aim was to determine, whether PDE-EVs can alter progression of peritoneal fibrosis.
Methods: PDEs were collected from children receiving continuous ambulatory peritoneal dialysis treatment in the Ist Department of Paediatrics, Semmelweis University. PDE derived EVs (EVs) were isolated by size exclusion chromatography following ultrafiltration and were characterized according to the recommendations of the International Society for Extracellular Vesicles. Their impact on the proliferation and collagen production of primary peritoneal fibroblasts (pPFs) was tested by MTT or Sirius Red assay, respectively. The effect of EVs was also studied in a chlorhexidine gluconate (CG) induced mice model of peritoneal fibrosis in vivo. Submesothelial thickness was analysed after Periodic Acid Schiff- and Masson's Trichrome staining.
Results: EVs showed spherical morphology in the 100 nm size range. The IR spectrum of EVs after buffer subtraction presented typical spectral features of EVs. Flow cytometry certified their CD9, CD63 and annexin positivity and lack of calnexin. PDE derived EVs entered into the cytoplasm of pPFs and significantly reduced PDE induced proliferation and collagen production of them. Finally, EVs penetrated into the peritoneal membrane of C57/BL6 mice after their *i.p.* injection, reduced the peritoneal thickness of CG treated mice almost to the control level.
Conclusion: PDE derived EVs have strong effect on PDE induced activation of pPFs *in vitro* and peritoneal thickness *in vivo*.
Support: This Project was supported by the FIKP 61830-69271, STIA-KFI-2020 (61830-69319), OTKA 124549 and OTKA 125470 funds.

Investigation of vibration spectroscopy of extracellular vesicles in central nervous system tumors using vibration spectroscopy

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Recently, numerous studies highlighted the diagnostic or prognostic value of extracellular vesicles (EVs) as biomarkers. The EVs are the mirrors of the pathophysiological properties of the human body, because they concentrate the disease-specific molecules as the enhancers or signal-multipliers of lesions.
The field of liquid biopsy (LB) has emerged as a great revolution in oncology, because it offers great opportunity for detecting and monitoring cancer. In addition to its minimal invasiveness, LB can provide information about all lesions in the patient's body, in contrast to the histological biopsy from a part of the heterogenic tumor tissue.
We combine Raman spectroscopy with machine learning to investigate the serum EVs and thereby the complex spectral pattern of central nervous system tumors, including glioblastoma multiforme (GBM), brain metastasis (BM) and meningioma (M). However, Raman spectroscopy is an inadequate method for molecule identification, the spectra based on our results could be informative for the pathophysiological status of the patients. We aimed to study the ratios and relations of the whole molecular composition present in the billions of circulating EVs, avoiding the need for specific biomarkers.
We collected the Raman spectra of EV samples from 138 subjects. Each sample was measured in 5 repilicates using short integration time. After the measurement, the post-processing of the spectra was the same for all samples. Every spectrum was baseline corrected and normalized by a standard normal variate method. Despite the challenge, CTRL vs. GBM subtraction spectrum, and FreeViz plot reveals that there are chemical differences between the patient groups.
Compared the CTRL group to the malignancies, most of the discriminative spectral differences were characteristic for carbohydrates, nucleic acids, lipids and amino acids.
We have found that this method is suitable to diagnose central nervous system tumors in a fast and cost-effective way. Because our method is a blood-based test, the high-risk skull surgery for a histological result is not necessary. Raman spectroscopy-based analysis of vesicles is an innovative method in biomarker research, what we aim to extend to additional tumor types in further studies.

Cardioprotection mediated by calcium-ionophore induced extracellular vesicles

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Background: Remote ischemic conditioning is a cardioprotective intervention which protects the heart against ischemia/reperfusion injury. Transient activation of Toll-like receptor 4 (TLR4) and its downstream regulators (TNFα and IL-6) have been implicated in cardioprotective interventions. Extracellular vesicles (EVs) play a role in cardioprotection through the activation of the TLRs. **Aim:** Isolation of EVs in high amounts with suitable purity from blood is a challenge, our aim was to develop a cellular model system from which TLR-inducing, cardioprotective EVs can be isolated in a reproducible manner.
Methods: EV release from HEK293 cells wa s induced by calcium-ionophore A23187. EVs were characterized, cytoprotection by EVs against simulated ischemia/reperfusion injury and its mechanism were investigated in H9c2 and AC16 cell lines.
Results: A23187 induction of HEK293 cell induced EV release and the isolates contained mostly large EVs. EVs decreased cytotoxicity and apoptosis due to 16h ischemia followed by 2h reperfusion in H9c2 and AC16 cells in a dose-dependent manner. EVs activated TLR4 and its downstream signaling pathway in H9c2 and AC16 cells as well as the expression of cytoprotective heme oxigenase 1 (HO-1) in H9c2 cells.
Conclusion: A23187-induced EVs exert cytoprotection in H9c2 and AC16 cells by inducing TLR4 signaling and HO1 expression. Therefore, EVs released via calcium-ionophore treatment may serve as a basis of an efficient carpdioprotective therapy.

Serum exosomal miRNA pattern of an active lifestyle, its correlation with anthropometric parameters and role in chronic disease prevention

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Background: While a sedentary lifestyle may accelerate biological aging, regular exercise is an important factor of chronic disease prevention. Accumulating evidence suggests the importance of tissue crosstalk via extracellular vesicles (especially exosomes) and their cargo. Investigating exosomal miRNAs (exomiRs) in the context of short and long-term exercise may help to identify novel targets for therapeutic intervention of age-associated diseases.

Aims and Methods:

In the present study we examined serum exomiR expression in healthy young, sedentary participants (n = 14; age: 23 ± 2 years) at baseline and following a half year-long regular exercise training. We also analyzed serum exomiR expression in older, healthy trained participants (seniors, n = 11; age: 62 ± 6 years) who engaged in endurance activities for at least 25 years. Following the isolation and enrichment of serum exosomes, their exomiR levels were determined using the amplification-free Nanostring platform.

Results:

Surprisingly, the majority of the exomiRs overlapped between the short-term (0.5 year in this study) and the long-term (25 + years in this study) trained groups. The top 12 significantly altered and overlapped exomiRs were used for KEGG pathway analysis. According to the pathway analysis a large portion of the exomiRs target chronic diseases including cancer, neurodegenerative and metabolic diseases and viral infections.

Conclusion:

Taken together, these findings demonstrate that exomiRs released during exercise have a great potential to enhance our understanding of how exercise ameliorates impairments associated with physiological aging.

A computational method to select the right molecular target for each cancer patient.

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Background: The current concept of precision oncology is to find predictive molecular biomarkers to personalize treatment decisions. This has led to the one biomarker, one drug paradigm. These predictive diagnostic tests have been co-developed and co-registered with targeted therapies as diagnostic medical devices. As of today, there are only 18 validated biomarkers compared to the more than 700 driver genes listed by the census of cancer genes. Even in the presence of a registered predictive diagnostic test, many patients fail to respond to the corresponding therapy due to the parallel presence of other driver alterations, which can confer resistance to targeted therapies.
Aim: Our aim was to develop a computational system to identify the most relevant molecular target in each cancer patient based on the individual molecular profile of their tumor.
Methods: We have built an AI-based rule-based expert system, called "Digital Drug-Assignment, DDA" that uses more than 12,000 algorithms to calculate, which molecular target is linked to the higher number and more important driver genes. The system has been recently described and clinically validated on the data of the SHIVA01 clinical trial (Petak I et al. NPJ Precis Oncol. 2021 Jun 23;5(1):59.). We have used real-world data of patients enrolled into precision oncology programs assisted by DDA to assess the unmet need for novel targeted therapies
Results: In real-world evidence using the system predicted resistance in 21% cases to currently registered targeted therapies.
Conclusion: DDA can potentially improve treatment decisions with current molecularly targeted therapies and help to identify novel targets of clinical relevance for future drug discovery.
This work was supported by the Hungarian Innovation Agency (NVKP_16-1-2016-0005, 2019-1.1.1-PIACI-KFI-2019-00367).

Metabolic ecosystem and plasticity in the progression of cancer tissues

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Each individual has unique biochemical characteristics in spite of almost identical genetic background. Similarly, tumour cells also have unique metabolic features in tissue microenvironment. The evolution in tumour tissues is weakly described, however, it has been recently highlighted that deterministic selection acts rather on phenotype than genotype. Gene mutation-centric views are dominated molecular cancer research. Additionally, the experimental results and the failures in targeted therapies suggest that after the revolution of molecular biology and genomics we need to analyse the relationship between metabolic network and oncogenic alterations in tumour tissues to have further development in cancer treatment. Tumour growth can only occur when there is enough available energy, building blocks and nutrient supports; therefore, tumours as ecosystems could have metabolic flexibility and plasticity in their progression. Environmental and additional metabolic adaptation, symbiosis, plasticity and fitness are critical for maintaining the survival and growth at starving conditions or during treatments. Otto Warburg described the altered metabolism in tumour cells about 100 years ago; Hanahan and Weinberg highlighted metabolic reprogramming in hallmark of cancers 10 years ago. Since the last decade growing evidences have suggested the importance of developing tumour metabolism research topics. The descriptions of cellular metabolic differences and their distributions in cancer tissues have been started to study in many working groups. Regarding to these results, the developing experimental models and the in situ imaging technologies can help to: a. define novel targets and find new or already used drugs; b. rationalise preclinical tests, phase trials and the therapy and c. finally decrease the mortality and increase the survival of cancer patients.

Study supports – FK128404 and ED_17-1-2017-00009 National Bionics Program (National Research, Development and Innovation Fund Hungary)

RAS: targeting the untargetable

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RAS is the most frequently mutated human oncogene in various cancers but till now it was considered undrugable unlike most of the other mutated human oncogenes. RAS has three isoforms, K, N and H and in human cancer KRAS is the most frequently involved one. Due to the abundacy of RAS proteins in various signaling pathways, the challenge was to have mutant-specific drugs to prevent toxicities. Partly influenced by the „Cancer Moonshot Program" (NCI), there were significant developments in the field by discovering novel 3D structural characteristics of KRAS and by development of various mutant allele-specific approaches. In 2021 the first mutant KRAS inhibitor, Sotorasib, was approved by FDA for the treatment of G12C mutant lung adenocarcinoma. Unfortunately, other G12C mutant tumors such as colorectal- or pancreatic cancers were not sensitive enough to get approval. Accordingly, the follow-up drug, Adagrasib, try to focus on colorectal cancer, where a combination of G12C mutant allele specific irreversible inhibitor is combined with anti-EGFR antibody therapy with curraging efficacy. It seems inevitable to use these novel drugs in combination. There are several approaches, the most interesting is the the combination of G12C inhibitors with immunotherapy. On the other hand, the disturbed RAS signaling pathway offers several combinatorial approaches like RAF and MEK inhibitors. Interestingly, novel studies suggest that in case of NRAS mutant human cancers both MEK and RAF inhibitors may be clinically effective. Last but not least, in mutant HRAS it seems that the long forgotten farnesyltransferase inhibitors seem to be clinically effective in case of head and neck- or bladder cancer. Therefore the last frontier in oncology, the mutant RAS containing tumors, started to be conqueried.

Effects of tofacitinib therapy on arginine and methionine metabolites in association with vascular pathophysiology in rheumatoid arthritis: a metabolomic approach

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Background: Rheumatoid arthritis (RA) has been associated with increased cardiovascular (CV) risk and metabolic changes.
Objectives: We wished to determine how the Janus kinase (JAK) inhibitor tofacitinib influences vascular pathophysiology and metabolites of the arginine and methionine-homocysteine pathways.
Methods: Thirty RA patients with active disease were treated with either 5 mg bid or 10 mg bid tofacitinib and evaluated at baseline and after 6 and 12 months. We determined DAS28, CRP, IgM rheumatoid factor (RF) and anti-cyclic citrullinated peptide (aCCP) levels. We assessed brachial artery flow-mediated vasodilation (FMD), carotid intima-media thickness (IMT) and pulse-wave velocity (PWV) by ultrasound. We also determined plasma L-arginine, L-citrulline, L-ornithine, inducible nitric oxide synthase (iNOS), asymmetric (ADMA) and symmetric dimethylarginine (SDMA), L-N-monomethyl-arginine (L-NMMA), cysteine, homocysteine, and methionine levels.
Results: Twenty-six patients completed the study. Tofacitinib treatment maintained FMD and PWV. Ten mg bid tofacitinib significantly increased L-arginine, L-ornithine, iNOS and methionine levels after 12 months. Tofacitinib transiently increased L-citrulline and L-NMMA and decreased homocysteine levels after 12 months. Based on L-citrulline, L-ornithine, ADMA and SDMA levels, L-arginine remained highly available for endothelial NO production. Multivariate analysis indicated variable correlations of L-arginine, L-citrulline, ADMA, L-NMMA, homocysteine and methionine with DAS28, CRP, ESR and RF but not with aCCP. Regarding vascular pathophysiology, only PWV and methionine correlated with each other after 12 months.
Conclusion: Tofacitinib suppressed systemic inflammation in RA yielding stabilization of vascular function. It may exert CV protective effects in RA, at least in part, by shifting L-arginine metabolism to high arginine availability and decreasing homocysteine levels.
Acknowledgements: This research was supported by the European Union and the State of Hungary and co-financed by the European Social Fund in the framework of TAMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program '(Z.S.); by the European Union grant GINOP-2.3.2-15-2016-00015 (Z.S.) and by the WI188341 investigator-initiated research (IIR) grant obtained from Pfizer US (Z.S.).

Intracellular signaling upon withdrawal of trophic serum factors in a cell line model expressing constitutively active RhoA monomeric G-protein

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Background: Serum deprivation in culturing medium results in the omission of growth factors. Upon growth factor withdrawal, the cell cycle progression in G1 restriction point is blocked at first. Later these quiescent cells start apoptosis.
The antiproliferative and the consequent apoptotic effects caused by either growth factor or complete serum starvation have been studied for a long time in a widely known cell line model, namely PC12 rat phaeochromocytoma cells.
RhoA monomeric G protein is known to be more than an important cytoskeleton regulator. It is a cell cycle progression and survival promoting molecule, as well. The role of RhoA in the apoptotic intracellular signaling processes has not been completely clarified so far.
Aim: Our aim in this project is to study the involvement of RhoA protein in growth factor depletion-induced intracellular signaling processes in PC12 cell line.
Methods: We performed biochemical viability tests, DNA fragmentation analyses, nuclear morphology inspection, caspase activation studies, Western blotting, immunocytochemistry and qPCR analyses.
Results: Based on our present results achieved from analysis of apoptotic processes, cell cycle regulation and stress signaling, we may refine the role of p38 and JNK stress MAP kinases, AKT kinase, MDM2 ubiquitin ligase and p53 transcription factor.
Conclusion: We can conclude that serum withdrawal-induced apoptosis is not prevented, only delayed by activated RhoA in this experimental system. It is also feasible that both p53-dependent and p53-independent apoptotic pathways are involved in serum starvation-induced cell death. The examination of MDM2 protein needs further analyses, but it seems to be a key participant.
This project is supported by EFOP-3.6.1-16-2016-00004 and Kispál Gyula scholarship.

The role of the nucleus in mediating tissue damage detection

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Inflammatory signals have long been known to arise upon cell lysis and necrosis during tissue damage. Previously we have shown that epithelial damage detection is also mediated by direct surveillance of barrier integrity. Upon tissue damage in zebrafish larvae, osmotic cues from the environment cause cell swelling at the wound margin, which leads to rapid leukocyte recruitment through the activation of cytosolic phospholipase A2 (cPLA2). Arachidonic acid released by cPLA2 is oxidized to pro-inflammatory eicosanoids by 5-lipoxygenase (5-LOX) on the nuclear envelope. Osmotic cell swelling activates cPLA2 by translocating it from the nucleoplasm to the nuclear envelope. Elevated cytosolic Ca^{2+} is necessary but not sufficient for cPLA2 translocation, and we now show that nuclear swelling is required as a parallel input. cPLA2 translocation upon nuclear swelling was reconstituted in isolated nuclei, and appears to be a simple physical process mediated by tension in the nuclear envelope. Our data show that the cell nucleus serves an unexpected role as the primary mechanosensor for transduction of cell swelling and lysis into pro-inflammatory eicosanoid signaling. Cell swelling and lysis are widely implicated in inflammatory pathology. We propose that the nucleus plays a general mechanosensory role in inflammation, and this explains why cPLA2 and 5-LOX localize to nuclei in many tissues.

The role of plasma membrane Ca^{2+} channels in the development of epithelial cell injury in exocrine pancreatic pathophysiology

József Maléth

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Regardless of its etiology, sustained intracellular Ca^{2+} overload is a well-known hallmark of acute pancreatitis (AP). Toxic Ca^{2+} elevation induces pancreatic ductal cell damage characterized by impaired ductal ion- and fluid secretion –essential to wash out the protein-rich fluid secreted by acinar cells while maintaining the alkaline intra-ductal pH under physiological conditions– and mitochondrial dysfunction. While prevention of ductal cell injury decreases the severity of AP, no specific drug target has yet been identified in the ductal cells. Although Orai1 –a store operated Ca^{2+} influx channel– is known to contribute to sustained Ca^{2+} overload in acinar cells, details concerning its expression and function in ductal cells are currently lacking. We demonstrate that functionally active Orai1 channels reside in the apical plasma membrane of pancreatic ductal cells. Selective Orai1 inhibition impairs Stim1-dependent extracellular Ca^{2+} influx evoked by bile acids or ethanol combined with non-oxidative ethanol metabolites. Furthermore, prevention of sustained extracellular Ca^{2+} influx protects ductal cell secretory function *in vitro* and decrease pancreatic ductal cell death. Finally, Orai1-inhibition partially restores and maintains proper exocrine pancreatic secretion in *in vivo* AP models. In conclusion, our results indicate that Orai1 inhibition prevents AP-related ductal cell function impairment and holds the potential of improving disease outcome.

New inflammatory mechanisms in chronic heart failure

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Background: Interleukin-1β (IL-1β) is an important pathogenic factor in cardiovascular diseases including chronic heart failure (HF). The CANTOS trial pointed out that inflammasomes as primary sources of IL-1 β are promising new therapeutic targets in cardiovascular diseases.
Aim: Therefore, we aimed to assess inflammasome activation in failing hearts to identify activation patterns of inflammasome subtypes as sources of IL-1β.
Methods and Results: Out of the 4 major inflammasome sensors tested, expression of the inflammasome protein absent in melanoma 2 (AIM2) and NLR family CARD domain-containing protein 4 (NLRC4) increased in human heart failure regardless of the etiology (ischemic or dilated cardiomyopathy) while the NLRP1/NALP1 and NLRP3 (NLR family, pyrin domain containing 1 and 3) inflammasome showed no change in HF samples. AIM2 expression was primarily detected in monocytes/macrophages of failing hearts. Translational animal models of HF (pressure or volume overload, and permanent coronary artery ligation in rat, as well as ischemia/reperfusion-induced HF in pigs) demonstrated activation pattern of AIM2 similar to that of observed in end-stages of human HF. In vitro AIM2 inflammasome activation in human THP-1 monocytic cells and human AC16 cells was significantly reduced by pharmacological blockade of pannexin-1 channels by the clinically used uricosuric drug probenecid. Probenecid was also capable to reduce pressure overload-induced mortality and restore indices of disease severity in a rat chronic HF model in vivo.
Conclusion: This is the first report showing that AIM2 and NLRC4 inflammasome activation contribute to chronic inflammation in heart failure and that probenecid alleviates chronic HF by reducing inflammasome activation. The present translational study suggests the possibility of repositioning of probenecid for HF indications.

Novel therapeutic options for heart failure with preserved ejection fraction targeting inflammation and oxidative

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Background: Oxidative stress, systemic, low-grade inflammation of metabolic risk contributed to diastolic left ventricular (LV) dysfunction and heart failure with preserved ejection fraction (HFpEF) through coronary microvascular endothelial activation, which alters paracrine signalling to cardiomyocytes and predisposes them to hypertrophy and high diastolic stiffness, but the underlying mechanisms are still elusive.
Aim: The aim of the study was to explore the mechanisms of inflammatory endothelial activation and its effects on oxidative stress, nitric oxide (NO) bioavailability, and cyclic guanosine monophosphate (cGMP)-protein kinase G (PKG) signalling in myocardial biopsies of HFpEF patients and thereby on cardiomyocyte function via titin modification. Our second aim was to investigate the therapeutic approaches to reverse these alterations.
Methods: We investigated in human myocardium from patients with HFpEF using immunoblots and ELISA inflammation and oxidative stress parameters. In addition, we studied the function of increased inflammation and oxidative stress in endothelial vasorelaxation and cardiomyocyte function.
Results: In HFpEF, we found higher oxidative stress-dependent activation of eNOS leading to PKGIα oxidation. Interestingly, immunofluorescence imaging and electron microscopy revealed that oxidized PKG1α in HFpEF appeared as dimers/polymers localized to the outer-membrane of the cardiomyocyte. We found increased cardiomyocytes and vascular stiffness and endothelial dysfunction in HFpEF. Monovariate linear regression analysis confirmed the correlation of oxidative stress and PKGIα-polymerization with increased cardiomyocyte stiffness and diastolic dysfunction in HFpEF. Acute empagliflozin and soluble guanylyl cyclase (sGC) both reduced inflammation, oxidative stress/eNOS-dependent PKGIα oxidation and improved cardiomyocyte and vascular stiffness and thereby reversed endothelial dysfunction in myocardium from HFpEF patients and murine ZDF obese rats.
Conclusion: HFpEF is associated with coronary microvascular endothelial activation and oxidative stress. These lead to a reduction of NO-dependent signalling in endothelial cells and cardiomyocytes, which contributes to the high cardiomyocyte stiffness. All these changes could be reversed upon treatment with empagliflozin and sGC.

Novel druggable targets of heart failure with reduced ejection fraction identified by droplet digital PCR and validated by Next Generation Sequencing

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Introduction: Heart failure with reduced ejection fraction (HFrEF) is a leading cause of mortality and hospitalization worldwide. Despite the currently available advanced therapies of HFrEF, identification of novel druggable targets is still needed to improve outcomes for patients' benefit. G-protein coupled receptors (GPCRs) represent the largest family of targets for already approved drugs providing a great opportunity for drug repurposing. Therefore, screening for differences in cardiac GPCR expression during HFrEF provides an efficient way of identifying novel drug targets for this disease. We hypothesized that droplet digital PCR (ddPCR) providing an absolute quantification of mRNAs in a highly sensitive manner can be used as a screening method to identify differentially expressed GPCRs in HFrEF vs. healthy hearts.
Aim: Here we aimed to investigate the differential expressions of 288 cardiac GPCRs by ddPCR and Next Generation Sequencing (NGS) in a rat model of HFrEF.
Methods: 8-10 weeks old, male Wistar rats were subjected to transverse aortic constriction (TAC, n=5) or sham (SHAM, n=5) surgery. 15-18 weeks after surgery, cardiac echocardiography was performed, and cardiac samples were collected for histological and gene expression analyses. Absolute quantification of the expression of 288 GPCR genes was performed by ddPCR, and was validated by bulk RNA sequencing using NGS with the depth of 40 million reads. Differential expression of GPCRs of TAC and SHAM hearts identified by both techniques was compared, and the results were correlated.
Results: TAC animals showed significantly deteriorated systolic and diastolic functions, accompanied by cardiac hypertrophy and fibrosis when compared to SHAM. Out of 288 GPCRs, ddPCR identified a total of 27 genes, and NGS identified a total of 69 genes to be significantly differently expressed in TAC vs. SHAM animals, 14 of which were identified by both methods, showing significant and clinically meaningful correlation.
Conclusions: This is the first demonstration of using ddPCR as a screening method for identifying GPCRs as novel druggable targets in a disease, and validated our results by NGS. As GPCRs are potent candidates for drug repurposing, we provide a list of easily testable drug targets for future basic research in the field of HFrEF.

Formation of a protein corona on the surface of extracellular vesicles in blood plasma

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Background: In this study we tested whether a protein corona is formed around extracellular vesicles (EVs), our endogenous nanoparticles in blood plasma. Artificial nanoparticles have been found to develop a protein corona altering their biodistribution and bioavailability.
Aim: Here we set the aim to study if a similar protein corona is formed at the surface of EVs in biofluids.
Methods: We isolated nascent EVs of THP1 cells and platelets, and incubated them in EV-depleted platelet-free plasma. EVs were subjected to differential centrifugation, size exclusion chromatography, or density gradient ultracentrifugation. EVs were then studied by mass spectrometry (MS/MS), electron microscopy, confocal microscopy, capillary Western immunoassay and flow cytometry. Controls included i) plasma without the addition of EVs; ii) EVs incubated in buffer. Also, in a functional analysis, we exposed dendritic cells (DCs) to EVs with different protein coronas after which the response of DCs was tested with flow cytometry and ELISA.
Results: Plasma protein-coated EVs had a higher density compared to nascent ones and carried numerous newly associated proteins. Interactions between plasma proteins and EVs were confirmed by electron microscopy, confocal microscopy, capillary Western immunoassay and flow cytometry. Based on our results and data published by others, we identified 9 shared proteins present in the coronas of THP1-and platelet-EVs, viruses and artificial nanoparticles in plasma. We found induction of protein aggregation by centrifugation of our EV-depleted plasma samples. The aggregate proteins showed high overlap with the EV protein corona and showed a unique appearance with confocal microscopy. However, in contrast to aggregates, coated EVs induced expression of TNFα, IL-6 and CD83 of dendritic cells.
Conclusion: Our data shed new light on the commonly reported plasma protein "contamination" of EV preparations. They also suggest the formation of a disease-dependent protein corona on EVs in blood plasma.
Acknowledgements: NKFIH-OTKA PD 111958; 120237; NVKP_16-1-2016-0017, ÚNKP-19-3-I-SE-45 –New National Excellence Program of the Ministry for Innovation and Technology; VEKOP-2.3.2-16-2016-00002, VEKOP-2.3.3-15-2016-00016

Is there a chance for next „cariprazine”?

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Background: 2015 was a very significant year for the Hungarian pharma industry. The drug called Vraylar (discovered at Richter, and from Phase II developed jointly with Forest/Actavis/Allergan/AbbVie) was approved by FDA.
Aim: Reveal the necessary circumstances for repetition this success in near future in any Hungarian company.
Methods: Thorough investigation of the “have to”-s in the past (from 1999 till 2015 and over) lead to the product, generating about 300 million USD/year royalty for Richter.
Results: Possibility exists, but further actions must be taken to reach this superb result again, including up-to-date scientific knowledge, better external cooperation and more business intelligence.
Conclusion: There is a natural and useful need to improve medical R&D in Hungary, and it depends on the community to act more and better to live up to expectations.

Disease modeling with iPSC-derived neurons: functional studies and alternative for drug development

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The use of induced pluripotent stem cells (iPSC) to model brain diseases is a powerful tool to study alterations in neuronal disorders. The iPSC-derived neurons (iPSC-N) have a genetic background of patients and manifest the disease-specific pathophysiology and phenotypes. Utilizing iPSC-N from patients and control individuals allow advanced drug development. Here, we utilized iPSC-N to study Autistic Spectrum Disorder (ASD) which is a complex neurodevelopmental disorder of the central nervous system characterized by difficulties in social interaction, communication, and repetitive behaviors. Currently the lack of optimal diagnosis at early developmental stages makes it difficult to study the disease pathology and to develop treatments for ASD patients. To model the autistic phenotype in vitro we cultured iPSC-Ns from both patients and healthy controls. We found significant decrease in spontaneous activity iPSC-N cell lines derived from individuals with ASD compared to healthy controls, suggesting the appearance of synaptic dysfunction in the autistic patients. Using chemogenetic approach we confirmed the reduced neuronal connectivity in iPSC-N cells from ASD individuals compared to healthy controls. Previously ASD has been linked to dopaminergic signaling and we tested the cellular responses to dopaminergic stimuli in our iPSC-N cells. We found that application of an in-house developed D_3 receptor partial agonist resulted in stimulation of the neuronal oscillatory network by increasing Ca^{2+} transients. Furthermore, using chemogenetic approach we demonstrated that D_3R-activation improves neuronal network connectivity in ASD NPC-Ns to the level of healthy controls, suggesting the D_3 receptor as a potential target for autism. Taken together, our in vitro approach of using iPSC-N cells from ASD individuals demonstrates that iPSC-N cells highly suitable alternative approach for drug discovery studies.

Disrupted Social Hierarchy in Prenatally Valproate-Exposed Autistic-Like Rats

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Using automated home-cages to study behavior is a relatively new concept. Rodents are kept in a social, relatively natural environment. These conditions allowed us to reveal new findings in psychogenic rodent models. We established mouse place preference learning and reversal learning methods and studied the effect of a known memory disrupting anticholinergic agent, scopolamine in mice. We found not only impaired reversal learning ability when treated with scopolamine but found difference in activity and lick behavior as well. Partly based on these findings, we designed a more complex set of methods to study autistic rats. Autism spectrum disorder (ASD) is characterized by impaired socio-communicational function, repetitive and restricted behaviors. Valproic acid (VPA) was reported to increase the prevalence of ASD in humans because of its use during pregnancy. VPA treatment also induces autistic-like behaviors in the offspring of rats after prenatal exposure; hence it is a preclinical disease model with high translational value. In the present study our aim was to characterize ASD relevant behaviors of socially housed, individually identified male rats in automated home cages. Natural behavior of rats was assessed by monitoring their visits to drinking bottles in an environment without human influence aiming at reducing interventional stress. Although rodents normally tend to explore their new environment, prenatally VPA-treated rats showed a drastic impairment in initial and long-term exploratory behavior throughout their stay in the automated cage. Furthermore, VPA rats displayed psychogenic polydipsia as well as altered circadian activity. In the competitive situation of strict water deprivation controls switched to an uneven resource sharing and only a few dominant animals had access to water. In VPA animals similar hierarchy-related changes were completely absent. While the control rats secured their chance to drink with frequent reentering visits, thereby ‘guarding’ the water resource, VPA animals did not switch to uneven sharing and displayed no evidence of guarding behavior.

Response-related sensorimotor rhythms in the Touchscreen Visual Discrimination test in rats: functional connections to cognitive impairment

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Background: The Touchscreen Visual Discrimination (VD) task is frequently used in preclinical cognitive research due to its high translational potential; when combining it with EEG, the underlying neuronal activity might reveal potential correlates of cognitive functioning. In humans, voluntary goal-directed movements such as touch or button pressing induce the desynchronization of sensorimotor mu rhythm (8-12 Hz), whose rodent homologue has been suggested. The mu rhythm is presumed to reflect the integration of sensory, motor, and cognitive processes; however its functional relevance has not been clearly established yet.
Aim: Our aim was to explore whether the touch response-related activity in the VD paradigm can be suitable to find sensorimotor rhythms that connect to the cognitive aspect using healthy and cognitively impaired rats.
Methods: Performance was disturbed by a single injection of different doses of scopolamine or MK-801; epidural EEG was recorded over the visual, somatosensory, and motor cortices and was time-stamped to touch responses.
Results: Arciform ~10 Hz oscillations appeared during visual processing, then characteristic α/β desynchronization-resynchronization pattern emerged in the period of correct touches predominating above the sensorimotor areas. Both drugs induced dose-dependent cognitive impairments and reduced the desynchronization of upper α frequencies.
Conclusion: Normal cognitive slowing positively correlated with α/β power activity, which was further increased under drug exposure. In conclusion, a mu homologue rhythm can be suspected, whose upper α component is regulated by both cholinergic and glutamatergic systems. The VD task can be utilized for the investigation of sensorimotor rhythms in rats, which indicate a potential EEG correlate of cognitive performance.
Acknowledgement: This work was financially supported by Gedeon Richter Plc. and 2017-1.2.1-NKP-2017-00002.

Adaptive thermogenesis: the diet strikes back

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Background. Although the secret weapon – caloric restriction– in the battle against excess weight is well known, most diets end without satisfactory results. Adaptive thermogenesis, the more than predicted decrease in energy expenditure during caloric restriction and weight loss, is a well-known effect; numerous studies has shown its role in the failure of diets. Metabolic adaptation persists even for years, even if the individual returns to their original caloric intake.
Aims. The aim of our study was to mimic diet-induced metabolic adaptation and consequential rapid weight regain in diet induced obese C57BL/6J mice.
Methods. Continuous metabolic measurement was performed on C57BL/6J mice using PhenoMaster metabolic cage system. A group of animals were restricted to 70% of the food intake of control group. Body weight, food intake, energy expenditure and activity were monitored. After reaching weight loss plateu (no change in body weight for one week), animals were 100% paired fed to the control group for another 4 weeks.
Results. Caloric restriction led to a pronounced decrease in energy expenditure in C57BL/6J mice. Body weight decreased rapidly in the first week of the diet. Animals reached their minimum weight after 21 days and remained on it for 7 days. After that, refeeding phase (100% paired feeding) was initiated. In the first two weeks of refeeding, despite of the normal caloric intake, energy expenditure of formerly restricted animals was significantly lower than that of controls. In accordance with this, animals rapidly gained weight during the first two weeks in the refeeding phase, ending up with a body weight higher than their initial weight.
Conclusions. Similar to humans, long lasting metabolic adaptation can be evoked in C57BL/6J mice. Diet-induced decrease in energy expenditure, reserved even after returning to normal caloric intake, leads to energy surplus and as a consequence, excess weight gain.

Cariprazine Alleviates Core Behavioral Deficits in the Prenatal Valproic Acid Exposure Model of Autism Spectrum Disorder

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Background: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social communication and interaction and restricted, repetitive behaviors. The unmet medical need in ASD is considerable since there is no approved pharmacotherapy for the treatment of these deficits in social communication, interaction, and behavior. Cariprazine, a dopamine D3-preferring D3/D2 receptor partial agonist, is already approved for the treatment of schizophrenia and bipolar I disorder in adults; investigation in patients with ASD is warranted.
Aim: The objective of this study was to investigate the effects of cariprazine, compared with risperidone and aripiprazole, in the rat prenatal valproate (VPA) exposure model on behavioral endpoints representing the core and associated symptoms of ASD.
Methods: To induce the ASD model, time-mated Wistar rat dams were treated with VPA during pregnancy. Male offspring were assigned to groups and studied in a behavioral test battery at different ages, employing social play, open field, social approach-avoidance, and social recognition memory tests. Animals were dosed orally, once a day for 8 days, with test compounds (cariprazine, risperidone, aripiprazole) or vehicle before behavioral assessment.
Results: Cariprazine showed dose-dependent efficacy on all behavioral endpoints. In the social play paradigm, only cariprazine was effective. On the remaining behavioral endpoints, including the reversal of hyperactivity, risperidone and aripiprazole displayed similar efficacy to cariprazine.
Conclusion: In the present study, cariprazine effectively reversed core behavioral deficits and hyperactivity present in juvenile and young adult autistic-like rats. These findings indicate that cariprazine may be useful in the treatment of ASD symptoms.
Funding: This study was financially supported by Allergan (prior to its acquisition by AbbVie) and Gedeon Richter Plc.

Research of α7 nACh receptor positive allosteric modulators in Richter

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Homomeric α7 nicotinic acetylcholine receptors (α7 nAChRs) are ligand-gated pentameric ion channels expressed in "cognitive areas" of the central nervous system including the prefrontal cortex, the hippocampus, and other subcortical limbic structures. A wide variety of nAChR ligands have been shown to improve cognitive functioning (*e.g.* memory or attention) in rodents – however, clinical trials using orthosteric agonists revealed only suboptimal efficacy. To avoid the potential efficacy-limiting effect of fast receptor desensitization, we developed multiple series of novel α7 nAChR-selective positive modulator compounds. One structural group was built upon the optimization of a well-known reference compound (A-867744), while other chemical clusters were based on the expansion of hit molecules emerging from a high throughput screening (HTS) campaign utilizing the Corporate Compound Collection. Selection of ligands was based on detailed in vitro characterization, followed by monitoring procognitive effects in rodent models. Compounds varied broadly in terms of their in vitro and in vivo effects, however, promising compounds emerged from each structural groups. Our data (together with the identified structural elements) provide chances for further compound optimization, suggest unique and promising properties of the positive modulation of the α7 nAChR and deepen our understanding of nicotinic actions at the receptor level. Further development of these compounds may provide an efficient strategy for the treatment of neurocognitive disorders.

Investigation of higher-order cognitive functions in pharmacological and natural models of cognitive decline in non-human primates

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Background: Early detection and effective intervention in progressive neurocognitive disorders is an unmet medical need posing several challenges for translational behavioral neuroscience.
Aim: We aimed to develop preclinical test batteries for non-human primates with high translational validity for testing various aspects of cognition from basic mechanisms to complex executive functions based on human non-verbal psychological tests.
Methods: Rhesus macaque monkeys were involved in the experiments. Alertness and basic attentional processes were tested in a modified version of the psychomotor vigilance paradigm. Short-term visual (working) memory (VWM) was assessed with several variations of the delayed matching to sample (DMTS) task. We used muscarinic antagonist scopolamine to pharmacologically induce transient and reversible amnesia. Furthermore, we devised a new version of the visuospatial paired associates learning task to induce and measure proactive interference (iPAL), a prominent limiting factor of VWM in healthy and diseased states.
Results: In the PVT task, we successfully measured reaction time correlates of alertness and temporal expectation, both impaired by scopolamine and only the latter reversed by donepezil. In the DMTS task, similar amnestic and partial rescue effects were observed only in medium delay conditions. In the iPAL task, distraction caused be re-introducing recent memory items interfered with task performance and remained highly stable over several months.
Conclusion: Results indicate that pharmacological models of AD in primates make a valid contribution to modeling certain symptoms of dementia. Taking within-trial delay length into account in the PVT and DMTS tasks can be a valuable tool to test the behavioral manifestations of temporal expectation and VWM maintenance and can foster further research on age-related cognitive impairments. Results in the iPAL paradigm performance under various challenge conditions allowed to investigate new hallmarks of VWM in a non-pharmacological model suggesting an effective new strategy in preclinical drug development against pathological cognitive decline.

Pericyte-secreted IGF2 promotes breast cancer brain metastasis formation

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Background: Brain metastases are life-threatening complications of triple negative breast cancer, melanoma, lung cancer and a few other tumor types. Poor outcome of cerebral secondary tumors largely depends on the microenvironment formed by cells of the neurovascular unit, among which pericytes are the least characterized**.**
Aim: The aim of the study was to unravel communication pathways between brain pericytes and metastatic cells with a special focus on IGF.
Methods: In vivo, in vitro techniques and human samples were used to explore the role of pericytes and the role of IGF mediated signalling in brain metastasis formation.
Results: Brain pericytes had a prompt chemoattractant effect on breast cancer cells and established direct contacts with them. By secreting high amounts of extracellular matrix proteins, pericytes enhanced adhesion of both melanoma and triple negative cancer cells, which might be particularly important in the exclusive perivascular growth of these tumor cells. In addition, pericytes secreted 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 signaling 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 tumors in mice inoculated with triple negative breast cancer cells**.**
Conclusion: Our results indicate that brain pericytes have significant pro-metastatic features, especially in breast cancer. Our study underlines the importance of targeting pericytes and the IGF axis as potential strategies in brain metastatic diseases.

Robert's Intragastric Alcohol–Induced Gastric Lesion Model as an Escalated General Peripheral and Central Syndrome, Coun-teracted by the Stable Gastric Pentadecapeptide BPC 157

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We redefined Robert's prototypical cytoprotection model, namely intragastric administration of 96% alcohol in order to generate a general peripheral and central syndrome similar to that which occurs when major central or peripheral veins are occluded in animal models. With the redefinition, we used Robert's model to examine the cytoprotective effects of the stable gastric pentadecapeptide BPC 157. The intragastric alcohol administration of alcohol induced gastric lesions, intracranial (superior sagittal sinus) hypertension, severe brain swelling and lesions, portal and vena caval hypertension; aortal hypotension, severe thrombosis, inferior vena cava and superior mesenteric vein congestion, azygos vein failure (as a failed collateral pathway), electrocardiogram disturbances, and heart, lung, liver and kidney lesions. The use of BPC 157 therapy (10 μg/kg or 10 ng/kg given intraperitoneally 1 min after alcohol) counteracted these deficits rapidly. Specifically, BPC 157 reversed brain swelling, superior mesenteric vein and inferior vena caval congestion, and helped the azygos vein to recover, which improved the collateral blood flow pathway. Microscopically, BPC 157 counteracted brain (i.e. intracerebral haemorrhage with degenerative changes of cerebral and cerebellar neurons), heart (acute subendocardial infarct), lung (parenchymal haemorrhage), liver (congestion), kidney (congestion) and gastrointestinal (epithelium loss, haemorrhagic gastritis) lesions. In addition, this may have taken pace along with the activation of the specific molecular pathways. In conclusion, these findings clarify and extend the theory of cytoprotection, offer an approach to its practical application and establish BPC 157 as a prospective cytoprotective treatment.

BPC 157: Lessons from the counteraction of the inferior caval vein syndrome, Pringle maneuver ischemia, reperfusion, Budd-Chiari syndrome, superior sagittal sinus occlusion, and superior mesenteric artery and/or vein occlusion in rats

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With the stable gastric pentadecapeptide BPC 157 regimens in rats, we attempt to introduce the therapy for the occluded essential vessel tributaries, both arterial and venous, peripherally and centrally, occluded superior mesenteric vein and artery in rats, consequent noxious syndrome, peripherally and centrally. Commonly, using BPC 157 regimen in the rats with occluded major vessel(s), counteracted were the superior sagittal sinus, portal and caval hypertension, aortal hypotension, progressing venous and arterial thrombosis peripherally and centrally, ECG disturbances attenuated. Markedly, the multiple organs lesions, heart, lung, liver, kidney, and gastrointestinal tract, in particular, as well as brain lesions, and oxidative stress in tissues were attenuated. The syndrome development and counteraction with BPC 157 therapy commonly appeared along with variety of the procedures and vessels occlusion, i.e., the inferior caval vein syndrome, Pringle maneuver ischemia, reperfusion, Budd-Chiari syndrome, superior sagittal sinus occlusion, and superior mesenteric artery and/or vein occlusion. The bypassing loops appeared as reliant on the corresponding injurious occlusion and re-established blood flow that compensates for vessel occlusion. There, we pointed out the left ovarian vein, the inferior mesenteric vein, the azygos vein in the porto-caval shunt, and in superior-inferior caval vein shunt. More specifically, there were the inferior and superior anterior pancreaticoduodenal, pyloric vein in the superior mesenteric vein-portal vein shunt, and inferior mesenteric artery and inferior anterior pancreaticoduodenal artery, and centrally, (para)sagittal venous collateral circulation. It was theorized that BPC 157 therapy would likely represent a 'bypassing key', rapidly activating bypassing pathways and abrogating the complex syndrome induced by simultaneous occlusion of essential arterial and venous tributaries. As therapy background, we hypothesized the cytoprotection theory maxim endothelium maintenance → epithelium maintenance upgraded to endothelium maintenance → epithelium maintenance = blood vessel recruitment and activation ('running') towards the site of injury, also described as bypassing occlusion via alternative pathways, and the stable gastric pentadecapeptide BPC 157, as novel and more relevant cytoprotection mediator, to rapidly activate collateral bypassing pathways, and rapidly alleviate vessel occlusion syndromes.

Structural elucidation of ligand binding mechanisms to somatostatin receptors

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Background: Somatostatin is an endogenous cyclic neuropeptide, which is widely expressed in the body. It plays an important role in regulation of the endocrine system, neurotransmission, cell proliferation, relieving pain and inflammation.
Aim: There are five sub-types of somatostatin receptors, of which the fourth sub-type is the most promising for development of a new analgetics and others have been targeted by antiproliferative compounds. However, experimental determination of structures of somatostatin receptors is still missing.
Methods: Pharmacoinformatic tools were applied to overcome this limitation.
Results: In the present lecture, we feature our recent work on structural elucidation of binding mechanism of active ligands to somatostatin receptors at atomic resolution.
Conclusion: Our findings show good agreement with available experimental results and provide a new pathway for the design of new, subtype-selective somatostatin analogues.
Acknowledgements: This work was funded by the Hungarian National Research, Development and Innovation Office (K123836, K134214), 2017-1.2.1 NKP-2017-00002 (NAP-2; Chronic Pain Research Group), EFOP-3.6.1.-16-2016-0004 and GINOP 2.3.2-15-2016-00050 “PEPSYS”. This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. We acknowledge the support from the Governmental Information Technology Development Agency, Hungary. The work was supported by the ÚNKP-21-5 and ÚNKP-21-3-II. New National Excellence Program of the Ministry for Innovation and Technology. Supported by the PTE ÁOK-KA No:2019/KA-2019-31. The project has been supported by the European Union, co-financed by the European Social Fund. Project name and code: Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, EFOP-3.6.1-16-2016-00004.

Participation of corticotropin-releasing factor in gastroprotection against indomethacin-induced gastric injury and somatic pain regulation through glucocorticoid involvement

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Background: Corticotropin-releasing factor (CRF) is a major mediator of stress response. CRF action is mediated by CRF receptors of subtype 1 and 2 (CRF-R1 and CRF-R2 receptors). CRF stimulates the ACTH release through CRF-R1 and, ACTH, in turn, stimulates glucocorticoid production. As we demonstrated previously, glucocorticoids released in response to stressor are gastroprotective hormones.
Aim: Here we studied whether glucocorticoids are involved in CRF-caused effects on the gastric mucosa and somatic pain sensitivity under ulcerogenic action of indomethacin (IM) in rats.
Methods: Gastric lesions and plasma corticosterone levels were examined 4 h after IM (35 mg/kg, ip) administration. Somatic pain sensitivity was evaluated by tail flick latency (tail flick test) or paw licking latency (hot plate test) before IM administration as well as 4 h after IM injection. CRF-R1/CRF-R2 antagonist astressin or ACTH was used to modulate corticosterone levels.
Results: Both CRF (5 µg/kg, ip) and ACTH (1 U/kg, ip) by itself caused an increase of plasma corticosterone levels as well as in tail flick latencies and paw licking latencies (analgesic effects). IM induced the formation of gastric erosion 4 h after injection. CRF or ACTH administration before IM reduced an area of gastric lesions (gastroprotective effect). IM-induced gastric injury was accompanied by an increase of tail flick latencies, which was prevented by CRF. Pretreatment with astressin (50 µg/kg, ip, 15 min before CRF) inhibited corticosterone levels and prevented the gastroprotective effect of CRF as well as normalization of somatic pain sensitivity. There were no any changes in paw licking latencies under circumstances of the gastric injury.
Conclusion: Thus, glucocorticoids released in response to CRF can be involved in the gastroprotective effects against ulcerogenic action of IM and somatic pain regulation.
Funding: The study was supported by grants from Russian Science Foundation (RSF) № 19-15-00430.

POSTER ABSTRACTS

Investigation of the anticancer potentials of the newly synthesized 13α-estrone derivatives

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Background: 13α-Estrone is an epimer of natural estrone with no hormonal activity. Certain 13α-estrone derivatives possess remarkable antineoplastic effects.
Aim: The present study aims to investigate the anticancer properties of a set of newly synthesized 13α-estrone derivatives as well as to understand their mechanism of action.
Methods: Analogs of 13α-estrone and its 17-deoxy counterparts were synthesized by Pd-catalyzed, microwave-assisted C–H activation reactions. The compounds were tested for anti-proliferative effects against a panel of five human adherent gynecological cancer cell lines (MCF-7, 231, HeLa, SiHa and A2780) using MTT assay. Investigation of other anticancer properties of the selected compounds was conducted using cell cycle analysis, caspase activity, migration assay and tubulin polymerization assay.
Results: Certain potent antiproliferative compounds were identified based on addition of an *N*,*N*-dimethylsulfamate pharmacophore as well as the 2-(4-chlorophenyl) moiety which improved the antitumor potential. Among the tested compounds, the newly synthesized sulfamate derivative 2-(4-chlorophenyl)-13α-estrone sulfamate displayed a remarkable antiproliferative activity against HeLa and SiHa (IC_{50}: 2.28, 2.71 respectively) and was used for further assessment. Cell cycle analysis showed a cell cycle disturbance. Its proapoptotic property was confirmed by a significantly increased caspase-3 activity. Migration assay displayed a statistically significant antimigration effects in a dose dependent manner. It also displayed a significant increase in tubulin polymerization.
Conclusion: The tested compound is considered the first 13a-estrone derivative with an outstanding potency against SiHa cancer cells. Therefore, it might provide further insights towards the design of anticancer agents targeting cervical carcinomas.
Support from National Research, Development and Innovation Office-NKFIH (Hungary) through project TKP2020-NKFIH-1279-2/2020 is gratefully acknowledged.

Investigation of antiproliferative properties of 16-triazolyl estranes and their 16-azidomethyl precursors

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Background: A large amount of experimental data indicates that steroidal compounds without hormonal activities may exert substantial anticancer activities. The estrane skeleton is especially suitable for this kind of structural diversification in order to perform lead-finding studies.
Aim: The aim of our current research is to investigate the antiproliferative and antimetastatic properties of a set of 16-triazolyl estranes and their 16-azidomethyl precursors.
Methods: The antiproliferative properties of the prepared compounds were characterized by colorimetric MTT assay against a panel of human cancer cell lines of gynecological origin (MDA-MB-231, MCF-7, HeLa and SiHa). Effects of selected agents on cell cycle distribution and migration capacity were investigated by flow cytometry and wound healing assay, respectively. Boyden-chamber assay was additionally used to describe antiinvasion effect of the compounds.
Results: Two out of 26 compounds exhibited outstanding cell growth inhibiting capacity; their calculated IC_{50} values were lower than that of reference agent cisplatin. These agents, two isomeric 16-azidomethyl precursors, elicited marked increase in subG1 population of MDA-MB-231. The migratory capacity of the same cell lines was decreased in a concentration and time-dependent way at subantiproliferative concentrations. The antimetastatic potency of the selected compounds has been confirmed by Boyden assay.
Conclusion: In conclusion, two of the investigated estrane analogs modified at position 16, isomeric 16-azidomethyl compounds, exerted outstanding antiproliferative action against human cancer cells. Besides this property, antimetastatic action was documented at lower concentration. Estrane analogs substituted at position 16 may be considered starting structures for further potential anticancer lead compounds.
Financial supports of National Research, Development and Innovation Office-NKFIH (Hungary) through project TKP2020-NKFIH-1279-2/2020 and Economic Development and Innovation Operative Program (GINOP-2.3.2-15-2016-00012) are gratefully acknowledged.

Induction of T-cell development from hematopoietic stem cells by exosomes

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Background: The development of early thymic progenitors starts in the bone marrow and continues in the thymus, where thymic epithelial cells trigger T-cell lineage commitment and further thymocyte development. Basically Wnt4 (along with FoxN1) has the major regulatory task during thymopoesis and maintains the cellular composition of TECs (thymic epithelial cells) in the young thymus. Wnt4 can be released by the cells via exosomes into the cell culture medium so they can be collected and isolated for further use.
Aim: Our goal was to create an in vitro model inducing T-cell commitment from human hematopoietic stem cells.
Methods: In our model study human thymic carcinoma cells (1889c) were seeded onto a 3D plate insert scaffold to provide a thymic stromal niche. Later MACS-enriched CD34+ (hematopoietic stem cells) from human Buffy-coat were seeded to create an artificial personalized human thymus. Also, exosomes were collected and isolated from cell culture media of Wnt4 over-expressing TEP1 (mouse thymus epithelial) cell line and added to the 3D cultures. Following long-term culture in human T-cell expansion medium qPCR and flow-cytometric evaluation was performed.
Results: Our data suggest enhanced T-cell development, as we were able to detect higher number of CD4 and CD8 single positive cells after adding Wnt4 exosomes to the cell cultures.
Conclusion: Results indicate that 3D cell culturing techniques provide an in vitro platform for thymocyte development. Also presence of Wnt4 exosomes enhances T-cell maturation and has beneficial effect to the cell culture environment. The increasing life expectancy of the aged population requires new solutions that support the reinforcement of the thymus. Therefore the T-cell development supporting effect of Wnt4 transgenic exosomes has a high therapeutic potential and our study could be a start of a new personalized immunotherapeutic strategy.
The research project was supported by UNKP-19-3 2019/2020, PTE PoP and EFOP-3.6.1-16-2016-00004.

In vitro evaluation of antiproliferative effect of D-ring modified estrane derivatives against human cancer cell lines with different HPV-status

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Human papillomavirus (HPV) infection is the leading cause of cervical cancers and an increasing proportion of oropharyngeal cancers. High-risk HPV-types, such as HPV-16 and -18 are involved in most of these carcinomas.
In this current study we aimed to determine the antiproliferative effect of 35 newly synthesized, D-ring modified steroid compounds on human cervical and oropharyngeal carcinoma cell lines, moreover we compared their activity based on the HPV-status of the utilized cell lines.
Human adherent cervical (HeLa, SiHa and C33A) and oropharyngeal carcinoma cell lines (UPCI-SCC-131 and UPCI-SCC-154) were used. To determine the antiproliferative activity of the compounds we used the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.
D-homo-estrone, but not its derivatives exerted substantial antiproliferative effect on the cervical HPV-18-positive cell line and had negligible effect on the other cell lines.
Artemisinin-hybrids displayed low antiproliferative effect on HPV-16-positive cervical and oropharyngeal cell lines, moderate effect on HPV-18-positive cells and substantial effect on HPV-negative cervical cells. Except for one hybrid, lower effect was demonstrated on the same HPV-status bearing oropharyngeal counterpart.
Both D-seco-oximes exerted substantial antiproliferative effect on the cervical HPV-18-positive, HPV-negative and oropharyngeal HPV-negative cell lines and had moderate effect on both HPV-16-positive cell lines. D-seco-estrones had no effect.
Both precursors of organophosphorus steroids exerted substantial antiproliferative effect regardless of origin or HPV-status. Their derivatives demonstrated moderate or low effect on the investigated cell lines, except for one compound, that exerted substantial antiproliferative activity on the HPV-negative oropharyngeal cell line.
Further investigation is required to understand the molecular mechanisms lying behind the antiproliferative effect of the investigated compounds and to understand the role of HPV-status in these carcinomas from a therapeutic point of view.
This work was supported by National Research, Development and Innovation Office-NKFIH (Hungary) through project TKP2020-NKFIH-1279-2/2020 and the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

Osteogenic and neurogenic differentiation capacity of dental progenitor cells

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Background: Cell-based therapies are emerging as promising tools for the treatment of various diseases. Previous studies have demonstrated that mesenchymal progenitor cells can be isolated even from adult human dental tissues, such as periodontal ligament (PDL) and dental pulp (DP).
Aim: The purpose of this study was to isolate PDL and DP derived progenitor cells and to investigate their neurogenic and osteogenic differentiation.
Methods: PDL and DP were collected from human impacted third molars and digested in a solution of collagenase type I (1 h, at 37°C). The cells were cultured in α-MEM medium under standard conditions (37°C, 100% relative humidity, 5% CO_2). We used the previously described neuro- and osteoinductive in vitro protocols to investigate the neurogenic differentiation potential of DP progenitor cells and the osteogenic differentiation potential of PDL cells. Morphological changes were monitored using a phase contrast microscope. Following induction, the appearance of neuron-specific antigens was examined by immunocytochemistry, and the mineralised lesions were analysed by von Kossa staining and immunocytochemistry.
Results: Primary cell cultures were successfully established from DP and PDL tissues and they were sustainable for a long time.
Upon completion of the neurogenic induction protocol, we were able to observe neuronal cell morphology on which neurogenic markers were detected.
The success of osteogenic differentiation was confirmed by von Kossa staining and by the detection of osteogenic immunocytochemical markers.
Conclusion: Our results support the idea that in the future the dental progenitor cells may be suitable for tissue replacement as well as for regenerative use.
Acknowledgements: The present work was supported by the Hungarian Human Resources Development Operational Program (EFOP-3.6.2-16-2017-00006).

Immunomodulatory properties of the octapeptide NAP in *Campylobacter jejuni* infected mice suffering from acute enterocolitis

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Background: Human infections with the food-borne zoonotic pathogen *Campylobacter jejuni* are progressively rising and constitute serious global public health and socioeconomic burdens. Hence, application of compounds with disease-alleviating properties are required to combat campylobacteriosis and post-infectious sequelae.
Aim: In our preclinical intervention study applying an acute *C. jejuni* induced enterocolitis model, we surveyed the anti-pathogenic and immune-modulatory effects of the octapeptide NAP which is well-known for its neuroprotective and anti-inflammatory properties.
Methods: Therefore, secondary abiotic IL-10$^{-/-}$ mice were perorally infected with *C. jejuni* and intraperitoneally treated with synthetic NAP from day 2 until day 5 post-infection.
Results: NAP-treatment did not affect gastrointestinal *C. jejuni* colonization but could alleviate clinical signs of infection that was accompanied by less pronounced apoptosis of colonic epithelial cells and enhancement of cell regenerative measures on day 6 post-infection. Moreover, NAP-treatment resulted in less distinct innate and adaptive pro-inflammatory immune responses that were not restricted to the intestinal tract but could also be observed in extra-intestinal and even systemic compartments. NAP-treatment further resulted in less frequent translocation of viable pathogens from the intestinal tract to extra-intestinal including systemic tissue sites.
Conclusion: For the first time we here provide evidence that NAP application constitutes a promising option to combat acute campylobacteriosis.

Pituitary Adenylate Cyclase-Activating Polypeptide – A Neuropeptide as Novel Treatment Option for Subacute Ileitis in Mice Harboring a Human Gut Microbiota

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Background: The neuropeptide Pituitary adenylate cyclase-activating polypeptide (PACAP) is well known for its important functions in immunity and inflammation. Data regarding anti-inflammatory properties of PACAP in the intestinal tract are limited, however.
Aim: In our present preclinical intervention study we addressed whether PACAP treatment could alleviate experimental subacute ileitis mimicking human gut microbiota conditions.
Methods: Therefore, secondary abioitic mice were subjected to human fecal microbiota transplantation and perorally infected with low-dose *Toxoplasma gondii* to induce subacute ileitis on day 0. From day 3 until day 8 post infection, mice were either treated with synthetic PACAP38 or placebo.
Results: At day 9 post-infection, placebo, but not PACAP treated mice exhibited overt macroscopic sequelae of intestinal immunopathology. PACAP treatment further resulted in less distinct apoptotic responses in ileal and colonic epithelia that were accompanied by lower T cell numbers in the mucosa and lamina propria and less secretion of pro-inflammatory cytokines in intestinal *ex vivo* biopsies. Notably, ileitis-associated gut microbiota shifts were less distinct in PACAP as compared to placebo treated mice. Inflammation-ameliorating effects of PACAP were not restricted to the intestines, but could also be observed in extra-intestinal including systemic compartments as indicated by lower apoptotic cell counts and less pro-inflammatory cytokine secretion in liver and lungs taken from PACAP treated as compared to placebo control mice, which also held true for markedly lower serum TNF and IL-6 concentrations in the former as compared to the latter.
Conclusion: Our preclinical intervention study provides strong evidence that synthetic PACAP alleviates subacute ileitis and extra-intestinal including systemic sequelae of T cell-driven immunopathology. These findings further support PACAP as a novel treatment option for intestinal inflammation including inflammatory bowel diseases.

Carvacrol ameliorates acute campylobacteriosis in a clinical murine infection model

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Background: The prevalence of human infections with the zoonotic pathogen *Campylobacter jejuni* is rising worldwide. Therefore, the identification of compounds with potent anti-pathogenic and anti-inflammatory properties for future therapeutic and/or preventive application to combat campylobacteriosis is of importance for global health. Results of recent studies suggested carvacrol (4-isopropyl-2-methylphenol) as potential candidate molecule for the treatment of campylobacteriosis in humans and for the prevention of *Campylobacter* colonization in farm animals.
Aim/Methods: To address this in a clinical murine infection model of acute campylobacteriosis, secondary abiotic IL-10$^{-/-}$ mice were subjected to synthetic carvacrol via the drinking water starting four days before peroral *C. jejuni* challenge.
Results: Whereas at day 6 post-infection placebo treated mice suffered from acute enterocolitis, mice from the carvacrol cohort not only harbored two log orders of magnitude lower pathogen loads in their intestines, but also displayed significantly reduced disease symptoms. Alleviated campylobacteriosis following carvacrol application was accompanied by less distinct intestinal apoptosis and pro-inflammatory immune responses as well as by higher numbers of proliferating colonic epithelial cells. Remarkably, the inflammation-ameliorating effects of carvacrol treatment were not restricted to the intestinal tract, but could also be observed in extra-intestinal organs such as liver, kidneys and lungs and, strikingly, systemically as indicated by lower IFN-γ, TNF, MCP-1 and IL-6 serum concentrations in carvacrol versus placebo treated mice. Furthermore, carvacrol treatment was associated with less frequent translocation of viable *C. jejuni* originating from the intestines to extra-intestinal compartments.
Conclusion: The lowered *C. jejuni* loads and alleviated symptoms observed in the here applied clinical murine model for human campylobacteriosis highlight the application of carvacrol as a promising novel option for both, the treatment of campylobacteriosis and hence, for prevention of post-infectious sequelae in humans, and for the reduction of *C. jejuni* colonization in the intestines of vertebrate livestock.

Significance of bile acids in pancreatic cancer

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Background: Pancreatic cancer (PC) is usually associated with obstructive jaundice (OJ), although the effect of bile acids (BAs) on tumour progression is less studied. MUC4 is an oncogenic mucin that upregulated in PC however, it's interaction with BAs is not completely clear. Therefore, **our aim** was to characterize the effect of BAs on tumour progression and to study the possible role of mucins in it.
Materials and methods: The serum concentrations of BAs were measured with high-performance liquid chromatography. The effects of BAs on tumour progression were investigated using different assays. Mucin expressions were studied in normal and pancreatic ductal adenocarcinoma cell lines (PDAC) and in human samples, using real-time PCR and immunostainings. Silencing of MUC4 was performed using specific siRNA.
Results: The levels of BAs were significantly higher in the PDAC+OJ group compare to the healthy, control group. Most of the BAs enhanced the rate of proliferation, migration, adhesion, colony forming and MUC4 expression in PDAC, whereas decreased the viability of normal cells. In patients, where PC is associated with OJ, strong MUC4 staining was detected. Silencing of MUC4 decreased carcinogenic processes in PDACs.
Conclusion: Normal cells respond by cell death to BAs treatment, that probably a protective mechanism in order to avoid malignant transformation. In PDAC, BAs promote tumour progression, in which the increased expression of MUC4 probably plays an important role. Our data indicate that in PC patients with OJ, the early treatment of biliary obstruction may improve life expectancy.
This study was supported by Bolyai Postdoctoral Fellowship of the Hungarian Academy of Sciences (HAS) to VV (00509/16), by the National Research, Development and Innovation Office (FK123982), the Economic Development and Innovation Operative Programme Grants (GINOP-2.3.2-15-2016-00015) and by the National Research, Development and Innovation Office, by the Ministry of Human Capacities (EFOP 3.6.2-16-2017-00006).

In vitro anti-proliferative activity and pharmacokinetic attributes of 2-aminomethylated estrone analogues on cancer cell lines of gynecological origin

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Although 2-methoxyestradiol (2ME2) exhibits a strong non-tumor specific and non-tumor selective antiproliferative activity, it possesses some disadvantageous pharmacokinetic properties as well. To overcome these non-beneficial characteristics the antiproliferative activities and pharmacokinetic properties of newly synthesized 2-aminomethylated derivatives of 2ME2 were investigated on selected gynecological (HeLa, MDA-MB-231 and A2780) cancer cell lines.

The antiproliferative activities of 12 test compounds were assayed using methyl thiazolyl tetrazolium bromide (MTT) assay while the kinetic aqueous solubility of the compounds (at pH 6.5 and 7.4), was performed using Multiscreen filter plates. *In vitro* gastrointestinal (GI)- and the blood brain barrier (BBB)-specific permeabilities of the compounds were also investigated using the parallel artificial membrane permeability assay (PAMPA). The central nervous system multi-parameter optimization desirability tool (CNS-MPO) was utilized to assess the appropriate pharmacokinetic attributes of the compounds.

Four benzylamino-estradiol derivatives displayed similar potency, tumor specificity and selectivity compared to 2ME2 (IC_{50} = 1.07-5.09 μM). Their kinetic aqueous solubility proved to be substantially better than that of 2ME2, however they possess weaker permeability characteristics related to 2ME2. Two estrone and one estradiol derivatives extensively inhibited proliferation of one or two cancer cell lines only, however with higher IC_{50} values than 2ME2. They slightly influenced non-cancerous cell proliferation. Their estimated pharmacokinetic properties are comparable or better than that of 2ME2, except of their GI permeability.

Three newly synthesized 2ME2 derivatives were identified with higher tumor specificity and improved tumor selectivity, however with slightly lower antiproliferative potency. Additionally, they portrayed desirable pharmacokinetic features. The research outcome underscores the immense potential in steroid-based synthetic analogues in treatment of various types of cancers.

This work was supported by National Research, Development and Innovation Office-NKFIH (Hungary) through project GINOP-2.3.2-15-2016-00038 and project TKP2020-NKFIH-1279-2/2020.

Urocortin stimulates ERK1/2 phosphorylation but reduces cell proliferation in MCF7 breast cancer cells

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Urocortins are ligands of corticotropin-releasing factor (CRF) receptors. Small amounts of them are present in the circulation and they are also produced locally in various tissues of higher vertebrates. Aside from regulating circulation, or food uptake they also influence, via auto- and paracrine mechanisms, cell proliferation.
Our aim was to investigate the effect of human urocortin onto mitogenic signaling via ERK 1/2 in MCF7 human breast cancer cells. We examined the expression and contribution of CRF receptors to the activation of ERK1/2, the involvement of PKA, PKB/Akt and MEK, the intracellular distribution of ERK1/2 and the expression of p53.
We carried out Western blotting to examine the activating phosphorylation of ERK1/2 by human urocortin 1 (HU). The intracellular localization of phosphorylated ERK1/2 was revealed by immunofluorescence. ATP measurement assay was used to determine the number of viable cells.
We could detect CRF-R1 and CRF-R2 expression in MCF7 cells. 10 nM HU induced transient ERK1/2 phosphorylation five minutes after the start of the treatment, accompanied by nuclear translocation of the enzyme, while a more prolonged PKB/Akt phosphorylation peaked at 3 hours. The MEK inhibitor U0126 could completely abolish the ERK1/2-phosphorylating effect of HU and the PKA inhibitor H89 markedly reduced ERK1/2 phosphorylation too.The CRF-R1-specific inhibitor Antalarmin significantly reduced ERK1/2 activation by HU. HU-treatment could markedly increase p53 protein expression and significantly decreased cell proliferation.
We demonstrated that HU treatment induced transient ERK1/2 phosphorylation via CRF-R1 - its effect depended also on the activity of PKA - and it was mediated by MEK. The same HU-treatment induced phosphorylation of PKB/Akt and increased p53 expression. The latter might be responsible for the observed downregulation of cell proliferation.
This work was supported by ÁOK KA-2021-31 and project no. TKP2020-IKA-08, the latter financed under the 2020-4.1.1-TKP2020 funding scheme.

Pharmacological investigation of a newly synthesized monoterpene derivatives on human cancer cell lines in vitro

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Background: Cancer is one of the major health problems worldwide. Tumours affecting the female reproductive organs (breast, uterus, cervix and ovaries) are found among the 10 most frequently diagnosed cancers. Natural products including terpenes are one of the most investigated group of compound in lead-finding studies. Monoterpenes from essential oils of many plants may prevent the carcinogenesis process and exert growth-inhibiting action against cancer cells.
Aim: The aim of the present study was the screening of a newly synthesized set of monoterpene-based 2,4-diaminopyrimidine type derivatives against gynaecological cancer cell lines. The mechanism of the most effective analogues was additionally tested to describe their mechanism of action.
Methods: The growth-inhibitory effects of the tested compounds were determined by MTT assay on a cell line panel (Hela, Siha, MDA-MB-231 and MCF-7, A2780 and NIH/3T3 fibroblast to characterize the cancer selectivity). Changes in the cell cycle phase distribution of the treated cells were determined by flow cytometry. Fluorescent staining have been performed to distinguish apoptotic and necrotic cells by their nuclear morphology and membrane integrity. The proapoptotic effects of the tested derivates were confirmed by caspase-3 activity assay.
Results: Two out of 20 monoterpene derivatives exhibited promising antiproliferative action with IC_{50} values below 10 µM. These analogues elicited substantial changes in the cell cycle distribution of A2780 ovarian cancer cells with increased number of cells in SubG1 and G2/M phases on the expense of G1 population. The proapoptotic potential of these agents were confirmed by propidium-Hoechst 33258 staining and caspase-3 assay.
Conclusion: Our results suggest that the new monoterpene compounds may be regarded as promising candidates in the development of new anticancer agents, against ovarian cancer cell lines.
Financial supports of the National Research, Development and Innovation Office (NKFIH, Hungary) through project TKP2020-NKFIH-1279-2/2020 and Innovation Operative Program (GINOP-2.3.2-15-2016-00012) are gratefully acknowledged.

Computational binding studies of a new bergamottin analog CYP3A4 inhibitor with antiproliferative activity

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Bergamottin is a naturally occurring furanocumarin, a known inhibitor of specific CYP450 enzymes responsible for various food-drug interactions. Besides its enzyme inhibitory effects, bergamottin was tested previously on various tumour cell lines, and proven to have a marked antitumour effect. A new derivate with a nitroxide moiety was synthesised (SL-bergamottin), and its enzyme inhibitory mechanism was computationally investigated in comparison with the parent compound and a known strong inhibitor of CYP3A4 enzyme, ketoconazole. The binding modes of bergamottin and SL-bergamottin were de novo described in the study. It was found that the three compounds occupy the same positions in the CYP3A4 active site. They are co-ordinated to the heme iron ion, ketoconazole with a N atom of the imidazole ring, bergamottin and SL-bergamottin with an O atom of a furo[3,2-g]chromen-7-one ring. SL-bergamottin was proven to be a stronger inhibitor of the enzyme, than the parent compound, with a better calculated binding free energy value. Synthetic modification of the geranyl sidechain of bergamottin can result in potent CYP3A4 eznyme inhibitors, with a potential strong and selective antiproliferative activity.

Acknowledgements: This work was funded by the Hungarian National Research, Development and Innovation Office (K123836, K134214), 2017-1.2.1 NKP-2017-00002 (NAP-2; Chronic Pain Research Group), EFOP-3.6.1.-16-2016-0004 and GINOP 2.3.2-15-2016-00050 “PEPSYS”. This work was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. We acknowledge the support from the Governmental Information Technology Development Agency, Hungary. The work was supported by the ÚNKP-21-5 and ÚNKP-21-3-II. New National Excellence Program of the Ministry for Innovation and Technology. Supported by the PTE ÁOK-KA No:2019/KA-2019-31. The project has been supported by the European Union, co-financed by the European Social Fund. Project name and code: Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs, EFOP-3.6.1-16-2016-00004.

An evaluation of the translational prediction value of model species in cardiac electrophysiology

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Background: Rodents are commonly used as models in electrophysiology. However, distinct differences exist between large animals and rodents in terms of their ion channel expression and action potential shapes, possibly limiting the translational value of findings obtained in rodents.
Aim: Direct comparison of the possible impact of selective inhibition of ion channels on the cardiac repolarization in preparations from human hearts and from model species.
Methods: We applied the standard microelectrode technique at 37 °C on cardiac ventricular preparations (papillary muscles and trabecules) from human (n=63), dog (n=47), guinea pig (n=53), rat (n=43), and rabbit (n=16) hearts, paced at 1 Hz. To selectively block the I_{Kur} current, 1 μM XEN-D101; I_{K1} current, 10 μM barium chloride; I_{Kr} current, 50 nM dofetilide; I_{Ks} current, 500 nM HMR-1556; and I_{to} current, 100 μM chromanol-293B were applied directly to the tissue bath.
Results: The block of I_{Kur} and I_{K1} elicited significantly more prominent prolongation of APD in rats (35.6% and 67.9%, respectively) when compared to the other species, including that of human (1.0% and 2.6% respectively). On the other hand, I_{Kr} block did not affect APD in rat preparations (1.6%), whereas it elicited marked prolongation in other species (9.0–47.7%), especially being pronounced in human preparations (60.3%). I_{Ks} inhibition elicited similar but minor APD prolongation (0.3–11.4%) in all species. Inhibition of I_{to} moderately lengthened APD in dog (22.3%) and rabbit (17.5%), but elicited no change of APD in human preparations. In contrast, block of I_{to} caused marked APD prolongation in rat preparations (33.2%).
Conclusion: Our findings suggest that the specific inhibition of various ion channels elicits fundamentally different effects in rodent ventricular action potential when compared to those of other species, including human. Therefore, from a translational standpoint, rodent models in cardiac electrophysiological and arrhythmia research should be utilized with great caution.

Immune checkpoint inhibition with PD-1 inhibitor induces cardiac dysfunction without overt myocarditis in C57BL/6 mice

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Background: Immune checkpoint inhibitors have revolutionized the treatment of several form of malignancies (including metastatic melanoma) by enhancing the cytotoxic effects of T cells against cancer cells. Cancer cells evade immune surveillence by increasing the expression of T cell inhibitory molecules, also known as immune checkpoints, such as programmed cell death-1 (PD-1). Pharmacological inhibition of these molecules by immune checkpoint inhibitors (ICI) will enhance the antitumor activity of T cells. However, enhanced T cell activity may cause immune related adverse effects, including cardiotoxicity.
Aims: We aimed to investigate the effect of PD-1 inhibition on cardiac function and the underlying mechanisms in mice.
Methods: 8-10 week old C57BL6/J mice were treated with isotype control or anti-PD-1 antibody for 2 or 4 weeks. Cardiac function and morphology was assessed by echocardiography and histology, while the transcriptomic changes were analyzed via RNA sequencing. Nitrosative stress in the heart was assessed by immunohistochemistry and qRT-PCR. Inflammatory gene expression alterations were determined by qRT-PCR in the heart and thymus.
Results: Small animal echocardiography revealed cardiac dysfunction even after 2 weeks of anti-PD-1 treatment, accompanied by transcriptomic changes in the heart. Nitrosative stress was found to be elevated in the myocardium due to anti-PD1 treatment, however, histological and qRT-PCR analysis did not reveal T cell infiltration into the myocardium. In contrast, there was significant increase in inflammatory gene expression in the thymus of anti-PD1-treated animals, where interleukin-17 increased most prominently.
Conclusions: These findings characterize cardiac dysfunction as a distinct form of ICI-induced cardiotoxicity, independent of myocarditis, likely related to increased remote thymic cytokine production.
Funding: The work was supported by the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 739593. TGG was supported by "Semmelweis 250+ Kiválósági PhD Ösztöndíj" (EFOP-3.6.3-VEKOP-16-2017-00009) and by Gedeon Richter Talentum Foundation's scholarship.

Responsiveness of the widely used cardiomyocyte cell platforms affected by simulated ischemia/reperfusion

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Ischemic heart disease is the leading cause of death worldwide; therefore, the development of cardioprotective therapies is currently a main focus of research. Cultured cell lines and primary cell cultures are highly used in cardiovascular research. However, there are many limitations when using these models, including variations in proliferation capacity, uncontrolled stress during cell isolation, poor reproducibility and low translational value. The aim of our study was to test the responsiveness of the most widely used cardiomyocyte platforms to simulated ischemia/reperfusion injury (SI/R) considering the effect of differentiation protocols.
Human and rat cell lines, furthermore primary cell cultures were used for in vitro viability assay with or without differentiation protocols. The cells were exposed to normoxic or simulated ischemia/reperfusion protocols: a simulated ischemic period of 6 hours was used for the differentiated cell cultures, while for the non-differentiated cells 16 hours of simulated ischemia followed by 2 hours of reperfusion was applied. The duration of simulated ischemia was determined based on preliminary experiment. Normoxic protocol was used for control series. The viability of the cells was measured by calcein assay.
Cell viability in non-differentiated human AC16 and rat H9C2 cardiac cell lines was significantly reduced (50%) by 16h SI / 2h R, which effect could be decreased by using cardioprotective positive controls (S-nitroso-N-acetyl-DL-penicillamine, insulin). In primary rat neonatal cardiac myocytes 6h SI / 2h R caused significant cell death (25%), while in primary human iPSC-derived cardiac myocytes the same protocol did not result in significant injury (10%). However, the 16h SI / 2h R protocol in primary human cells lead to a 37% of cell death. Responsiveness of different cell types to SI/R ranged between 40-100%.
It was presented that primary cardiac cells and non-differentiated cardiac cell lines respond significantly differently to simulated ischemia/reperfusion. Differentiation protocols in cell lines markedly affects their response to simulated ischemia / reperfusion as well as their response to positive control cardioprotective drugs. Comparative analyses of different types of cardiomyocytes can provide a good basis for accurate design of in vitro cardioprotective test systems.
Acknowledgements: this study was supported by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017 National Heart Program). Higher Education Institutional Excellence Programme of the Ministry of Human Capacities in

Hungary, within the framework of the Therapeutic Development thematic programme of the Semmelweis University. "Research Excellence Programme of the National Research, Development and Innovation Office of the Ministry of Innovation and Technology in Hungary (TKP/ITM/NKFIH)". The work was supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 739593.

Helium conditioning increases cardiac fibroblast migration which effect is not transferable via soluble factors or extracellular vesicles

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Background: Helium inhalation induce cardioprotection against ischemia/reperfusion injury, of which cellular mechanism is not fully elucidated. Extracellular vesicles (EVs) are cell-derived, nano-sized membrane vesicles which play role in cardioprotective mechanisms, but their function in helium-conditioning (HeC) has not been studied, yet.
Aim: We investigate how HeC affects cardiac fibroblasts, and if their HeC-induced EVs or other secreted factors propagate cardioprotection between cardiac cells.
Methods: Neonatal rat cardiac fibroblasts (NRCF) were exposed to glucose deprivation and HeC rendered by four cycles of 95% helium + 5% CO2 for one hour, followed by one hour normal culturing condition. 40 hours after HeC NRCF activation was analyzed with Western blot (WB) and migration assays. From cell supernatant, medium extracellular vesicles (mEVs) were isolated with differential centrifugation and analyzed with WB and nanoparticle tracking analysis. Supernatant from HeC-treated NRCF was transferred to naïve NRCF and immortalized human umbilical vein endothelia cells (HUVEC/TERT2) and migration and angiogenesis assay was performed.
Results: HeC accelerated the migration of NRCFs. Meanwhile, HeC did not increase the expression of markers of fibroblast activation, or secretion of mEVs from NRCF. HeC tend to decrease mEV secretion of NRCFs, but supernatant of HeC or CTRL NRCF did not accelerate the migration of naïve NRCF or affect angiogenic potential of HUVEC/TERT2.
Conclusion: Since HeC increased the migration of NRCF but did not induce myofibroblast transformation and since this effect was not transferable by EVs or soluble factors, HeC can activate potential cardioprotective mechanisms, but may not have prolonged effect on long-term fibrosis or on angiogenesis.

Does small-conductance Ca^{2+}-activated potassium channel contribute to sinus-node action potential?

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Background: Sinus pacemaking is based on tight cooperation of the intracellular Ca^{2+} handling and surface membrane ion channels. Spontaneous, rhythmical discharges of the sarcoplasmic reticulum (Ca^{2+} clock) induces inward Na^{+}/Ca^{2+} exchanger current that contributes in the diastolic depolarization together with the membrane currents (membrane clock). An important player of this synergistic cooperation could be the small-conductance Ca^{2+}-activated K^{+}-channels (I_{SK}) that may contribute to fine tuning of the repolarization in response of the intracellular Ca^{2+} changes, however, there is no data in the literature regarding the role of I_{SK} in sinus-node cells under normal condition.

Methods: In normal rabbit sinus-node cells, SK2 channel expression was investigated by immunoblot technique and confocal microscopy. Ionic currents were measured by patch-clamp technique.

Results: We found that SK2 channels expressed in sinus-node cells. In the presence of constant 500 nM Ca^{2+}_{i} in the pipette, we found apamin-sensitive current that showed increasing current density toward positive voltages. In the presence of 1 µM forskolin, we found apamin sensitive current during a sinus-node action potential.

Conclusion: Our data may suggest that I_{SK} contribute in the sinus-node repolarization, especially under high Ca^{2+}_{i} conditions. It may indicate an important role of I_{SK} under β-adrenergic stimulation.

Acknowledgement: This work was supported by grants from the National Research Development and Innovation Office (NKFIH PD-125402 (for NN), FK-129117 (for NN), the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (for NN), GINOP-2.3.2-15-2016-00006, the Ministry of Human Capacities Hungary (20391-3/2018/FEKUSTRAT and and EFOP-3.6.2-16-2017-0006).

The electrophysiological effects of cannabidiol on action potential and transmembrane potassium currents in dog and rabbit cardiac preparations: investigation of possible proarrhythmic risk

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Background: Cannabis use is associated with known cardiovascular side effects such as cardiac arrhythmias or even sudden cardiac death. The mechanisms behind these adverse effects are unknown.
Aim: The aim of the present work was to study the cellular cardiac electrophysiological effects of cannabidiol (CBD) on action potentials and several transmembrane potassium currents, such as the rapid (I_{Kr}) and slow (I_{Ks}) delayed rectifier, the transient outward (I_{to}) and inward rectifier (I_{K1}) potassium currents in rabbit and dog cardiac preparations.
Methods: Transmembrane potential and currents were recorded using conventional microelectrode and voltage clamp techniques, respectively.
Results: CBD increased action potential duration (APD) significantly in both rabbit (from 211.7±11.2. to 224.6±11.4 ms, n=8) and dog (from 215.2±9.0 to 231.7±4.7 ms, n=6) ventricular papillary muscle at 5 µM concentration. CBD decreased I_{Kr}, I_{Ks} and I_{to} (only in dog) significantly with corresponding estimated IC_{50} values of 4.9, 3.1 and 5 µM, respectively, without changing I_{K1}.
Conclusion: Although the IC_{50} value of CBD was found to be higher than literary C_{max} values after CBD smoking and oral intake, our results raise the possibility that potassium channel inhibition by lengthening cardiac repolarization might have a role in the possible proarrhythmic side effects of cannabinoids in situations where CBD metabolism and/or the repolarization reserve is impaired.
Keywords: Cannabidiol; Electrophysiology; Action potential; Potassium currents; Rabbit; Dog

Transcriptomic analysis and comparative characterization of rat H9C2, human AC16 and murine HL-1 cardiac cell lines

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Background: Cardiac cell lines and primary cell cultures are widely used to model various cardiovascular diseases *in vitro*. Despite the increasing number of publications using these models, limitations of these cell lines are still undetermined. The aim of our study was to compare the most commonly used cardiac cell lines to primary cultures and to mature cardiac tissues by transcriptomic analysis and morphological characterization.
Methods: H9C2 (rat), AC16 (human) and HL-1 (mouse) cardiac cell lines were differentiated towards a phenotype more resembling cardiomyocytes, by methods most widely used in the literature, and cells were harvested at stages of proliferation and differentiation. Whole left ventricular tissue, neonatal primary cardiac myocytes isolated from mice and rats, or human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) were applied as references. Transcriptome analysis and immunocytochemical detection of cardiac structural proteins were performed on all cell models.
Results: RNA expression of cardiac markers (e.g. *Tnnt2*, *Ryr2*, *Tnni3*) was markedly lower in cell lines compared to primary cells or hiPSC-CM and adult tissue controls. Differentiation procedures induced a significant increase in cardiac- and decrease in embryonic markers in AC16 and H9C2 lines; however, the overall expression pattern of investigated genes in all cell lines showed significant differences in comparison to corresponding myocardium or primary cultures. Immunocytochemistry confirmed low expressions of structural protein alpha-actinin and troponin I in cell lines.
Conclusion: Expression patterns of cardiomyocyte markers and mRNA profile indicates low-to-moderate similarity of cell lines to primary cells/cardiac tissues regardless the differentiation protocol used. These limitations should be taken into account while choosing cells as *in vitro* platforms to model cardiomyocytes and cardiovascular diseases.
Funding: The work was supported by the European Union's Horizon 2020 [No 739593], National Research, Development and Innovation Office [2020-4.1.1.-TKP2020, NVKP_16-1-2016-0017, FK134751, K128369], VEKOP-2.3.2-16-2016-00002, VEKOP-2.3.3-15-2016-00016, EFOP-3.6.3-VEKOP-16-2017-00009.

Cardiac ischemic postconditioning in pig: protective role of matrix metalloproteinase-9 and biglycan

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Background: Matrix metalloproteinase-2 (MMP-2) has been shown to be activated during myocardial ischemia-reperfusion (IR) injury and degrade cardiac intracellular contractile proteins. We previously found in a porcine model of acute myocardial infarction (AMI), that ischemic postconditioning (IPoC) did not decrease myocardial necrosis compared to ischemic control, however, significantly reduced the severity of myocardial microvascular obstruction (MVO).
Aim: Our aim was to investigate changes in cardiac MMP-2 and -9 activities and the levels of their extracellular substrate, biglycan in a porcine AMI model.
Methods: AMI was induced in domestic pigs (25-35 kg) with balloon catheter occlusion of the left descending coronary artery for 90 min. Animals were divided into sham-operated, ischemic control and IPoC (6× 30 s ischemia/reperfusion after 90-min occlusion) groups. At the end of the 3-hours or 3 days of reperfusion infarcted left ventricular tissue were taken to determine MMP-2, -9 activities and BGN levels.
Results Cardiac MMP-2 activity showed significant increase in IPoC group compared to ischemic control after 3h reperfusion but after 3d reperfusion no significant change was observed between the groups. MMP-9 activity changed similarly to MMP-2, a significant increase was seen at 3h reperfusion in IpoC group, that did not appear after 3d reperfusion. Cardiac BGN level in IPoC was significantly decreased compared to ischemic control after 3h reperfusion, which difference was abolished after 3d reperfusion due to the decrease of BGN level of ischemic control group.
Conclusion: IPoC decreased MVO and BGN level and increased MMP-2 and -9 activities in the infarcted left ventricle during early reperfusion. Further increase was found in MMP activities after 3 days in both groups, which affected BGN level only in ischemic control group. Early decrease in cardiac BGN level may contribute to the beneficial effect of IPoC on MVO.
Funding: GINOP-2.3.2-15-2016-00040; NVKP 16-1-2016-0017; EFOP 3.6.3-VEKOP-16-2017-00009

Characterization of acute stress by gastrointestinal and cardiac electromyography in awake rats

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Background: Stress and neuropsychiatric disorders are often associated with gastrointestinal (GI) symptoms and heart rate changes. The simultaneous detection of the myoelectrical slow-waves of the GI tract with the changes in heart rate and other parameters may provide more accurate information on patients' acute anxiety and psychological status, but such a method is currently not available in the clinical practice.
Aims: Investigation of acute stress response and stress reducing drugs with simultaneous detection of GI, cardiac, plasma corticosterone and body temperature changes in wakeful rats.
Methods: The sensor was placed under the abdominal skin of male SPRD rats (300-310 g) to record simultaneously the GI tract myoelectric signals, cardiac signals, and body temperature. The primer GI records were analyzed by fast Fourier transformation.
The rats were also treated with diazepam (5 mg/kg) or haloperidol (1 mg/kg) intraperitoneally. The changes in plasma level of corticosterone were determined by ELISA.
Results: Acute stress induced a significant increase in the electromyographic signals of each segments of the GI tract, as well as corticosterone plasma levels, body temperature and heart rate of the animals. Diazepam and haloperidol reduced stress-related parameters, except heart rate, as these agents cause tachycardia. The hypothermic action of diazepam masked the body temperature alterations in the treated rats.
Conclusion: Acute stress can be measured with a single sensor for simultaneous detection of GI- and cardiac myoelectric activity, plasma corticosterone levels and body temperature. During psychopharmacological studies in rats, the change in stress level can be accurately followed by GI electromyography that shows correlation with the changes in stress hormone levels. The other investigated stress parameters did not fully reflect the changes. Our method may open new perspectives in the diagnosis and treatment of psychosomatic disorders.
This work was supported by the Ministry of Human Capacities [Hungary grant 20391-3/2018/FEKUSTRAT] and EFOP-3.6.1-16.

The role of reverse Na^+/Ca^{2+} exchanger in the sinus node spontaneous automaticity

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Introduction: Recent results suggest a tight cooperation between the intracellular Ca^{2+}-handling ('Ca^{2+}-clock') and surface membrane ion channels ('membrane clock') in the mechanism of the spontaneous cardiac pacemaking, which is referred as coupled-clock mechanism. The key role of depolarizing forward Na^+/Ca^{2+} exchanger (NCX) is experimentally proved, because spontaneous local Ca^{2+}-releases activates the forward NCX which accelerates the diastolic depolarization. Since experimental evidence regarding the role of reverse NCX in the sinus node pacemaking was missing so far, our aim was to investigate the role of reverse NCX current by applying the selective NCX inhibitor ORM-10962.
Methods: Experiments were performed on spontaneously beating rabbit sinus node cells. Ion currents were measured by whole cell configuration of patch clamp, using canonic action potentials measured with perforated patch-clamp technique as command potentials. Ca^{2+} transients were monitored by applying Fluo-4AM fluorescent dye.
Results: The difference current calculated after the application of 1 µM ORM-10962 supports the existence of reverse NCX current in the early phase of the action potential. The lack of reverse NCX decreases the Ca^{2+}_i and selective NCX inhibition exerts better improvement in Ca^{2+}_i when reverse NCX is abolished.
Conclusion: Here we demonstrate the first direct evidence regarding the presence and the role of reverse NCX current in the sinus node. Our experimental results suggest that reverse NCX has an important role in the cardiac pacemaking, since the Ca^{2+}-influx mediated by the reverse mode can contribute to the refueling of sarcoplasmic reticulum and Ca^{2+}-clock. Thereby, reverse NCX indirectly facilitates the forward NCX and can accelerate the spontaneous automaticity.
Funding: This work was supported by grants from the National Research Development and Innovation Office (NKFIH PD-125402 (for NN), FK-129117 (for NN), the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (for NN), GINOP-2.3.2-15-2016-00006, the Ministry of Human Capacities Hungary (20391-3/2018/FEKUSTRAT and EFOP-3.6.2-16-2017-0006 (for N.N.), EFOP 3.6.3-VEKOP-16-2017-00009 (for N.T).

Investigation of cardiotoxicity by dipeptidyl-peptidase-4 inhibitors on a human cardiomyocyte cell line as well as on samples from chronic heart failure patients

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Background: Dipeptidyl-peptidase-4 (DPP4) inhibitors are new therapeutic tools for type 2 diabetes. The SAVOR-TIMI-53 clinical trial has revealed increased HF-associated hospitalization in saxagliptin treated patients. Although this critical side effect could limit the therapeutic use considerably, the mechanism by which DPP4 inhibitors damage the heart is still unclear.
Aims: We aim to set up a relevant cellular platform to investigate mechanistically DPP4 inhibition, and the role of potentially important substrates (e.g. Substance P and Neuropeptide Y). Moreover, we aim to determine the expression of DDP4 and its neuropeptide substrates in human and cellular samples.
Methods: Western blot and radioimmunoassay were used to investigate the expression of DPP4 and its neuropeptide substrates in human hearts and in AC16 cell-line. Viability measurements with calcein staining and scratch assay were used to test the potentially toxic effect of DPP4 inhibitors. The localization of DPP4 mRNA was determined with RNA Scope *in situ* hybridization.
Results: Expression of DPP4 protein decreased in left ventricular samples of patients with HF compared to healthy controls. AC16 human cardiomyocyte cell line expresses DPP4 enzyme. In human hearts DPP4 mRNA is detectable in cardiomyocytes, while other cell types (endothelial cells, fibroblasts, macrophages, and smooth muscle cells) show negligible expression. Treatment with the tested DPP4 inhibitors alone or combined with neuropeptides don't affect cellular survival; although, in scratch assay experiments treatments with neuropeptides decreased cell migration speed in isolated neonatal rat cardiomyocyte-fibroblast co-culture.
Conclusion: Decreased activity of DPP4 may play a role in the pathomechanism of end-stage congestive heart failure. The DPP4 enzyme could be important as a compensating mechanism against the elevated sympathetic activity in HF and for the altered neuropeptide tone. Inhibition of DPP4 could decrease this adaptive mechanism thereby exacerbating myocardial damage.
Funding: Horizon 2020 research and innovation programme No 739593, EFOP-3.6.3-VEKOP-16-2017-00009, VEKOP-2.3.3-15-2016-00016, TKP, NKFIA, NVKP-16-1-2016-0017

Rofecoxib alleviates hypercholesterolemia-induced myocardial dysfunction

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Background: Hypercholesterolemia (HC) is a well-known risk factor for development of cardiovascular diseases, but little is known about its direct cardiac effects and treatment of HC-induced myocardial dysfunction. We have previously shown that the COX-2 inhibitor rofecoxib improved cardiomyocyte contractility in vitro. Nitrosative stress plays a central role in the development of HC-induced myocardial dysfunction. Elevated levels of peroxynitrite may increase COX-2 protein expression, however the role of COX-2 inhibition on nitrosative stress is not known.
Aim: In the present study we aimed to investigate the effect of rofecoxib on HC-induced cardiac dysfunction and nitrosative stress in an in vivo rat model.
Methods: Male Wistar rats were fed with high cholesterol and cholate-enriched or standard chow for 12 weeks. After 8 weeks on chow, animals were treated with 5.12 mg/kg/day rofecoxib or its vehicle. Following treatment, hemodynamic measurements were performed using a pressure-volume catheter and the extent of nitrosative stress was determined by immunohistochemical staining for 3-nitrotyrosine.
Results: HC caused mild systolic and marked diastolic dysfunction (ejection fraction [EF]: NC+vehicle: 55.7±1.6%, HC+vehicle: 47.8±3.8%; end-diastolic pressure [EDP]: NC+vehicle: 4.7±0.3 mmHg, HC+vehicle: 6.9±1.0 mmHg, $p < 0.05$). This effect was significantly improved by rofecoxib (HC+rofecoxib: EF: 56.1±2.2%, EDP: 4.8±0.6 mmHg, $p < 0.05$). HC increased the level of 3-nitrotyrosine in left ventricular cardiac tissue which was not affected by rofecoxib treatment.
Conclusion: Rofecoxib improved HC-induced cardiac dysfunction. This effect was independent of reduction of nitrosative stress. Therefore, further experiments may reveal the relevance of COX-2 inhibitors in treatment of hypercholesterolemia-induced myocardial dysfunction.
Acknowledgements: This study was supported by the National Research, Development and Innovation Office of Hungary (NKFIA; NVKP-16-1-2016-0017 National Heart Program).

Diabetes increases secretory activity of pancreatic ductal epithelial cells

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Objectives. Preexisting diabetes mellitus is considered to be an important risk factor for acute and chronic pancreatitis. The aim of this study is to characterize exocrine functions of ductal epithelial cells of the pancreas under diabetic conditions. Identifying abnormalities in secretion can help in the development of both preventative therapy and disease treatment for diabetic patients with acute or chronic pancreatitis.
Methods. Diabetes was induced in WT and CFTR KO mice by ip. administration of streptozotocin and disease development was confirmed by glucose tolerance test. Intra-interlobular pancreatic ductal fragments were isolated by enzymatic digestion. Pancreatic ductal fluid, Cl^- and HCO_3^- secretion was measured by *in vivo* fluid secretion measurements and fluorescence microscopy. Pancreas tissue sections were prepared and immunohistologically stained against CFTR to observe expression and ductal morphology.
Results. Pancreatic ductal fluid, Cl^- and HCO_3^- secretion significantly increased in diabetic mice. This increase is also present in diabetes induced CFTR KO mice. Fluorescence tissue staining does not reveal difference in CFTR expression between control and diabetic mice.
Conclusion. Our results suggest that pancreatic ductal Cl^- and HCO_3^- secretion is increased in diabetes, independently from CFTR Cl^- channel activity.
This project was supported by CFRD-SRC Grant (No.: SRC 007), the National Research, Development and Innovation Office (FK123982) and the Economic Development and Innovation Operative Programme Grants (GINOP-2.3.2-15-2016-00015) and by the National Research, Development and Innovation Office, by the Ministry of Human Capacities (EFOP 3.6.2-16-2017-00006).

DNA damage induced by novel synthetic all-trans retinoic acid analogues in CHO cells

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Background: All-trans-retinoic acid (ATRA), the active metabolite of vitamin A, plays a pivotal role in cell differentiation, proliferation and embryonic development. It is an effective therapy for dermatological disorders and malignancies, but neurodegenerative diseases have been suggested as potential indications. Therefore, novel ATRA derivatives were synthesized for more beneficial pharmacokinetic and safety profiles.
Aims and methods: We selected a non-cytotoxic concentration range of novel diphenylacetylene-based retinoids and evaluated their genotoxicity. DC360 is a fluorescent ATRA analogue and DC324 a non-active derivative of DC360. EC23, DC525, DC540, DC645 and DC712 are promising ATRA analogues. Cell viability was determined by ATP assay,and genotoxicity was tested by comet assay after 24 hours treatments of CHO cells.
Results: No cytotoxicity was observed in the 10^{-6}-10^{-5} M concentration range. All compounds induced DNA migration similar to ATRA, but DC324, DC360 and EC23 did so to a greater extent, particularly at higher concentrations. These three compounds have the most hydrophobic structures, therefore, they may exhibit stronger off-target interactions with proteins and DNA. The dihydroquinoline region of DC360 could be reactive towards cellular components, thus, it may be the cause of the stronger genotoxic effects.
Conclusion: ATRA and its synthetic derivatives induce DNA strand breaks showing possible genotoxic mechanism. Identification of the molecular basis of genotoxicity as a result of retinoid treatment is important to understand the complex biological activities of these compounds.
Funding: This work was supported by the 2017-1.2.1-NKP -2017-00002 (NAP-2; Chronic Pain Research Group) GINOP-2.3.2-15-2016-00050 “PEPSYS”, EFOP-3.6.2-16-2017-00006 and EFOP-362-16-2017-00008. Angéla Kecskés was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences. Project no. TKP2020-IKA-08 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2020-4.1.1-TKP2020 funding scheme.

Acknowledgements: The authors thank Cecília Disztl for technical assistance.

Cardiac electrophysiological effects of rofecoxib following simulated ischemia-reperfusion in hypercholesterolemic rats

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Background: In our previous studies, we found that rofecoxib possessed cardiac electrophysiological adverse effects only revealed during ischaemia/reperfusion, manifested as a proarrhythmic effect. However, rofecoxib decreased the infarct size and increased the survival of adult rat cardiac myocytes. Interestingly, studies suggest, that diet-induced hypercholesterolemia reduces post-ischemic infarct size after myocardial ischemia- reperfusion injury.
Aim: Our aim was to investigate whether hypercholesterolemia and rofecoxib may have a protective effect in preventing reperfusion arrhythmias**.**
Methods: Two groups of rats were fed a high cholesterol diet and a normal diet for 3 months. The conventional microelectrode technique was used to record action potentials from rat left ventricular papillary muscles. Under normoxic and ischaemic conditions, the following parameters were measured in the presence or absence of 1µM or 10 µM rofecoxib: conduction time, action potential amplitude, action potential duration at 75% and 90% repolarization (APD_{75}, APD_{90}). The effects of rofecoxib during test ischaemia and reperfusion and the influence of hypercholesterolemia were studied.
Results: Under normoxic conditions, there was no significant difference in the measured parameters between the hypercholesterolemic and normal-fed groups. Rofecoxib did not influence electrophysiological parameters in normoxic conditions. In both groups following 30 minute ischaemia, APD_{90} was significantly increased during reperfusion compared to APD_{90} in baseline. However, there was no significant difference between the normal-fed and hypercholesterolemic groups under the effect of vehicle and 1 µM rofecoxib. In the presence of 10 µM rofecoxib, APD_{90} decreased to a lesser extent after test ischemia in hypercholesterolemic rats and increased to a lesser extent after reperfusion compared to rats fed a normal diet. However, the difference was not statistically significant.
Conclusion: Our preliminary experiments did not convincingly demonstrate that hyprcholesterolemia and rofecoxib had a preventive effect against reperfusion arrhythmias. However, repolarization increased lesser extent under the influence of rofecoxib in the hypercholesterolemic group which may have an antiarrhythmic effect. Further studies are needed to clarify this issue.
Supported by OTKA K-128851.

Investigation of the role and influence of sex hormones on hepatic metabolism of diclofenac in a rat liver perfusion model

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Background and aim: There is limited knowledge of Cross-Sex Hormone Therapy (CSHT), particularly regarding drug metabolism. We investigated the role and influence of sex hormones on hepatic metabolism of diclofenac in male, female, castrated, ovariectomized and CSHT treated rats. An *ex vivo* isolated rat liver perfusion was used for this study. This method has several advantages, such as the architecture of the organ is saved, compare to *in vivo* model higher dose of drugs can be tolerated and large number of perfusion samples can be collected.
Methods: After cannulation and removal, the liver of male, female, castrated, ovariectomized or CSHT treated SPRD rats (300-320 g) was placed into the porous chamber filled with buffer vapor. Oxygenated perfusion buffer was pumped into the organ in a recirculating pattern with constant flow rate. The pressure, pH of buffer and the level of lactate dehydrogenase were controlled during experiment continuously. The concentration of diclofenac and its main metabolites were determined via targeted reversed-phase LC-MS/MS method. ELISA kit was used for measuring the level of hepatic enzymes CYP2C8 and CYP2C9.
Results: The viability of the perfused liver was around 2-4 hours. The dynamic alterations of the concentrations of 4′-hydroxydiclofenac (Phase I. metabolite), and diclofenac-1-O-acyl glucuronide (Phase II. metabolite) were determined in the perfusion fluid. Suppression of testosterone leaded to significant increase in the level of Phase I and II. metabolites compare with males and CHST treated males. But interestingly, elimination of female sexual hormones induced significant decrease in the level of both phases' metabolites. The modification in CYP2C8 and CYP2C9 enzymes confirmed these results.
Conclusion: The CSHT modified the metabolism of diclofenac. Our results suggest that gender modification processes may influence the drug metabolism.
Acknowledgements: EU-funded Hungarian grant EFOP 3.6.1-16-2016-00008 is acknowledged. This work was supported by the Ministry of Human Capacities. [Hungary grant 20391-3/2018/FEKUSTRAT]

The influencing role of leptin and adiponectin systems in pregnant rat uterine contractility

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Background: Certain adipokines are known to influence smooth muscle function. Leptin reduces uterine contractility but no data is available about the ontogeny of leptin receptors during the gestational period. No information is available regarding the uterine action of adiponectin during the whole pregnancy.
Aim: Our aims were to investigate the modifying roles of leptin and adiponectin on the contractility of intact and endometrium-denuded pregnant uteri and to determine the changes in receptor expressions during gestation.
Methods: In an isolated organ bath, we performed contractility measurements with uterine rings taken from pregnant Sprague-Dawley rats on different gestational days. The intact or denuded uterine contractions were stimulated with KCl and cumulative concentrations of leptin (10^{-12}–10^{-8} M) or adiponectin (10^{-13}-10^{-9} M) were added to chambers. The expressions of leptin and adiponectin receptors were determined by RT-PCR, Western blot analysis and fluorescent immunohistochemistry.
Results: The utero-relaxant effect of leptin decreased towards the end of gestation. However, leptin inhibited the contractions of endometrium-denuded uteri throughout pregnancy. In intact uteri, the relaxing effect of adiponectin increased until day 18 of gestation, then ceased on the last day. When the endometrial layer was removed, a gradual reduction was seen in its effect. Leptin and AdipoR1 receptor presence were strong throughout gestation, the uterine AdipoR2 receptors diminished near the end of pregnancy. Immunostaining confirmed these changes in receptor densities and revealed the differences in the myometrial and endometrial receptor expressions.
Conclusion: Both adipokines reduced the contractility of intact and denuded uteri in early- and mid-pregnancy. These adipokines play a role in pregnant uterine relaxation, but their endometrial receptors may regulate these actions. We suggest that leptin and adiponectin participate in the maintenance of uterine quiescence, and they might also modify the outcome of gestational period.
Acknowledgements: Ministry of Human Capacities, Hungary grant 20391-3/2018/FEKUSTRAT is acknowledged.

Potentiation of the uterus relaxing effects of sildenafil with terbutaline and nifedipine: in vitro and in vivo studies on pregnant rat myometrium

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Background: Preterm birth is among the greatest challenges in obstetrical practice. Phosphodiesterase5-inhibitors (PDE5-Is) are expansively used for erectile dysfunction. However, the presence of PDE5 was proven in the myometrium.
Aim: The aim of this study was to investigate how other tocolytic agents influence the uterus relaxant effects of sildenafil *in vitro* and *in vivo*.
Methods: In the *in vitro* studies contractions of uterine rings from 22-day-pregnant Sprague-Dawley rats were measured in an organ bath. The 25 mM KCl stimulated contractions were inhibited by the cumulative concentrations of sildenafil citrate (10^{-10} − 10^{-4} M). The effects of sildenafil were also investigated in the presence of terbutaline (10^{-8} M) or nifedipine (10^{-9} M). The *in vivo* electromyographic studies were carried out under isoflurane anesthesia with a subcutaneous disk electrode pair placed above the myometrium. The rats were treated with 0.15 µg/kg terbutaline or nifedipine intravenously with raising doses (from 0.05 to 5 mg/kg) of sildenafil in cumulative bolus injection.
Results: Both *in vitro* and *in vivo* contractions were inhibited concentration-dependently by sildenafil on 22-day pregnant rat uteri. The sildenafil-induced relaxation was potentiated by the presence of β_2-adrenergic agonist terbutaline or the Ca-channel blocker nifedipine. The in vivo relaxing effect of sildenafil (4.2 µg/kg) was sixfold increased by terbutaline (0.15 µg/kg). Similar potentiation was found between sildenafil and nifedipine.
Conclusion: Both terbutaline and nifedipine were able to enhance the relaxing effect of sildenafil in small concentration, especially at low concentration range. Therefore, the combination of sildenafil and terbutaline or nifedipine may have clinical significance. Additionally, these combinations are supported by the fact that the pharmacodynamic, pharmacokinetic parameters and risks of these agents are well-known individually.
Acknowledgement: This work was supported by the Ministry of Human Capacities. [Hungary grant 20391-3/2018/FEKUSTRAT] and the Albert-Szent-Györgyi Scholarship of Albert Szent-Györgyi Medical School, University of Szeged.

The effect of citral on uterine contraction in rat

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Background: The aquaporin (AQP) water channels are small hydrophobic integral membrane proteins. Most of them are expressed in the female reproductive tissues and they supposed to play important role during pregnancy. In our previous studies, AQP5 expression was found to be predominant during pregnancy in rats, although it was significantly down regulated at the last gestational day. We hypothesized that an osmotic pathway - through AQP5 - may modify the transient potential vanilloid 4 **(**TRPV4) function and uterine contraction.
Aim: The aim of our study was to investigate the effect of citral on the cooperation between AQP5 and TRPV4 in the late-pregnant rat uterus in vitro and in vivo to identify their mutual influences on myometrial contraction.
Methods: In vitro uterine contractions were evoked by KCl and the response was modified with citral. The expressions of TRPV4 and AQP5 were measured by RT-PCR and Western blot techniques. The lengths of gestational periods were determined in normal and LPS-induced (preterm) births after citral treatment, in vivo.
Results: Citral significantly decreased the uterine contraction on day 22 of pregnancy. AQP5 expression was significantly increased after citral incubation; however, TRPV4 expression did not show any significant changes. After citral pretreatment, the gestational period was extended both in normal and LPS-induced (preterm) births.
Conclusion: Our results suppose that the down regulation of AQP5 may initiate hypertonic stress activating TRPV4 and uterine contraction on the last day of the gestational period. The clinical translation of our results might open a new possibility for the therapy and prevention of preterm birth targeting AQP5 and TRPV systems.
Acknowledgement: This work was supported by the National Research, Development and Innovation Office, Hungary (grant NKFI-FK19-132499).

Label-free protein and lipid quantification of extracellular vesicles by infrared spectroscopy

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Background: Extracellular vesicles (EVs) are in the focus of scientific interest: it was recognized that cells emit nano- and micro-sized structures bounded by a phospholipid bilayer, which play a significant role in intercellular communication between cells. Infrared (IR) spectroscopy, completed with standardized measurement conditions and data processing procedures, was recently introduced to characterize EVs.
Aim: Since IR spectroscopy provides information about proteins and lipids and/or other EV components simultaneously, a single assay quantification protocol for both proteins and lipids (phospholipids) might be feasible.
Methods: EVs (microvesicles) formed ex vivo in erythrocyte concentrates were examined by IR spectroscopy. The used ATR FT-IR technique requires small sample amount (~3µl) without any sample preparation and enables the measurement of aqueous suspensions with low concentration.
Results: The integrated area of the amide I band proved to be proportional to the protein quantity in the EV samples (up to 1 mg/ml), regardless of its secondary structure. Our results based on a calibration with bovine serum albumin was further affirmed also by multivariate modelling on raw spectra using Partial Least Squares regression[1]. Lipids are essential molecular components of EVs, but at the moment only limited knowledge about their quantification is available[2]. To extend the possibilities of IR spectroscopy, an effort has been made to elaborate an adequate lipid calibration, by using reference vesicles of bovine serum albumin and synthetic lipids.
Conclusion: A fast, label-free method based on the area of selected IR bands might gain an important role in EV research. Compact ATR-FTIR instruments are already available and the proposed spectral analysis protocols can be readily automated.
Acknowledgement: This study was funded by the National Research, Development and Innovation Office, Hungary under grants NVKP_16-1-2016-0007 and K131594. ZV is supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

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Non-genomic uterorelaxant actions of corticosteroids

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Background: The non-genomic effects of corticosteroids on uterine contractions are unknown.
Aim: We aimed to identify the short-term effects of a glucocorticoid and a mineralocorticoid on non-pregnant and pregnant rat uterine contractions to estimate their tocolytic potential.
Methods: The in-vivo contractility studies were performed with uterine tissues from non-pregnant and 22-days-pregnant SPRD rats. Cumulative dose-response of fludrocortisone (FLU) and dexamethasone (DEX) were measured alone or in the presence of steroid receptor antagonists (mifepristone – MIF- or spironolactone). [35S]GTPγS and cAMP immune assay were carried out to detect the activated G-proteins and cAMP, respectively. In-vivo uterine action of single doses of FLU and DEX were measured by smooth muscle electromyography. Results were statistically analyzed with unpaired t-test.
Results: FLU and DEX elicited relaxing effect both on pregnant (31 and 29%) and non-pregnant (41 and 54%) uteri in vitro, respectively. Endometrium removal did not modify their actions. MIF completely inhibited the relaxing effect of both drugs except the effect of DEX in non-pregnant uterus. GTPγS studies showed a MIF sensitive elevation in activated G-proteins both in pregnant and non-pregnant uteri by DEX, whereas FLU did not change the activation. DEX and FLU relaxed the pregnant uteri in vivo in a MIF sensitive way.
Conclusion: During the 30 min experiments, moderate relaxing actions of both drugs were observed proving their non-genomic effect on uteri also supported by the increased G-protein activation. Non-pregnant uterus seems to be more sensitive to FLU and DEX in vitro, but only the pregnant uteri responded to non-genomic steroid action in vivo.
Fast actions of FLU and DEX lead to pregnant uterine relaxation. Their single high doses may have significance in the treatment of threatening premature labor.
Acknowledgements: This work was supported by the Ministry of Human Capacities. [Hungary grant 20391-3/2018/FEKUSTRAT].

The effect of dimethyl trisulfide in the forced swim test is independent of the NK1 receptor

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In our previous study potential central nervous system depressant effect of dimethyl trisulfide (DMTS) was identified. DMTS is a chemically stable polysulfide that readily crosses the blood-brain barrier. Literature suggests antidepressant effect of sulfide. Antidepressant effect of NK1 receptor antagonists is weel documented in rodents. Based on the clinical demand for rapid-acting antidepressant, we set out investigate the activity of DMTS in the forced swim test and the possible involvement of NK1 receptors.

Experiments were conducted with male C57BL/6 and NK1 receptor gene-knockout (KO) mice. Animals remained naïve or were administered either vehicle (1.5% m/v polysorbate 80 in physiological saline) or DMTS (50 mg/kg i.p.). Forced swim test was performed in 24 °C water 30 min after drug application. Video recordings of the tests were processed with Noldus EthoVision XT 15 software. Highly active duration and activity frequency parameters were evaluated. Duration of inactivity was calculated by subtracting highly active duration from the total duration of observation.

DMTS treatment elevated activity frequency in NK1 receptor KO animals compared to naïve and vehicle-treated animals. Inactive duration and highly active duration showed a trend to decrease and increase, respectively. DMTS did not change the parameters in C57BL/6 mice.

DMTS ameliorated depression-like behavior in NK1 KO mice in the forced swim test. The effect was not larger than the one seen in other mouse strains. Substance P and NK1 receptors can be ruled out as mediators of the effect of DMTS.

The study was financed by the grant OTKA FK 132454 from the National Research, Development and Innovation Office.

Neuroprotective effect of estrogen-like ANGELS compounds

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Background: Neuronal loss of basal forebrain cholinergic neurons (BFC) can be prevented by estrogen (E2) in an animal model of Alzheimer's disease (AD). However, E2 might have several side-effects. Recently promising new estrogen-like compounds were identified, called "Activators of Non-Genomic Estrogen Like Signaling" (ANGELS).
Aim: Based upon *in silico*, and *in vitro* testing we aimed to find ANGELS compounds with neuroprotective potential *in vivo*, in an animal model of AD, but without estrogen-like side effects.
Methods: As estrogen receptor α (ERα) is responsible for the neuroprotective effect of E2, the assortment of the steroid molecules was achieved by computational docking to ERα. Eight molecules were tested for classical effects *in vitro*, investigating their estrogen responsive element (ERE) activating potential on ERE-luciferase transfected MCF-7 cells. The most effective candidates were tested for uterotropic effects by measuring the uterus weight on ovariectomized mice. Finally, the neuroprotective effects of ANGELS compounds were examined *in vivo* after $A\beta_{1-42}$ microinjection to the nucleus basalis magnocellularis (NBM). The $A\beta_{1-42}$-induced cholinergic fiber loss in the somatosensory cortex (SC) and cholinergic cell loss in the NBM were determined with quantitative histochemistry and immunohistochemistry.
Results: From the tested ANGELS we found three molecules without any classical ERE mediated actions and uterotropic effects. These compounds significantly restored the $A\beta_{1-42}$-induced cholinergic fiber loss in SC and they did not affect the number of cholinergic cell bodies in NBM.
Conclusions: Combining *in silico* computational screening with *in vitro* and *in vivo* testing proved to be useful for selection of potential neuroprotective compounds against $A\beta_{1-42}$-induced neurotoxicity. We believe that these compounds provide a novel approach in AD therapy.
Funding: This work was supported by the Hungarian Brain Research Program (KTIA_NAP_13-2014-0001,20017-1.2.1-NKP-2017-00002), the Hungarian Scientific Research Fund (OTKA; 112807), the European Union, and co-financed by the European Social Fund and the University of Pécs.

Interleukin-1 mediates chronic restraint stress-induced neuropathic hyperalgesia in a mouse model

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Background: Chronic stress is an etiological factor of fibromyalgia, and worsens a broad range of several pain conditions, such as chronic arthritis and neuropathy. The treatment is unsolved, therefore, it is essential to reveal the pathophysiological mechanisms and identify novel therapeutic target(s).
Aim: Cytokines are known to regulate immunological and inflammatory processes both in the periphery and the central nervous system. Interleukin-1αβ (IL-1) is involved in neuroinflammation related to both stress and pain, but the relationship between these pathways are unclear. Therefore, we investigated the role of IL-1 in a mouse model of chronic restraint stress-induced pain.
Methods: Female IL-deficient and C57Bl/6J wildtype (WT) mice were restrained in a plastic tube for 6 hours daily for 4 weeks. The thermo- and mechanonociceptive thresholds of the paw were determined weekly by increasing temperature hot plate and dynamic plantar esthesiometry, cold tolerance by withdrawal latency from icy water. Light-dark box, tail suspension and forced swim tests were performed to evaluate behavioral changes induced by restraint. Thymus and adrenal-gland weights were measured at the end of the experiments.
Results: Restraint stress induced significant, approximately 15-20% mechanonociceptive threshold decrease (hyperalgesia) after 2 weeks in WT, but not in IL-deficient mice. Cold tolerance decreased by 70% from the end of the first week similarly in both stressed groups. Chronic stress induced anxiety- and depression-like behaviors together with decreased thymus and increased adrenal gland weights after 2 weeks of restraint in both groups, which vanished by the end of the 4th week demonstrating adaptation.
Conclusion: We provide evidence that IL-1 mediates chronic stress-induced mechanical hyperalgesia suggesting that neuroinflammation and central sensitization might be the main factors in stress-related pain. These results point out analgesic potentials of IL-1 blocking drugs, such as anakinra, in these conditions.
Acknowledgements: National Brain Research Program 2017-1.2.1-NKP-2017-00002 (NAP-2; Chronic Pain Research Group), GINOP-2.3.2-15-2016-00050 (PEPSYS), EFOP 3.6.2-17-2017-00008 N (2017-2019)

Evidence for the effect of lipid raft disruption in mouse model of pain with different mechanism

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Background: Transient Receptor Potential (TRP) Vanilloid 1 and Ankyrin 1 (TRPV1/TRPA1) are nocisensors expressed in primary sensory neurons. Capsaicin, resiniferatoxin, noxious heat activate TRPV1, while formaldehyde, noxious cold, mechanical stimuli activate TRPA1. The specialized microdomains, lipid rafts of plasma-membrane rich in cholesterol, spihngomyelins and gangliosides, form functional complexes with TRP channels. Sphingomyelinase (SMase), myriocin (Myr), or synthetic products, as our carboxamido-steroid (C1) and methyl-β-cyclodextrin (MCD) are useful tool to disrupt rafts and investigate their effect on TRP channel.
Aim: Aim of this study is to prove antinociceptive effect of lipid raft disruption in mouse model of pain with different mechanism based on our earlier *in vitro* results.
Methods: Animals were pretreated locally with 50 mU SMase, 1 mM Myr, 100 or 500 µM C1 and 15 mM MCD. Eye-wiping movements were counted after 30 µg/ml capsaicin instillation into the right eye of the animal. Resiniferatoxin (0,1 µg/ml) was injected into the right hindpaw, and the development of thermal allodynia and mechanical hyperalgesia was measured with an increasing temperature HotPlate and Dynamic Plantar Aestesiomether, respectively. Intraplantar administered formalin (2,5%) evoked nocifensive behavior time were measured in two phases.
Results: In the "eye-wiping" test all compounds reduced the number of wipings, furthermore Myr and C1 had prolonged effect. SMase and Myr alleviated the RTX-induced thermal allodynia that developing mainly by peripheral mechanisms, and SMase and C1 diminished mechanical hyperalgesia that involves both peripheral and central components. Only Myr did not reduce duration of nocifensive behaviour after formalin injection in the second phase related to neurogenic inflammatory mechanisms.
Conclusion: We proved that not only cholesterol but also sphingolipids have important role in the lipid raft integrity and targeting them might be a novel pharmacological method in the pain management.
Support: National Brain Research Program 20017-1.2.1-NKP-2017-00002, GINOP-2.3.2-15-2016-00050, GINOP-2.3.2-15-2016-00048 and EFOP-3.6.2-16-2017-00008, János Bolyai Fellowship.

Angiotensin IV's neuromodulation of the glutamate neurotransmission in the prefrontal cortex

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Background: More than 40 million people are diagnosed with dementia worldwide and most types of dementia can't be cured. Proper fine-tuning of the glutamatergic neurotransmission in the prefrontal cortex appears to play a crucial role in normal cognitive function. Overactivity of the N-methyl D-aspartate (NMDA) receptors can lead to apoptosis and neurodegeneration, therefore it is a potential target in the treatment of dementia.
Aim: In this work we aimed to investigate the effect of the Angiotensin IV on the NMDA receptor function in the pyramidal neurons of the layer V of the PFC and map the cell-type specific localization of the angiotensin receptor 4 (AT4R), known to be identical to the insulin-regulated aminopeptidase (IRAP).
Methods: AT4R expression was analyzed by immunohistochemistry in tissue sections from 9-12 days old and 6 months old Wistar rats. Whole-cell patch clamp experiments were performed on pyramidal cells located in the layer V of the PFC from 9-12 days old Wistar rats. 30 µM NMDA produced reproducible inward cation currents. Following a control NMDA current, Ang IV was used to explore its effect, other IRAP inhibitors were added to enlighten the pathway, and synaptic isolation was applied to investigate the role of interneurons.
Results: AT4R expression was prominent in both pyramidal cells and GABAergic interneurons, while no expression was detected in glial cells. Ang IV inhibited the NMDA-induced ion currents in the pyramidal cells. Ang IV had kept its modulatory effect when synaptic isolation was applied, which suggests a direct impact on the pyramidal cells without interneuronal mediation.
Conclusion: Angiotensin IV has an inhibitory effect on the NMDA receptors in the pyramidal cells of the PFC, therefore its receptor may be a potential pharmacological target in the treatment of dementias.

INVESTIGATION OF PACAP1-38 EYE-DROPS TREATMENT IN GLAUCOMA

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Introduction: Approximately 4.5 million people worldwide are blind due to glaucoma, which makes it the second most common cause of irreversible blindness. This progressive condition develops by the blockage of the aqueous humor drainage system leading to intraocular hypertension. Progression of the condition causes the loss of the retinal ganglion cells and their axons. PACAP has shown protection against retinal degenerations in several diseases, such as excitotoxicity, hypoxia, or diabetic retinopathy. Also we proved that PACAP passes through ocular barriers and so, retinoprotection can be achieved also by eye drops. Accordingly, the aim of the present study was to examine the possible neuroprotective effects of topically administered (eye drops) PACAP in glaucoma.
Methods: We used 20 adult, male *Sprague-Dawley* rats for this study. Polystyrene microbeads (10µl, 10µm) were injected into the anterior chamber of the right eyes with 33G Hamilton syringe, while the control group recived the same volume of PBS serving as control. After the microbeads injections we treated the eyes with PACAP1-38 eye drops for 4 weeks. Intraocular pressure (IOP) was monitored with tonometer and retinal morphological changes were followed with Optical Coherence Tomography.
Results: In the PACAP1-38 treated group we observed a lower IOP and less severe damage in the retinal thickness and GCL compared to the microbeads injected, control animals.
Conclusion: Based on our results, we proved that topical administration of PACAP is neuro protective in glaucoma, providing the basis for future therapeutic administration
Supports: NKFIH129190, Bolyai Scholarship, NAP 2017-1.2.1-NKP-2017-00002, GINOP-2.3.2-15-2016-00050 “PEPSYS”, MTA-TKI 14016, EFOP-3.6.2-16-2017-00008, EFOP 3.6.1-16.2016.00004, Centre for neuroscience, PTE AOK-TANDEM 2019 Grant, “The role of neuro-inflammation in neurodegeneration: from molecules to clinics”. Higher Education Institutional Excellence Program of the Ministry of Human Capacities in Hungary, within the framework of the FIKPII. TUDFO/47138-1/2019-ITM FIKP program.

Utilization of the Noldus EthoVision XT 15 for characterization of the effect of dimethyl trisulfide on locomotor activity, anxiety-like and depression-like behavior in mice

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Based on our previous data on dimethyl trisulfide (DMTS), we set out to investigate the influence of the drug on stress and depression-like behavior. We decided to employ automated evaluation of behavioral animal experiments. Applicability of Noldus EthoVision XT 15 software was investigated in the open field (OFT) and forced swim tests (FST).
Wild-type (WT) of TRPA1 ion channel gene-knockout mice and C57Bl/6 animals were used in OFT. Beside these, TRPA1 knockout (KO) and NK1 receptor KO animals were used in FST. Animals were treated with DMTS (50 mg/kg, i.p.) 30 min before tests. Mice were introduced into the center of the open field arena and were filmed. The floor of the arena was changed between experiments. Mice were put into 24 °C water during FST and were filmed. Video recordings were evaluated with Noldus EthoVision XT 15.
In TRPA1 WT animals DMTS did neither reduce total distance moved nor duration of movement in OFT. Time spent in the center was smaller compared to naïve animals. DMTS did not alter the parameters of C57BL/6 mice. In the FST, DMTS reduced inactive duration and increased activity frequency in TRPA1 WT animals compared to naïve and vehicle-treated mice. In NK1 KO mice, activity frequency was elevated by DMTS application. No changes were found in TRPA1 KO and C57BL/6 animals. Using inactive duration and activity frequency, average duration of individual hyperactive periods was calculated. This parameter did not differ between various treatment groups and animal strains.
Noldus EthoVision XT 15 was suitable to detect differences between treatment groups and mouse strains in OFT. In the FST we discovered that the average duration of highly active bouts does neither differ between treatment groups nor between animal strains. In our setting, activity frequency is the most suitable parameter to analyze the outcome of FST.

Changes in motor activity and underlying molecular mechanism in the 3xTg-AD mice model of Alzheimer's disorder

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Background: Alzheimer's disease (AD) is an age-related neurodegenerative disease with progressive memory decline, which places a heavy burden on society. Its transgenic mice models are promising tools in understanding the underlying mechanisms. Among the early signs impaired fine motoric function might serve as a biomarker.
Aim: Using 6-month-old 3xTg-AD male mice we wanted to confirm the impaired motor skills and muscle strength in comparison to their age-matched controls. As possible underlying mechanism, the expression of genes involved in muscle atrophy (myostatin, activinA) in the gastrocnemius (GAS) muscle was measured.
Methods: Motor activity was investigated by open field test, while muscle strength by the grip test and qPCR technique was used to quantitatively compare the expression levels of genes in GAS.
Results: The 3xTg-AD animals moved less and had reduced muscle strength during the test of motor activity. These results were supported by increased mRNA expression of myostatin. However, paradoxically the activinA mRNA level was decreased in the transgenic mouse model.
Conclusion: In line with an early disturbance in fine motoric in humans, our 3xTg-AD mice model also showed impaired motor function at the starting point of the appearance of pathological accumulation of hallmark proteins (beta-amyloid and tau; at the age of 6 months). The elevated expression of myostatin, an inhibitor of muscle growth, may contribute to the observed behavioural phenotype. However, activinA, another negative regulator of muscle development, might be decreased as a possible attempt to compensate myostatin signaling. Nevertheless, molecular changes in the muscles might underline the fine motor disturbances, which might serve as an early biomarker for AD preogression.

Effect of a checkpoint inhibitor on hearing in mice

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As a weapon for cancer immunotherapy, an increasing number of immune checkpoint inhibitors (ICIs) appear on the pharmaceutical market (e.g. pembrolizumab, nivolumab). Besides the effectiveness of these medications, adverse events can occur on organ systems of the body which healthy function is maintained by the balanced immune system, among others. Although immune response plays an important role in the pathomechanism of many forms of hearing losses, potential hearing impairment less comes to the fore during the exploration of the side effect profile of ICIs. Development of hearing loss has been described in a case report following the application of programmed death receptor-1 (PD-1) inhibitor pembrolizumab, however, thorough preclinical examinations haven't been performed yet.

Our aim was to investigate the effect of PD-1 inhibition on hearing in mice using both in vivo and in vitro approaches.

C57/BL6 mice (8-10w) were assigned to either control or anti-PD-1 antibody treated group. Antibodies were injected intraperitoneally at a dose of 200 µg/mouse for four weeks (3/week). Hearing thresholds at 4-32 kH frequencies were measured by auditory brainstem response (ABR). Number of hair cells (HCs), spiral ganglion cells (SGNs) and macrophages were counted in the apical-, middle- and basal turns of the cochleae by using phalloidin, hematoxylin/eosin and Iba1 staining, respectively.

Hearing function showed no change for ICI treatment. Number of SGCs showed no difference either in any turns between groups. Surprisingly, outer HC survival was increased in the basal part in the ICI-treated group along with the enhanced macrophage number in this turn.

These results indicate that a 4w ICI-treatment does not affect functional and morphological integrity of the inner ear in the most relevant hearing range (4-32 kHz; apical-middle turns), but a noticable preservation of outer HCs and macrophage number elevation appeared in the high frequency (>32 kHz) basal part of the cochlea.

In vitro treatment with the proteasome inhibitor MG-132 has a biphasic effect in rat pheochromocytoma cells

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Background: The Ubiquitin-Proteasome System (UPS) plays a critical role in the regulation of the activity of various signaling molecules involved in cell cycle control, inflammation, differentiation or apoptosis. The dysfunction of the UPS is involved in numerous pathological conditions, such as cancer, inflammation or neurodegeneration. Therapeutically used proteasome inhibitors cause peripheral neuropathy as their most pronounced side effect and a decreased function of the proteasome was observed in certain neurodegenerative diseases (e.g. Parkinson's disease).
Aim: To clarify the effect of proteasome inhibition on neuronal cells, PC12 (rat pheochromocytoma) cells were treated with the proteasome inhibitor MG-132.
Methods: We performed nuclear staining, flow cytometry and WST-1 assay, analyzed signal transduction pathways involving ERK1/2, Akt, p38 MAPK, JNK, c-Jun and caspase-3. We applied kinase inhibitors and dominant negative H-Ras mutant-expressing PC12 cells in order to decipher connections of the examined signaling pathways.
Results: Upon MG-132 treatment, initially, neuronal differentiation was induced, but after 24 hours signs of morphological deterioration, most likely apoptosis, became apparent. The latter was coupled to a gradual shift in MG-132-induced signaling towards increased activity of stress kinases (JNK and p38) and caspase-3 from the initial stimulation of pro-differentiation/survival molecules (ERK1/2 and Akt). Finally, we showed that some kinase inhibitors could potentiate the effects of the proteasome inhibitor, MG-132, which could be the basis of possible therapeutic combinations in the future.
Conclusion: Here we demonstrate that treatment with the proteasome inhibitor MG-132 has a biphasic nature in PC12 cells: initially, it induces neuronal differentiation, but prolonged treatments lead to apoptosis. This long term proteasome inhibitor-induced apoptosis could contribute to the better understanding of the neurological side effects of proteasome inhibitors and the pathogenesis of neurodegenerative diseases as well.
This work was supported by ÁOK KA-2021-31 and TKP2020-IKA-08 financed under the 2020-4.1.1-TKP2020 funding scheme.

The "supernormal" heart: Effects of long-term endurance training on cardiac remodeling in newly developed large- and in small animal models of the human athlete's heart

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Background: Although the positive effects of regular physical activity is uncontroversial, a number of sudden cardiac death (SCD) due to long-term intensive training among top athletes have been reported in recent years. Structural and functional changes, including repolarization abnormalities, might also lead to SCD.
Aim: The aim of our present study was to elucidate the long-term heavy training-induced functional electrophysiological changes in animal models of human athlete's heart.
Methods: Twenty-four dogs and twenty-six guinea pigs were randomized into sedentary ('Sed') and trained ('Tr') groups (n = 12-12; n = 13-13). The latter groups underwent a long-term intensive training session. ECG and echocardiography measurements were performed. Following heart removal, fibrotic changes were quantified. Ventricular myocytes were enzymatically dissociated via retrograde perfusion. The transmembrane ionic currents were recorded using the whole-cell configuration of the patch-clamp technique. The action potentials were measured by the perforated patch-clamp technique.
Results: The repolarization reflected as the 90 percent of action potential duration. It was significantly lengthened in the left ventricular myocytes isolated from 'Tr' vs. 'Sed' dogs (472.8±29.6 ms; n=29 vs. 369.3±31.4 ms; n=24, p=0.023) and no change was observed in the case of guinea pigs. The amplitude of the transient outward current, which is not expressed in the guinea pig heart, was significantly smaller in the 'Tr' vs. the 'Sed' animals (7.6±0.6 pA/pF, n=54 vs. 10.2±1.0 pA/pF, n=42, p<0.05). Under the currently used protocols, no differences were detected in the magnitude of other ionic currents.
Conclusion: Our results correspond to the human endurance trained athlete's heart. These results suggest that long-term endurance training-induced mild ventricle fibrosis, increased repolarization inhomogeneity may underline the development of life-threatening arrhythmias. However, further studies are warranted to clarify this issue in more details.
Funding: It was supported by NKFIH grants (K-19992, K-135464, GINOP-2.3.2-15-2016-00047).
Key words: Athlete's heart; repolarization reserve; I_{to}-current

Mesenteric adipocytes, vascular endothelium, and fibroblasts promote metaflammation and represent a potential treatment target for Hydrogen Sulfide

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The mesentery is an important organ for gastrointestinal cellular homeostasis, while its contribution to metaflammation (MF) and their changes during increased bioavailability of Hydrogen Sulfide (H_2S) is unknown. Mesentery white adipocytes (MWA) have been reported to have not only a poor blood capillary supply but also intrinsically low antioxidant enzyme defenses which make them vulnerable to hypoxia and free radical damage.

Aim: To determine, whether increased bioavailability of H2S can protect MWA against age and high-fructose-diet-induced injury, and to determine the underlying molecular mechanisms with a focus on mitochondria, thiobarbituric acid reactive substances (TBARS), and activities of cystathionine γ-lyase (CSE) and cystathionine β-synthase (CBS), thiosulfate-dithiol sulfurtransferase (TST), and sulfite oxidase (SO)

Methods: We studied: 1) MWA cell injury by electron microscopy; 2) TBARS level, CSE, CBS, TST, SO activities by spectrophotometry in adult and aged male rats on a standard diet (SD) or 4-week high-fructose-diet (HFD) without or with exposition to acute water-immersion restraint stress (WIRS) with pretreatment of sodium hydrosulfide (NaHS, 5.6 mg/kg/day).

Results: In both groups of adult and aged rats the treatment with NaHS also protected MA mitochondria, microvascular endothelial and sub-endothelial structures, and fibroblasts versus the vehicle-treated group that had signs of damage. HFD increased MA injury and mitochondrial changes in both aged and adult rats. Severe HFD-associated metaflammation with low activities of CSE, CBS, TST, SO, and increased TBARS were in aged vs adult rats. Finally, we demonstrated that increased bioavailability of H2S abolished MWA mitochondria, vascular endotheliocyte, and fibroblast alterations in aged rats exposed to HFD and WIRS, lowered TBARS, and enhanced H_2S enzyme activities in contrast to the vehicle-treated group.

Conclusions: Mesenteric MWA, vascular endothelium, and fibroblasts dysfunction is regulated by H_2S signaling. Mitochondrial integrity alterations and redox imbalance are key targets of mesenteric adipose tissue damage during advanced age and metaflammation. Increased bioavailability of the H_2S approach represents a potential therapeutic option to treat metaflammation.

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Cigarette smoking alters NHE function of guinea pig oesophageal epithelial cells and human oesophageal cell lines

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Introduction: Clinical studies indicate that smoking predisposes the consumers to oesophageal inflammatory and malignant diseases, but the cellular mechanism is not completely clear. Ion transporters play an important protective role in the oesophageal epithelial cells (OECs). Our aim was to examine the effect of smoking on the oesophageal epithelial ion transport mechanisms, especially focusing on the activity of Na^+/H^+ exchanger (NHE), which has a great impact on pH_i homeostasis.
Methods: Study was performed on human oesophageal cell lines (CP-A, CP-D and OE-33) and primer OECs isolated from guinea pig (gpOECs). Cells were treated with cigarette smoke extract (CSE) (1, 10, 100 µg/ml) for 1 h and NHE activity was estimated by the NH_4Cl pulse technique, using BCECF-AM. In order to investigate the chronic effect of smoking, guinea pigs were exposed to cigarette smoke for 1, 2 and 4 months and NHE activity was also determined. Metaplastic (CP-A), dysplastic (CP-D) and oesophageal adenocarcinoma (OE-33) cell lines were treated with CSE for 6, 24 and 72 hours and the mRNA expression of NHE-1, and alterations in viability and proliferation was investigated. Silencing of NHE-1 was performed using specific siRNAs.
Results: NHE activity and expression decreased in metaplasia-dysplasia-adenocarcinoma sequence and the absence of NHE-1 increased proliferation, especially in CP-A and OE-33 cells. Incubation with CSE increased the NHE activity in gpOECs and CP-A cells, whereas decreased in CP-D cell line. Smoking time-dependently increased the activity of NHE in gpOECs. CSE treatment downregulated the expression of NHE1 and increased the proliferation in CP-D and OE-33 cells. In contrast, incubation of CP-A cells with CSE did not affect NHE expression and reduced cell proliferation.
Conclusion: Our results indicate that NHE plays a protective role in normal and metaplastic cells in order to avoid malignant transformation.
Grants: FK123982, GINOP-2.3.2-15-2016-00015, EFOP 3.6.2-16-2017-00006.

High-dose salicylic acid blocks GI passage inducing the activity of MMPs

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Background: It is known that salicylic acid (SA) can cause gastric damage and intestinal ulceration already in therapeutic dose. At the same time, we found that the high-dose of SA induced gastric diverticulum and block of GI passage. The matrix metalloproteinases (MMPs) play a key role in tissues remodelling and they can influence the smooth muscle motility.
Aim: We hypothesized that the GI passage block might be associated with the alteration of activities of MMPs. Thus, the aim of our study was to investigate how high-dose salicylic acid treatment modifies the activity of MMPs and the GI smooth muscles motility *in vitro* and *in vivo.*
Methods: Female Sprague-Dawley rats were treated to 3 days with high-dose 400 mg/day salicylic acid suspension by oral gavage. The alterations of smooth muscle contractions were determined in GI (*intestine, stomach, caecum*) tissues by *in vitro* isolated organ bath and *in vivo* electromyography. The activity of MMPs was measured with gelatin zymography and IVIS Lumina imaging system.
Results: SA administration decreased the spontaneous contractions of *stomach*, *ileum* and *coecum*. The intensity of GI electromyographic signal was enhanced on each day of the treatment, the highest values were reached on 2nd day in all samples. Both gelatin zymography and IVIS Lumina imaging system demonstrated that the activity of MMPs was reduced in parallel with the SA treatment.
Conclusion: Our results suggest that the high-dose SA can provoke gastric hypermotility at the beginning of the treatment while at the end the motility is reduced, and the GI passage is blocked. It seems that in GI tissues the high-dose SA treatment can decrease the MMPs activities which may lead to the gastrointestinal tissues transformation and impaired smooth muscle function.
This work was supported by the Ministry of Human Capacities. [Hungary grant 20391-3/2018/FEKUSTRAT]

Anti-inflammatory effects of the octapeptide NAP in human microbiota associated mice suffering from subacute ileitis

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Background: The octapeptide NAP is well known for its neuroprotective properties
Aim: We here investigated whether NAP treatment could alleviate pro-inflammatory immune responses during experimental subacute ileitis.
Methods: To address this, mice with a human gut microbiota were perorally infected with one cyst of *Toxoplama gondii* (day 0) and subjected to intraperitoneal synthetic NAP treatment from day 1 until day 8 postinfection (p.i.).
Results: Whereas placebo (PLC) control animals displayed subacute ileitis at day 9 p.i., NAP treated mice exhibited less pronounced pro-inflammatory immune responses as indicated by lower numbers of intestinal mucosal T and B lymphocytes and lower IFN-γ concentrations in mesenteric lymph nodes. The NAP-induced anti-inflammatory effects were not restricted to the intestinal tract, but could also be observed in extra-intestinal including systemic compartments, given that pro-inflammatory cytokines were lower in liver, kidney and lung following NAP as compared to PLC application, whereas at day 9 p.i., colonic and serum IL-10 concentrations were higher in the former as compared to the latter. Remarkably, probiotic commensal bifidobacterial loads were higher in the ileal lumen of NAP as compared to PLC treated mice with ileitis.
Conclusion: Our findings thus further support that NAP might be regarded as future treatment option directed against intestinal inflammation.

The octapetide NAP alleviates intestinal and extra-intestinal inflammatory sequelae of acute experimental colitis

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Background: The octapeptide NAP has been shown to exert neuroprotective properties and reduce neuro-inflammatory responses.
Aim: The aim of the present study was to investigate if NAP provides anti-inflammatory effects in acute murine colitis.
Methods: To address this, C57BL/6j mice were challenged with 3.5% dextran sulfate sodium from day 0 until day 6 to induce colitis, either treated intraperitoneally with NAP or placebo (NaCl 0.9%) from day 1 until day 6 post-induction (p.i.) and subjected to in depth macroscopic, microscopic and immunological evaluations.
Results: Whereas NAP application did not alleviate macroscopic (i.e. clinical) sequelae of colitis, lower numbers of apoptotic, but higher counts of proliferating/regenerating colonic epithelial cells could be observed in NAP as compared to placebo treated mice at day 7 p.i. Furthermore, lower numbers of adaptive immune cells such as T lymphocytes and regulatory T cells were abundant in the colonic mucosa and lamina propria upon NAP versus placebo treatment that were accompanied by less colonic secretion of pro-inflammatory mediators including IFN-γ and nitric oxide at day 7 p.i. In mesenteric lymph nodes, pro-inflammatory IFN-γ, TNF and IL-6 concentrations were increased in placebo, but not NAP treated mice at day 7 p.i., whereas interestingly, elevated anti-inflammatory IL-10 levels could be observed in NAP treated mice only. The assessed anti-inflammatory properties of NAP were not restricted to the intestinal tract, given that in extra-intestinal compartments such as the kidneys, IFN-γ levels increased in placebo, but not NAP treated mice upon colitis induction. NAP induced effects were accompanied by distinct changes in intestinal microbiota composition, given that colonic luminal loads of bifidobacteria, regarded as anti-inflammatory, "health-promoting" commensal species, were two orders of magnitude higher in NAP as compared to placebo treated mice and even naive controls.
Conclusion: NAP alleviates intestinal and extra-intestinal pro-inflammatory sequelae of acute experimental colitis and may provide novel treatment options of intestinal inflammatory diseases in humans.

Vitamin D in acute campylobacteriosis – results from an intervention study applying a clinical *Campylobacter jejuni* induced enterocolitis model

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Background: Human *Campylobacter* infections are progressively rising and of high socioeconomic impact.
Aim: In the present preclinical intervention study we investigated anti-pathogenic, immunomodulatory and intestinal epithelial barrier preserving properties of vitamin D applying an acute campylobacteriosis model.
Methods: Therefore, secondary abiotic IL-10$^{-/-}$ mice were perorally treated with synthetic 25-OH-cholecalciferol starting four days before peroral *Campylobacter jejuni* infection.
Results: Whereas 25-OH-cholecalciferol application did not affect gastrointestinal pathogen loads, 25-OH-cholecalciferol treated mice suffered less frequently from diarrhea in the midst of infection as compared to placebo control mice. Moreover, 25-OH-cholecalciferol application dampened *C. jejuni* induced apoptotic cell responses in colonic epithelia and promoted cell-regenerative measures. At day 6 post-infection, 25-OH-cholecalciferol treated mice displayed lower numbers of colonic innate and adaptive immune cell populations as compared to placebo controls that were accompanied by lower intestinal concentrations of pro-inflammatory mediators including IL-6, MCP1 and IFN-γ. Remarkably, as compared to placebo application synthetic 25-OH-cholecalciferol treatment of *C. jejuni* infected mice resulted in lower cumulative translocation rates of viable pathogens from the inflamed intestines to extra-intestinal including systemic compartments such as the kidneys and spleen, respectively, which was accompanied by less compromised colonic epithelial barrier function in the 25-OH-cholecalciferol as compared to the placebo cohort.
Conclusion: Our preclinical intervention study provides evidence that peroral synthetic 25-OH-cholecalciferol application exerts inflammation-dampening effects during acute campylobacteriosis.

Vitamin C alleviates acute enterocolitis in *Campylobacter jejuni* infected mice

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Background: Human foodborne infections with the zoonotic pathogen *Campylobacter jejuni* are on the rise and constitute a significant socioeconomic burden worldwide. The health-beneficial, particularly anti-inflammatory effects of vitamin C (ascorbate) are well known.
Aim: In our preclinical intervention study, we assessed potential anti-pathogenic and immunomodulatory effects of ascorbate in *C. jejuni*-infected secondary abiotic IL-10$^{-/-}$ mice developing acute campylobacteriosis similar to humans.
Methods: Starting 4 days prior peroral *C. jejuni*-infection, mice received synthetic ascorbate via the drinking water until the end of the experiment.
Results: At day 6 post-infection, ascorbate-treated mice harbored slightly lower colonic pathogen loads and suffered from less severe *C. jejuni*-induced enterocolitis as compared to placebo control animals. Ascorbate treatment did not only alleviate macroscopic sequelae of infection, but also dampened apoptotic and inflammatory immune cell responses in the intestines that were accompanied by less pronounced pro-inflammatory cytokine secretion. Remarkably, the anti-inflammatory effects of ascorbate pretreatment in *C. jejuni*-infected mice were not restricted to the intestinal tract but could also be observed in extra-intestinal compartments including liver, kidneys and lungs.
Conclusion: Due to the potent anti-inflammatory effects observed in the clinical murine *C. jejuni*-infection model, ascorbate constitutes a promising novel option for prophylaxis and treatment of acute campylobacteriosis.

Intestinal microbiota changes in mice lacking Pituitary adenylate cyclase activating polypeptide – Bifidobacteria make the difference

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Background: Pituitary adenylate cyclase activating polypetide (PACAP) constitutes a neuropeptide that is widely distributed in the host exerting essential cytoprotective properties, whereas PACAP$^{-/-}$ mice display increased susceptibility to distinct immunopathological conditions. The orchestrated interplay between the gut microbiota and the host is pivotal in immune homeostasis and resistance to disease. Potential pertubations of the intestinal microbiota in PACAP$^{-/-}$ mice, however, have not been addressed so far.
Aim/Methods: For the first time, we performed a comprehensive survey of the intestinal microbiota composition in PACAP$^{-/-}$ and WT mice starting 2 weeks *post-partum* until 18 months of age applying quantitative culture-independent techniques.
Results: Fecal enterobacteria and enterococci were lower in PACAP$^{-/-}$ than WT mice aged 1 month and ≥6 months, respectively. Whereas *Mouse Intestinal Bacteroides* were slightly higher in PACAP$^{-/-}$ versus WT mice aged 1 and 6 months, this later in life held true for *Bacteroides / Prevotella* spp. (≥12 months) and lactobacilli (>15 months of age). Strikingly, health-beneficial bifidobacteria were virtually absent in the intestines of PACAP$^{-/-}$ mice, even when still breast-fed.
Conclusion: PACAP deficiency is accompanied by distinct changes in fecal microbiota composition with virtually absent bifidobacteria as a major hallmark that might be linked to increased susceptibility to disease.

Murine fecal microbiota transplantation lowers gastrointestinal pathogen loads and dampens pro-inflammatory immune responses in *Campylobacter jejuni* infected secondary abiotic mice

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Background: Conventional mice are protected from *Campylobacter jejuni* infection by the murine host-specific gut microbiota composition.
Aim: We here addressed whether peroral fecal microbiota transplantation (FMT) might be an antibiotics-independent option to lower even high gastrointestinal *C. jejuni* loads in the infected vertebrate host.
Methods: Therefore, secondary abiotic mice were generated by broad-spectrum antibiotic treatment and perorally infected with *C. jejuni* by gavage.
Results: One week later, mice were stably colonized with more than 10^9 *C. jejuni* and subjected to peroral FMT from murine donors on three consecutive days. Two weeks post-intervention, gastrointestinal *C. jejuni* loads were up to 7.5 orders of magnitude lower following murine FMT versus mock challenge. Remarkably, FMT reversed *C. jejuni* induced colonic epithelial apoptosis, but enhanced proliferative and regenerative responses in the colon thereby counteracting pathogenic cell damage. Furthermore, FMT dampened both innate and adaptive immune cell responses in the large intestines upon *C. jejuni* infection that were accompanied by less *C. jejuni*-induced colonic nitric oxide secretion.
Conclusion: Our study provides strong evidence that novel probiotic formulations developed as alternative option to FMT in severe intestinal inflammatory morbidities including *Clostridoides difficile* infection might be effective to treat campylobacteriosis and lower pathogen loads in colonized vertebrates including farm animals.

Chronic treatment with the selective cyclooxygenase-2 inhibitor celecoxib does not cause small intestinal dysbiosis in rats

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Background. There are several lines of evidence that nonsteroidal anti-inflammatory drugs (NSAIDs) change the composition of intestinal microbiome, and this dysbiosis contributes to the development of small intestinal damage (enteropathy). It is still not clear whether chronic administration of selective cyclooxygenase-2 (COX-2) inhibitors is also associated with enteropathy and dysbiosis.
Aims. Here we aimed to analyse the effect of a 4-week treatment with celecoxib on the small intestinal mucosa and microbiome composition in rats.
Methods. Male Wistar rats were treated intragastrically with either vehicle or celecoxib (30 mg/kg) once daily for four weeks. The non-selective COX inhibitor, indomethacin (5 mg/kg) was used as a positive control. The severity of mucosal damage was assessed by macroscopic and histological analysis, and by measuring the intestinal levels of different inflammatory markers. The composition of microbiota was assessed by sequencing of bacterial 16S rRNA.
Results. 1. Indomethacin caused severe enteropathy with adhesions, ascites and significant mortality. Mucosal inflammation was confirmed by histology, and by elevated tissue levels of myeloperoxidase (MPO) and COX-2. By contrast, celecoxib did not cause any macroscopic or histological damage to the small intestine, and did not increase the level of inflammatory markers. 2. Indomethacin-induced enteropathy was associated with dysbiosis, which was characterized by a loss of Firmicutes and bloom of Proteobacteria. Celecoxib treatment had no significant impact on the composition of small intestinal microbiome.
Conclusion. Our present study suggests that chronic treatment with celecoxib does not cause small intestinal injury and dysbiosis in rats. Grant support:NKFI FK 124878.

Investigating the efficacy of anti-IL-1ß monoclonal antibody treatment in an aged mouse-model of non-alcoholic steatohepatitis

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Background: Non-alcoholic fatty liver disease (NAFLD) is a chronic, progressive pathology, comprising distinctive stages: steatosis, steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma. Critical inflammatory processes of NASH include inflammasome activation and subsequent interleukin-1ß production.
Aims: We aimed to investigate the hepatic and cardiac effects of anti-IL-1ß monoclonal antibody treatment in an aged animal model of NASH.
Methods: Aged male C57Bl6/J mice were fed with choline sufficient (CON) diet or choline deficient (CDAA) diet and were treated with isotype control or anti-IL-1ß mAb for 8 weeks. Cardiac functions were assessed by echocardiography. Liver samples were analyzed by immunohistochemistry and qRT-PCR.
Results: In comparison to the high mortality of the CON group, we detected better survival upon IL-1ß blockade. Echocardiography revealed improved cardiac functions in anti-IL-1ß treated mice. Histological and gene expression analyses showed marked hepatic fibrosis in CDAA-fed group, but IL-1ß inhibition did not ameliorate it, except for *Col3a1* expression. Hepatic *Ccl2* expression was increased due to NASH and was unaffected by the treatment. PCNA staining and qRT-PCR analyses showed marked hepatocyte proliferation in CDAA-fed animals, that was not influenced by IL-1β neutralization. IL-1ß inhibition led to increased hepatic expression of *Pd-1* and *Ctla-4*, while in the CDAA-fed group we found increased expression of *Pd-l1*.
Conclusion: IL-1ß inhibition improved cardiac function; however, it did not ameliorate key features of NASH and even promoted hepatic immune-checkpoint expression. This might give rise to malignant transformation, along with the NASH promoted hepatocellular proliferation. Surprisingly, none of these hepatic changes are affected by IL-1ß blockade.
Grants: European Union's Horizon 2020 research and innovation programme under grant agreement No 739593, Semmelweis University's excellence programme (EFOP-3.6.3-VEKOP-16-2017-00009), New National Excellence Program of the Ministry of Human Capacities (ÚNKP-21-3-II).

Functional expression and upregulation of Transient Receptor Potential Ankyrin 1 and Vanilloid 1 receptors in oral squamous cell carcinoma

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Oral squamous cell carcinoma (OSCC) is often diagnosed in an advanced stage. The oral mucosa is exposed to various stimuli, e.g. hot, cold, spicy foods, cigarette smoke and alcohol, which are all activators of the Transient Receptor Potential Ankyrin 1 (TRPA1) and Vanilloid 1 (TRPV1) receptors. They are suggested to play a role in malignant cell transformation in breast, digestive, prostate and head and neck cancers, however, there are no conclusive data on their expression in OSCC.

Therefore, we investigated local TRPA1 and TRPV1 expressions, their alterations, activation and its consequences in OSCC.

Buccal, lower lip, gingiva, tongue, sublingual regions, hard palate and mouth floor surgical samples of OSCC patients were studied. *TRPA1/V1* mRNA expression was quantified by quantitative polymerase chain reaction, their tissue localizations were determined by *in situ* hybridization RNAScope technique. Receptor activation in response to the TRPA1 agonist mustard oil and the TRPV1 agonist capsaicin and function was determined by ^{45}Ca uptake measurement and viability assay on PE/CA-PJ41 cell line.

TRPA1 and *TRPV1* mRNA expression was significantly, 4.5- and 2-fold higher in OSCC samples, respectively, compared to the healthy mucosa. Scattered *TRPA1* and *V1* transcripts were observed in epithelial cells of the tumour samples. Both TRPA1 and TRPV1 were functionally active in PE/CA-PJ41 cells, mustard oil and capsaicin induced concentration-dependent ^{45}Ca-uptake. The viability of the cells gradually decreased, 50% viability was measured after 100 nM AITC and 45μM capsaicin treatment.

We provided the first evidence for the presence and up-regulation of TRPA1 and confirmed the expression of TRPV1 in OSCC. We mapped the distribution of these receptors in human samples and proved that they both functionally active and their sustained activation reduce the viability of OSCC cells, however their exact role needs to be elucidated.

NAP-2 20017-1.2.1-NKP -2017-463 00002, FIKPII-17886-4/23018/FEKUTSTRAT, GINOP-2.3.2-15-2016-00050, EFOP-362-464-16-2017-00008, EFOP-3.6.1-16-2016-00004, János Bolyai Research Scholarship

Smooth muscle electromyography may detect the gastric acid overproduction in a rat model

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Background: The overproduction of gastric acid is a common disorders inducing the risk of gastric ulcer or reflux disease. The instrumental detection of the gastric acid overproduction is currently not solved.
Aim: Our aim was to develop an instrumental method for reflux prediction using smooth muscle electromyography (SMEMG).
Methods: A pH sensor was implanted into the stomach of anesthetized female SPRD rats (240-280g) along with a cannula for the administration of histamine. A bipolar disc electrode pair was placed under the abdominal skin to detect the myoelectric changes. Additionally, a strain gauge was put on the surface of the stomach to detect the mechanical contractions. The rats were treated with a single dose of histamine-hydrochloride (50 mg/kg) solution to induce acid secretion. The myoelectric signals, the pH and the mechanical contractions were simultaneously recorded for 90 min after histamine treatment.
Results: The administration of histamine increased the electromyographic activity without the modification of the gastric contractions and the pH during the first 15 min. The decrease in pH value was significant after 30 min accompanied with a still increased myographic signal. However, the pH was reduced gradually reaching the lowest value after 75 min without any increase in electromyographic or contractility activities.
Conclusion: Our rat model suggest that the initiation of gastric acid secretion is detectable by smooth muscle electromyography, but the electromyographic signal may 30-90 min ahead the real low pH value. We suppose that the application of smooth muscle electromyography may detect the overproduction of gastric acid and even may predict reflux episodes.
Acknowledgements: This work was supported by the Ministry of Human Capacities [Hungary grant 20391-3/2018/FEKUSTRAT] and UNKP-21-3-SZTE-80.

The role of interleukin (IL)-1 in thermoregulatory changes associated with severe systemic inflammation

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Background: Interleukin (IL)-1, a pyrogenic inflammatory cytokine, is considered to have diverse physiological functions and to play an important role in systemic inflammation. It is known, that inhibition of the IL-1 signaling results in attenuated fever response to a low dose of bacterial lipopolysaccharide (LPS), however the thermoregulatory role of IL-1 in severe forms of sepsis is not fully elucidated.
Aim: The aim of our study was to discover if IL-1 plays a role in the mediation of the thermal changes associated with severe forms of systemic inflammation.
Methods: Adult mice genetically lacking the gene of IL-1 α and β (KO) and their wild-type littermates (WT) were compared in two models of severe systemic inflammation: 1) aseptic systemic inflammatory response model induced by intraperitoneal injection of a high dose (5 mg/kg) of bacterial LPS and 2) polymicrobial sepsis model induced by cecal ligation and puncture (CLP). Deep body temperature of the mice was registered in both models, while the survival time was also recorded in polymicrobial sepsis.
Results: LPS administration resulted in a marked drop of body temperature in both genotypes. The LPS-induced hypothermia was markedly more pronounced in the WT mice (with nadir of 32.9±0.9°C) than in the KO mice (with nadir of 34.7±0.6°C; $p<0.05$ for inter-genotype difference). After CLP, the occurrence of death was delayed in KO mice (~22 hours) compared to WT controls (~18 hours). The highest difference in the survival rate was observed at 37 hours: 57.9% in KO versus 5.9% in WT mice.
Conclusion: These findings suggest that IL-1 contributes to the development of hypothermia and death in severe forms of systemic inflammation. When IL-1 is absent, the hypothermic response is less pronounced and the chance for survival increases in severe sepsis.
Support: NKFIH FK124483, Janos Bolyai Scholarship of the Hungarian Academy of Sciences

Questionable translational significance of IL-17A in imiquimod-induced psoriasiform mice model

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Background: Interleukin-17A is a member of IL17 cytokine family, mainly produced by T helper-17 cells, γδT cells and multiple cell lineages of the innate immune system. It acts as a pro-inflammatory cytokine and is critically involved in several autoimmune diseases. Monoclonal IL17A antibody is used for clinical treatment of psoriasis with a great effectiveness. Imiquimod (IMQ)-induced psoriasiform mouse model is widely accepted in basic research, however it's cytokine profile does not completely mimic the human conditions.
Aim: Therefore, our aim was to investigate the IL-17A cytokine concentration of the skin samples in IMQ-induced psoriasiform dermatitis model at different time-points of the 7-day-long experiment.
Methods: Localized psoriasiform skin inflammation was induced in Balb/c mice according to our refined model described in: Horváth S, Komlódi R, et al. Sci Rep. 2019 Mar 6;9(1):3685. doi: 10.1038/s41598-019-39903-x. Skin samples were collected on each day of the experiment and homogenized in cold PBS-PMSF buffer. Concentration of IL-17A was measured by Luminex xMAP technology and conventional sandwich ELISA in parallel.
Results: We could not detect any significant increase of IL-17A concentration in the IMQ-treated skin samples compared to the vaseline-treated control skin area with Luminex or ELISA methods. The highest concentration of IL-17A was measured on day-2 but there was no correlation between cytokine concentrations and the severity of psoriatic alterations in the skin.
Conclusion: According to our findings the role of IL-17A in the IMQ-induced psoriasiform murine model seems to be less consistent, therefore the translational relevance of IL-17A measurement is questionable.

Á.K was supported by the János Bolyai Research Scholarships of the Hungarian Academy of Sciences and the ÚNKP-20-5 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

TRPA1 receptors expressed in the brain influence the sensation of odours and social behaviour of mice

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Background: Transient receptor potential ankyrin 1 (TRPA1), a non-selective cation channel, contributes to several (patho)physiological processes including pain sensation, development of neuropathic pain and neurodegenerative disorders [1]. In addition, it seems to influence the innate-fear responses of mice [2]. Here, we aimed to reveal its role in olfaction and social behaviour.

Methods: The presence of Trpa1 mRNA was studied along the olfactory tract of mice by real-time polymerase chain reaction and combined RNAscope in situ hybridization and immunohistochemistry. The aversive effects of fox and cat odour were examined parallel with stress hormone levels. In vitro calcium imaging tested if these substances can directly activate the TRPA1 receptors. The role of TRPA1 in social behaviour was investigated by comparing Trpa1 wild type and knockout mice (KO).

Results: Trpa1 mRNA was detected in the olfactory bulb and piriform cortex, while its expression was weak in the olfactory epithelium. Fox, but not cat odour activated directly the TRPA1 channels. Accordingly, KO animals showed less aversion against fox, but not cat odour. The social interest of KO mice was reduced during social habituation-dishabituation and social interaction, but not during resident-intruder tests.

Conclusions: TRPA1 may contribute to odour processing at several points of the olfactory tract and may play an important role in the social behaviour of mice. Thus, TRPA1 may serve as a novel drug target in the treatment of behavioural disorders.

Funding: ELKH Research Network, TKP-2020, KTIA Hungarian Brain Research Program 2.0.

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Keywords: TRPA1, innate fear, social behaviour, piriform cortex, olfactory bulb, olfactory epithelium

Development of the retinopathy in a diet-induced type 2 diabetic rat model

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Background: Type 2 diabetes (T2D) accounts for 90% of the population with diabetes, and these patients are also related with diabetic retinopathy (DR). DR is one of the most common cause of new onset blindness in people between 20-74 years in developed countries. A high-fat–fed/streptozotocin (HF-STZ) induced hyperglycemic rat model is usually used for neural complications screening, but the development of DR in this animal model is not clear yet. Our aim was to investigate the development and the progression of DR in T2D in rats.
Methods: 3-month-old male Wistar rats were divided in a control and a HF-STZ groups. To induce T2D, HF-STZ animals were injected STZ (i.p.30mg/kg). Control group was kept on a regular rat chow while the HF-STZ group was kept on a high-fat diet. Each rat was followed with different methods (optical coherence tomography (OCT); electroretinography (ERG)) in vivo to screen the morphological and functional changes of the retina at specified intervals (0 day, 15th weeks and after 1 year). After 1 year rats were sacrificed and optic nerves were collected for further morphological analysis.
Results: OCT results demonstrated that the retinas exposed to diabetes showed significant changes in the retina structure, edema, hard excaudate and microaneurysm were observed. The total retinal thickness in diabetic group were significantly thinner from the 15th weeks compared to control once. ERG responses of diabetic rats were disturbed (amplitudes of waves and implicit time) compared to control animals. Routine histology of the optic nerve showed less oligodendrocytes cell number in the central retinal area and larger nerve diameter in the diabetes group compared to the controls.
Conclusion: These results clearly demonstrate that diabetic retinopathy is successfully developing in these animals, therefore it will be suitable for further ophthalmic research in the future.

Keywords: type 2 diabetes, diabetic retinopathy, OCT
This work supported by NKFIH FK129190, GINOP-2.3.2-15-2016-00050"PEPSYS", NAP 2017-1.2.1.-NKP-2017-00002, PTE-AOK TANDEM, MTA-TKI 14016, Bolyai Scholarship, EFOP-362-16-2017-00008, New national Excellence Program of the Ministry of Human Capacities, FIKPII.

Protective role of PAC-1 receptor in endotoxin-induced retinal inflammation

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Background: Lipopolysaccharide (LPS) administration is used for the induction of a model of neuroinflammation in the retina. Pituitary adenylate cyclase activating polypeptide (PACAP) has anti-inflammatory and anti-apoptotic effects in the retina, which are mostly mediated by the PAC-1 receptor. Our aim was to examine the retinoprotective role of the PAC-1 receptor agonist maxadilan in LPS induced inflammation by using different methods.
Methods: CD-1 IGS wild type mice were used in the experiment. Mice received intraperitoneal injection of LPS (6 mg/kg). Right eye was injected with maxadilan (1 µM) intravitreally and left eye served as a control (PBS injection). Half of the animals were investigated 24h after LPS injection and their retina samples were labelled with glial fibrillary acidic protein (GFAP). In the other half of the groups were investigated in vivo by optical coherence tomography (OCT) for 5 weeks to monitor the histological changes of the different retinal layers. After anesthesia, animals were decapitated, the eyes were removed, and retinas were stained with toluidine blue dye for further morphometric analysis.
Results: GFAP labelling showed remarkable changes in LPS injected animals. Expression was less intense in the entire retina in LPS+maxadilan-injected ones compared to the LPS alone. OCT results demonstrated that retinas exposed to LPS showed significant changes in the structure. Histological analysis showed significant differences in the maxadilan-treated retinas compared to the LPS-injected ones. All the retinal layers were significantly thinner in LPS-injected mice compared to the treated animals.
Conclusion: In summary, PAC-1 receptor has important retinoprotective role in endotoxin (LPS)-induced inflammation.
Keywords: endotoxin, maxadilan, neuroprotection
This work supported by NKFIH FK129190, GINOP-2.3.2-15-2016-00050 PEPSYS, NAP 2017-1.2.1.-NKP-2017-00002, PTE-AOK TANDEM, MTA-TKI 14016, Bolyai Scholarship, EFOP-362-16-2017-00008, New National Excellence Program of the Ministry of Human Capacities, FIKPII.

CHARACTERIZATION OF TRANSGENIC MICE EXPRESSING THE HUMAN SOMATOSTATIN RECEPTOR SUBTYPE 4

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Background: Somatostatin receptor subtype 4 (sst_4) mediates analgesic, anti-depressant and anti-inflammatory functions of somatostatin without endocrine effects, making it a promising drug target.
Aims: Our goal is to generate and test mice with humanized sst_4 receptor, which we designed to be a model for preclinical testing of novel sst_4 receptor agonist drug candidates.
Methods: We inserted the human chromosomal fragment containing the SSTR4 gene in a PiggyBac transposon vector, then we added the coding sequences of luciferase and tdTomato reporter proteins. Transgenesis was performed in sst_4 receptor deficient mice. Multiple copies of the transgene were identified and located via ligation mediated PCR. The breeding process separated the different copies of the transgene into distinct mouse lines. We verified by sequencing that the inserted transgene is intact in these transgenic mice. Expression pattern was mapped by in vivo imaging of luciferase activity, by RNA in situ hybridization (RNAscope) and qRT-PCR.
Results: In vivo imaging showed the strongest signal of luciferase luminescence in the CNS, especially in the area of the cerebrum. RNAscope results show that the expression pattern of the human sst_4 receptor is slightly different in the transgenic mice compared to mouse sst_4 receptor in wild type mice.
Conclusion: Results show that the human SSTR4 transgene is expressed. Our data suggest that further characterization of the receptor expression pattern by RNAscope and testing the receptor function via electrophysiology are needed to prove that these humanized mice are useful for preclinical research of novel sst_4 receptor agonists.
Acknowledgements for support:
Richter Gedeon PhD scholarship
KTIA_NAP_13-2-2014-0022 and KTIA_NAP_13-1-2013-0001
GINOP-2.3.2-15-2016-00050, EFOP-362-16-2017-00008
20765-3/2018/FEKUTSTRAT

The thermoregulatory role of transient receptor potential vanilloid-1 channels in acute and chronic acidosis in mice

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Background: Antagonists of the transient receptor potential vanilloid-1 (TRPV1) channel were reported to alter deep body temperature (T_b) in animals and humans. It is presumed that in the peritoneal cavity, TRPV1 channels are tonically activated by protons and drive the reflectory inhibiton of thermogenesis.
Aim: Our aim was to investigate the thermoregulatory role of TRPV1 channel in acute and chronic metabolic acidosis in mice.
Methods: In adult C57BL/6 and TRPV1 knockout (KO) mice, we developed an acute acidosis model by using intraperitoneal infusion of NH_4Cl (6 M; 0.1 ml/10g). To study the effect of chronic metabolic acidosis, we administered 0.28 M NH_4Cl for 12 days in drinking water and in intraperitoneal injections of 0.5 ml on days 1 and 6. We measured the alterations of colonic temperature (a form of deep T_b) with thermocouple thermometry, while blood pH measurements were performed after blood collection with cardiac puncture.
Results: Administration of NH_4Cl resulted in markedly lower blood pH both acutely and chronically ($P < 0.05$ for both). Acute acidic load caused a drop in T_b by ~3°C compared to vehicle in C57BL/6 mice. Similarly to acute acidosis, the chronic administration of NH_4Cl also significantly ($P<0.05$) lowered the basal T_b in the mice. The acute acidosis-induced hypothermia was exaggerated by more than a degree (to a drop of ~4.5°C) in the TRPV1 KO mice.
Conclusion: We conclude that systemic acidosis leads to a drop in T_b, which is less pronounced in chronic acid load. The exaggeration of the hypothermia in TRPV1 KO mice suggests that TRPV1 channels play a limiting role in the thermal changes associated with severe, acute forms of acidosis.
Support: NKFIH FK124483, Janos Bolyai Scholarship of the Hungarian Academy of Sciences

Role of Mac-1 in the formation of neutrophil granulocyte derived extracellular vesicles

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Background: Previously, our group characterized distinct populations of extracellular vesicle (EV) released from neutrophilic granulocytes: EV formed spontaneously (sEV), during apoptosis (apoEV) and upon activation with opsonized particles (aEV). The aEV differs in protein cargo and its ability to inhibit bacterial growth. We described that Mac-1 integrin (CR3 receptor) plays key role in the aEV production.
Aim: In the present work, our aim was to investigate whether Mac-1 activation on its own are sufficient for the initiation of the aEV biogeneis.
Methods: We isolated neutrophil derived EVs from peripheral human blood by two-step centrifugation and filtration. We quantified the vesicles by flow cytometry, determined their protein content by Bradford assay. We also determined the concentration and size distribution of the EVs by NTA. We examined their effect on bacterial survival of *S. aureus*, and we performed ELISA for the assessment of cytokine production of PMNs during 3 hours co-incubation with the different type EVs.
Results: On C3bi coated surface, we observed an increased EV production. These EVs possessed antibacterial capacity and pro-inflammatory effect. However, in soluble condition, C3bi did not induce further EV production, and these EVs did not show any antibacterial property or IL-8 release inducing effect.
Conclusion: Mac-1 activation is not just crucial, but sufficient in initiation of the aEV biogenesis and the clustering of this integrin is possibly also required.
Funding: EFOP-3.6.3-VEKOP-16-2017-00009, NKFIH K119236, János Bolyai Research Scholarship of the Hungarian Academy of Sciences, and the ÚNKP-20-5 New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund.

Determining signaling pathways by transcriptomic analysis of the dorsal root ganglia in a translational mouse model of Complex Regional Pain Syndrome

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Background: Complex Regional Pain Syndrome (CRPS) is a severe chronic pain condition accompanied by hypersensitivity, swelling and autonomic dysfunctions after a small injury. Autoimmunity, complex sensory-immune-vascular interactions and neuroinflammation were suggested to be involved in its pathophysiology. Since its therapy is unsatisfactory, there is a great need to explore the main mediators and identify novel drug targets.
Aim: We performed here the transcriptomic analysis of dorsal root ganglia (DRG) samples obtained from a novel passive transfer-trauma mouse model to identify key signaling pathways.
Methods: Female C57Bl/6 mice were treated daily with purified serum-IgG from CRPS patients or healthy volunteers following plantar skin-muscle incision. The mechanonociceptive threshold was measured by dynamic plantar aesthesiometry, and RNA was isolated from the lumbal 3-4-5 DRGs on day 5. RNA sequencing was performed with Illumina NextSeq 550, differentially expressed genes and signaling pathways were determined by bioinformatic analysis.
Results: Significantly greater incision-induced mechanical hyperalgesia developed in CRPS IgG-treated mice. There were 125 differentially expressed genes in DRGs of the incision-side in response to CRPS IgG compared to healthy. Twelve up and 12 down regulated genes affecting the innate immune system and autoimmune processes, cytokine responses including tumor necrosis factor (TNF), interleukin 1 (IL-1) and JAK-STAT pathways, chemokine secretion and neuropeptide signaling.
Conclusion: Targeting neuroinflammatory mechanisms with specific emphasis on TNF, IL-1 and JAK-STAT signaling could open new therapeutic perspectives to treat CRPS-related pain.
Support: National Brain Research Program (2017-1.2.1-NKP-2017-00002); GINOP-2.3.2-15-2016-00050 „PEPSYS”; EFOP-3.6.1-16-2016-00004 FK132587 és ELIXIR Hungary.

RESOLVIN D1 AND D2 INHIBIT TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 AND ANKYRIN 1 ION CHANNEL ACTIVATION ON SENSORY NEURONS VIA LIPID RAFT MODIFICATION

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Transient Receptor Potential Vanilloid 1 and Ankyrin 1 (TRPV1, TRPA1) cation channels are expressed in nociceptive primary sensory neurons, and regulate nociceptor and inflammatory functions. Resolvins are endogenous lipid mediators. Resolvin D1 (RvD1) is described as selective inhibitor of TRPA1-related postoperative and inflammatory pain in mice acting on the G protein-coupled receptor DRV1/GPR32. Resolvin D2 (RvD2) is a very potent TRPV1 and TRPA1 inhibitor in DRG neurons, decreases inflammatory pain in mice acting on the GPR18 receptor, via TRPV1/TRPA1-independent mechanisms. We provided evidence that resolvins inhibited neuropeptide release from the stimulated sensory nerve terminals by TRPV1 and TRPA1 activators capsaicin (CAPS) and allyl-isothiocyanate (AITC), respectively. We showed that RvD1 and RvD2 in nanomolar concentration significantly decreased TRPV1 and TRPA1 activation on sensory neurons by fluorescent calcium-imaging and inhibited the CAPS- and AITC-evoked ^{45}Ca-uptake on TRPV1- and TRPA1-expressing CHO cells. Since CHO cells are unlikely to express resolvin receptors, resolvins are suggested to inhibit channel opening through surrounding lipid raft disruption. Here we proved the ability of resolvins to alter the membrane polarity related to the cholesterol composition by fluorescence spectroscopy. It is concluded that targeting lipid raft integrity can open novel peripheral analgesic opportunities by decreasing the activation of nociceptors.
Support: 2017-1.2.1-NKP -2017-00002 (NAP-2), GINOP-2.3.2-15-2016-00050, EFOP-362-16-2017-00008, 17886-4/23018/FEKUTSTRAT. É. Szőke and É. Sághy were supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences, ÚNKP-19-4 New National Excellence Program of the Ministry of Human Capacities grant

Serum growth factor and cytokine levels show positive correlations with the intensity of certain pain parameters in endometriosis patients

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Endometriosis is a complex estrogen-dependent inflammatory disease, which affects 10-12 % of women in reproductive age, causing chronic pain and infertility. Complex sensory-vascular- and immune reactions play a role in its development and progression, but the underlying mechanism and therapy remain to be clarified. Therefore, here we investigated inflammatory cytokines, chemokines and growth factors in serum and tissue samples to determine their potential involvement in the pathophysiology of the disease.
Serum samples of endometriosis patients with minimal/mild (n=9) and severe (n=56) endometriosis (luteal and secretory phases) and rectal lesions (n=8) from severe endometriosis patients were examined. The concentrations of epidermal growth factor (EGF), vascular-endothelial growth factor (VEGF), macrophage migration inhibitory factor (MIF), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6 and IL-8 were measured by immunoassays. Pain parameters (dysmenorrhoea, dyspareunia, dyshchesia, dysuria, chronic pelvic pain: CPP) were determined by visual analog scale (VAS).
In severe endometriosis patients serum EGF levels significantly increased in the secretory phase compared to both age-matched healthy women and the luteal phase of these patients. However, their serum IL-6 and MIF concentrations were significantly lower in the secretory than in the luteal phase. Although IL-8 did not change in the serum, it was significantly elevated in rectal endometriosis tissues compared to healthy controls. Other investigated factors did not significantly change either in the serum or tissue. Serum EGF, IL-8, TNF-α and VEGF levels showed low positive correlations with dysmenorrhoea intensity, EGF and IL-8 with dysuria, and MIF with dyschesia. No correlations were found with dyspareunia and CPP.
EGF, MIF and IL-6 might be involved in endometriosis pathogenesis, particularly in the luteal phase. EGF, IL-8, TNF-α, VEGF and MIF possibly contribute to certain pain parameters, but more patients are needed to be included to draw strong conclusions.
NAP-2 20017-1.2.1-NKP -2017-463 00002, FIKPII-17886-4/23018/FEKUTSTRAT, EFOP-362-464 16-2017-00008, EFOP-3.6.1-16-2016-00004, Bolyai Research Scholarship.