RECOOP HST ASSOCIATION

Student Conference

RECOOP - Frigyes Korányi Science Forum
March 9, 2018
Hotel Gellert, Budapest, Hungary

Cedars - Sinai Medical Center -
RECOOP HST Association Research Center

CRRC

SEQUERE LUCEM

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Knowledge transfer began with the Sumerians, the earliest known civilization in the Bronze Age. Sumerians developed the initial documentation of their life in writing around 3500–3200 BC. The next step was the production of papyrus in Egypt, as early as 3000 BC; later papyrus was sold to the ancient Greeks. The Mycenaeans, an early tribe of Greeks between 1500 and 1200 BC, adapted the Minoan syllabary to write an early form of Greek. The Romans kept very good written records of their life and history starting in the 7th Century BC in a language that was called Latin. The written and spoken Latin language was used for hundreds of years after the Roman Empire fell and many European languages still use the Roman alphabet today. Sanskrit is the primary liturgical language of Hinduism and was written around the 6th-4th Centuries BC. In China, the educated people knew Chinese characters at about 4,000 BC. Various current Chinese characters have been traced back to about 1200–1050 BC. Little is known of the prehistory of the Japanese language. The earliest known examples of Japanese writing date back to the 5th and 6th Centuries AD.

The Ancient Egyptians made significant advances in astronomy, mathematics and medicine, traced back to 3000 BC. Traditional medicine has been developing for thousands of years in the Ancient Greek society and China. Just 600 years ago, the scientific revolution took place in Europe during the Renaissance and in the modern-day, science has become professionalized.

Scientific work contributes to the development of abilities for creative work and for the acquisition of knowledge needed by every university graduate; it is also useful in the selection of future researchers and teachers. Science is not perfect and sometimes produces controversial results, but at the same time it teaches analytical thinking skills and is very valuable in modern societies. Scientists should share their raw data in ways that are easily accessible and digestible, and it is necessary for their findings to be reanalyzed or replicated by others. Scientists need to publish the methods and findings more completely and science should be more transparent. Therefore, the scientific activity of students is an important factor for bringing a “fresh view” into everyday scientific work.

Cedars – Sinai Medical Center, as the leader of the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) realized the importance of student scientific research in the progress of medicine. Four years ago, CSMC – RECOOP engaged with Frigyes Korányi College for Advanced Studies which has many years of tradition in students’ research works. RECOOP - Frigyes Korányi Science Forum in 2018 underscores the important role played by student organizations like Frigyes Korányi College for Advanced Studies in the success of scientific work by students, because university science will stagnate and decline without the scientific work of students and their tutors.

CSMC – RECOOP would like to promote the involvement of students in RECOOP research activities. We would like to provide an opportunity for the student to prepare their graduate or Diploma Thesis from the research they are doing at our Cedars-RECOOP Research Centers (CRRC). See the list of the RECOOP Member Organizations at https://www.cedars-sinai.edu/Research/Research-Administration/Recoop/

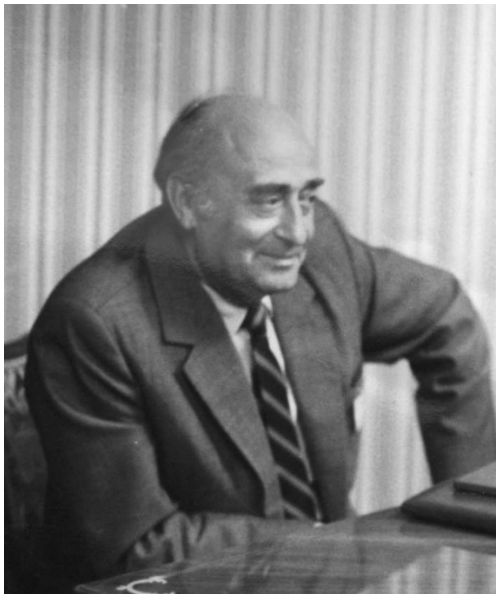
Dr. Sandor G. Vari, MD
Director of International Research and Innovation in Medicine Program
Cedars - Sinai Medical Center, Los Angeles, CA, USA &
President of the Regional Cooperation in the Fields of Health, Science and
Technology Association (RECOOP HST Association)

Agenda

Student Conference
RECOOP - Frigyes Korányi Science Forum 2018

In memoriam of Professors Frigyes Kulka and Zoltan Szabo iconic figures of the Hungarian medical education in the 20th Century.

The purpose of their life can be summarized in three words ***Ut Prosim* Aliis**
"*May* I be *useful* to others".



Professor Frigyes Kulka organized and founded the Division of Thoracic Surgery of the Department of Surgery, University of Szeged in 1959. Most of the surgical procedures were lung resections for tuberculosis patients until the end of the 60s. From 1979 he served as Chair of the Department of Thoracic Surgery of the Postgraduate Medical University, Budapest. In 1981 became the Director of the 1st Department of Surgery, and in 1986 he was elected Rector of Postgraduate Medical University. He also served as president of both the Hungarian and the European Society of Surgery.

Professor Zoltan Szabo served as the Director of the Varosmajor Heart and Vascular Centre from 1981 until 1992. He was an innovative surgeon, in 1967 pioneered the pacemaker implant in Hungary, and in January 1992 he performed Hungary's first successful heart transplant following years of research. Professor Szabo between 1979 and 1985 was dean of the Faculty of Medicine of the Semmelweis University and served as Vice-Rector for General Affairs for 3 years.

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Balint Egyed, Medical Student, Member of the Science Committee of Frigyes Korányi College for Advanced Studies, Semmelweis University Budapest
http://semmelweis.hu/szakkollegium/en/

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General Information

Venue: March 9, 2018 Danubius Gellert Hotel, http://www.danubiushotels.hu/gellert

Arrival on March 8, 2018 (Thursday) until 6 pm
Departure on March 10, 2018 (Saturday)

Access to the Spa on Thursday afternoon or Saturday free of charge.
Accommodation March 8 and 9 for two nights

March 8, 2018

Arrival until 6 pm.

20:00 pm Buffet Dinner – Duna Room

Registration desk at Tea Saloon Foyer, time interval for registration 15:00 – 19:00

March 9, 2018

Registration desk at Tea Saloon Foyer 7:30 – 12:00

Interactive Poster Plenary Session – Tea Saloon
Poster Display and Discussion - Gobelin Room - Poster size Height 120cm Width 90cm
Coffee Breaks – Gobelin Foyer

08:30 – 08:45 Welcome
Sandor G. Vari

08:45 – 09:50 Interactive Poster Plenary Session - Cardiovascular Diseases (CVD) – Tea Saloon

Session Chairs
Ines Drenjancevic
Jan Pitha
Katarina Sebekova
Bela Szekacs
Oana Tautu

Effect of ACE inhibition combined with blockade of soluble epoxide hydrolase on the course of congestive heart failure in a rat model
Petr Kala, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Prevention of the development of HFpEF by the PDE-5A inhibitor Vardenafil in rats with type 2 diabetes
Balint Andras Barta, Semmelweis University, Budapest, Hungary

Assessment of left ventricular reverse remodeling by cardiac CT after transcatheter aortic valve implantation
Borbala Vattay, Semmelweis University, Budapest, Hungary

Sustained potentiation of α1-adrenergic vasoconstriction by sphingosine-1-phosphate
Rita Cecilia Panta, Semmelweis University, Budapest, Hungary

Sexual differences in the biomechanics and vasoreactivity of coronary resistance arteries in exercise induced left ventricular hypertrophy
Eszter Horvath, Semmelweis University, Budapest, Hungary

Effects of juvenile-onset depression on the preclinical signs of cardiovascular diseases – preliminary results
Karoly Havlik, Semmelweis University, Budapest, Hungary

Reendothelisation and freezing methods aimed for vascular tissue engineering
Kristof Csepi, Semmelweis University, Budapest, Hungary

Importance of cardiac screening for master athletes over age 35 due to cardiovascular risk
Mate Babity, Semmelweis University, Budapest, Hungary

Improved cognitive performance following transcatheter aortic valve implantation
Mirjam Turani, Semmelweis University, Budapest, Hungary

The effect of myocardial bridge on the presence of coronary artery disease: case-control study
Sara Papp, Semmelweis University, Budapest, Hungary

Increased visceral arterial tortuosity in Marfan syndrome: a possible new approach to predict aortic dissection
Noemi Daradics, Semmelweis University, Budapest, Hungary

Heritability of left ventricular morphology: A classical twin study
Sarolta Borzsak, Semmelweis University, Budapest, Hungary

New possibilities in aortic valve surgery
Alexandra Balint, University of Pécs, Hungary

09:50 – 10:30 Interactive Poster Plenary Session - Drug Development and Effects (DDE) - Tea Saloon

Session Chairs
Robert Gaspar
Tamas Tabi
Judit Hajagos-Toth

Modeling doxorubicin-induced endothelial toxicity *in vitro;* endogenous and exogenous preventive and protective mechanisms
Kristof Gyorgy Csiko, Semmelweis University, Budapest, Hungary

Rofecoxib is cardioprotective but paradoxically shows hidden cardiotoxic properties
Tamas Gergely, Semmelweis University, Budapest, Hungary

Nimodipine treatment preserves the efficacy of neurovascular coupling in the ischemic rat cerebral cortex
Dora Hantosi, University of Szeged, Hungary

Selected concepts and investigations among 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones as biologically active compounds
Olha-Maria Fedusevych, Danylo Halytsky Lviv National Medical University, Ukraine

Impact of acquired drug resistance on the anticancer activity of the experimental anticancer lanthanum complex KP772
Nataliia Molochii, Ivan Franko National University of Lviv, Institute of Cell Biology of National Academy of Sciences of Ukraine

10:30 – 11:30 Poster Display and Discussion - Gobelin Room

Cardiovascular Diseases (CVD)
Drug Development and Effects (DDE)

11:30 – 12:00 Coffee Break - Gobelin Foyer

12:00 – 12:40 Interactive Poster Plenary Session - Neurodegenerative Diseases (NDD) Tea Saloon

Session Chairs
Maria Heffer
Senka Blazetic
Sandor G. Vari

Isolating Neural Stem Cells of Subependymal Zone from Cerebrospinal Fluid
Rana Rasool, University of Zagreb, School of Medicine, Croatia

E-cadherin as potential biomarker for progression of intracranial meningioma
Petar Brlek, School of Medicine, University of Zagreb, Croatia

Study of the Phylogenetic Background of Neurotransmitters in the Unicellular Organism *Tetrahymena Pyriformis*
Andria Tziakouri, Semmelweis University, Budapest, Hungary

Multifractal properties of dynamic functional brain connectivity in the resting state
Orestis Stylianou, Semmelweis University, Budapest, Hungary

The rapid effect of 17-β-estradiol on diffusion dynamics of p75 receptor in live neurons
Andras Straub, University of Pécs, Hungary

Neuroprotective effect of estradiol in basal forebrain cholinergic neurons in vivo:
results and perspectives
Daniel Schranz, University of Pécs, Hungary

Hypoperfusion in response to anoxic depolarization is caused by the failure of autoregulation in the cerebral cortex
Borbala Eva Szepes, Institute: University of Szeged, Szeged, Hungary

12:40 – 13:00 Neurodegenerative Diseases (NDD) Poster Display and Discussion - Gobelin Room
13:00 – 14:00 pm Buffet Lunch – Duna

14:00 – 15:30 Interactive Poster Plenary Session - Translational Medical Research (TMR) - Tea Saloon

Session Chairs
Eszter Ducza
Shubhada Bopegamage
Lesya Kobylinska
Livia Simon Sarkadi
Rostyslav Stoika

Aging-related changes in staining optical density of Insulin Receptor α, Insulin-like Growth Factor 1b Receptor and Leptin Receptor in male murine brain
Matija Fenrich, J. J. Strossmayer University of Osijek, Croatia

The Expression of Inflammation Markers in Fatty Tissue of Female Sprague Dawley Rats Following Chronic Stress
Milorad Zjalic, J. J. Strossmayer University of Osijek, Croatia

Changes of inflammation markers in visceral adipose tissue of Sprague Dawley rats fed with HFHS diet after antidiabetic therapy
Aleksandar Katic, J. J. Strossmayer University of Osijek, Croatia

Biocompatibility of D-mannose-coated maghemite nanoparticles tested on neural stem cells
Hrvoje Mlinaric, University of Zagreb, School of Medicine, Croatia

Neural stem cell culture on bacterial nanocellulose and polylactic acid scaffolds
Jana Leskovar, University of Zagreb, School of Medicine, Croatia

Inhibition of the tyrosine-kinase c-Src has no effect on TASK-1 K+ channel
Sofiya Bilodid, Semmelweis University, Budapest, Hungary

Analysis of Immunoglobulin Heavy Chain Variable Region Mutational Status in Chronic Lymphocytic Leukemia
Ediz Eyupoglu, Semmelweis University, Budapest, Hungary

Characterization of lymphedema in a transgenic experimental mouse model
Gabor Kovacs, Semmelweis University School of Medicine, Budapest, Hungary

Relevance of endothelial and neuronal NOS in the recovery of the cerebrocortical blood flow after unilateral carotid artery occlusion in mice
Laura Simeos Dobrydnio, Semmelweis University School of Medicine, Budapest, Hungary

High-efficiency isolation of extracellular vesicles from blood plasma using iodixanol density gradient ultracentrifugation
Laura Almasi, Semmelweis University, Budapest, Hungary

Inhibition of Mitochondrial Substrate-Level Phosphorylation Kills Glutaminolytic Cancer Cells
Lennart Apenberg, Semmelweis University, Budapest, Hungary

Phylogenetical Molecular Evolution of CCL2 Chemokines – Investigations Of Chemotactic Ability of 47R, 72K AND 89H in Tetrahymena Model-Cell
Michika Hamada, Semmelweis University, Budapest, Hungary

Identification of potentially cardioprotective microRNAs in porcine acute myocardial infarction model after ischemic conditioning
Nabil Sayour, Semmelweis University, Budapest, Hungary

MicroRNA-mRNA network alterations under hypercholesterolemia in rat myocardium
Tamas Daniel Csury, Semmelweis University, Budapest, Hungary

Renal effects of acute postnatal hyperglycaemia in rats – preliminary study
Dorottya Balika, University of Pécs, Hungary

Role of Tocopherol Prooxidant-Antioxidant Balance in Liver of Rats Under Water-Immobilization Stress
Mariia Voronovska, Danylo Halytsky Lviv National Medical University, Ukraine

Chronic stress and ovariectomy affect the liver antioxidative status of adult rats
Sara Sormaz, J.J. Strossmayer University of Osijek, Croatia

15:30 – 16:15 Translational Medical Research (TMR) Poster Display and Discussion - Gobelin Room

16:15 – 16:40 Coffee Break - Gobelin Foyer

16:40 – 18:00 Interactive Poster Plenary Session - Clinical Research (CLR) - Tea Saloon

Session Chairs
Tibor Ertl
Imre Fehervari
Janina Kulka
Andrea Gaspar Suranyi
Charles F. Simmons

Relationship between blood eosinophil level and early re-exacerbations in COPD
Balazs Csoma, Semmelweis University, Budapest, Hungary

In silico cell model of Chronic Lymphocytic Leukaemia
Nora Aniko Kiss, Semmelweis University, Budapest, Hungary

In vitro model of the functional alterations of human neutrophil granulocytes in severe sepsis
Anna Parkanyi, Semmelweis University, Budapest, Hungary

Enhanced cell killing in B16F10 melanoma cell line by combining chemotherapy with conventional or modulated electro-hyperthermia
Eniko Major, Semmelweis University, Budapest, Hungary

Nanofabricated poly(vinyl alcohol) scaffolds for abdominal hernia repair
Constantinos Voniatis, Semmelweis University, Budapest, Hungary

The effects of music therapy and Kangaroo Mother Care (KMC) method on preterm babies
Eszter Tarjanyi, Semmelweis University, Budapest, Hungary

Protocol for studying the effect of modulated electrohyperthermia on melanoma in a mouse model
Alen Mathew, Semmelweis University, Budapest, Hungary

Exhaled carbon monoxide in obstructive sleep apnea
Martina Meszaros, Semmelweis University, Budapest, Hungary

Genetic variants of the Krebs cycle enzyme encoding genes in pheochromocytoma and paraganglioma
Sara Zakarias, Semmelweis University, Budapest, Hungary

Examination of genotypic-phenotypic correlations in Marfan-syndrome in order to predict the severity of cardiovascular manifestations
Roland Stengl, Semmelweis University, Budapest, Hungary

Frequent subclonal TP53 mutations in chronic lymphocytic leukemia detected by next generation sequencing
Viktoria Fesus, Semmelweis University, Budapest, Hungary

Cell surface receptor SLAMF5 enhances DC-autophagy via IRF8
Dora Bencze, University of Debrecen, Hungary

Rheumatoid Arthritis – a Mathematical Model
Nicolae Moise, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Viral Infections and Pregnancy
Alexandra Souli Bakaloglou, Slovak Medical University in Bratislava, Slovak Republic

Pathomorphological Review and Differential Diagnostics of Osteogenesis Imperfecta in Maroteaux-Lamy syndrome
Khrystyna Yuskiv, Danylo Halytsky Lviv National Medical University, Ukraine

18:00 – 18:45 Interactive Poster Plenary Session - Medical Imaging (MI) - Tea Saloon

Session Chairs
Viktor Berczi
Laszlo Matyus
Srecko Gajovic

Modeling of DTI parameters for optimal image quality and downstream processing
David Andrijevic, J. J. Strossmayer University of Osijek, Croatia

Mouse bioluminescent neurons for in vivo imaging
Magdalena Valenta, University of Zagreb, School of Medicine, Croatia

In vivo localization of deep brain electrodes in mouse neurophysiology experiments
Balint Kiraly, Semmelweis University, Budapest, Hungary

Artificial Intelligence in the Diagnosis of Cancer
David Juhasz, Semmelweis University, Budapest, Hungary

The effect of segmentation experience on the values of cardiovascular radiomic features of coronary atherosclerotic lesions
Natasa Javorszky, Semmelweis University, Budapest, Hungary

Assessment of the small intestinal blood flow by indocyanine green fluorescence using color-fluorescence laparoscope
Shohei Yoshida, Semmelweis University, Budapest, Hungary

3D Computer Tomography is a Predictor of Osteopenia caused by Maxillary Alveolar Bone Functional Asymmetry in Young People?
Sofia Anna Marynets, Danylo Halytsky Lviv National Medical University, Ukraine

18:45 – 19:45 Poster Display and Discussion - Gobelin Room
Clinical Research (CLR)
Medical Imaging (MI)

19:45 – 20:00 Closing Remarks
Sandor G. Vari
Announcement of winners who will attend the RECOOP 13th Bridges in Life Science Annual Conference on April 12 – 15, 2018, Hotel International Zagreb, Miramarska 24, Zagreb 10000, Croatia http://www.hotel-international.hr

20:00 – 22:00 Buffet Dinner – Duna

March 10, 2018

Departure

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Cardiovascular Diseases (CVD)

Effect of ACE inhibition combined with blockade of soluble epoxide hydrolase on the course of congestive heart failure in a rat model.

Kala Petr[1, 2], Sedláková Lenka[1], Škaroupková Petra[1], Kopkan Libor[1], Červenka Luděk[1, 2]

[1] Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.
[2] Department of Pathophysiology, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.

Corresponding author: Kala Petr (petrkala@gmail.com), 6th year student of General Medicine, 2nd Faculty of Medicine, Charles University, Prague, Czech Republic.

Key words: congestive heart failure, angiotensin-converting enzyme, aorto-caval fistula, epoxyeicosatrienoic acids, soluble epoxide hydrolase.

Introduction: Congestive heart failure (CHF) represents a serious medical problem. New treatment strategies that would prevent development of progression of CHF to the fatal end are needed. It has been found that tissue deficiency in the epoxyeicosatrienoic acids (EETs), paracrine vasodilatory and natriuretic agents, that are metabolites of cytochrome P-450-dependent epoxygenase pathway of arachidonic acid metabolism, contributes to the pathophysiology of progression of CHF. Therefore, it has been suggested that pharmacological interventions that increase tissue EETs (blockade of a soluble epoxide hydrolase - sEH) could provide a new approach to effective treatment of CHF. Here we examined if sEH inhibition added to renin-angiotensin system (RAS) blockade (as a golden-standard of CHF therapy) would further enhance protection against CHF induced by aorto-caval fistula (ACF) in hypertensive rats (TGR).
Methods: The well defined ACF-induced CHF animal model was used in TGR compared to the sham-operated normotensive rats (HanSD). Treatment regimens (placebo/ACEi/ACEi+sEHi; n=36 in each group) were started one week after ACF creation and the follow-up period was 50 weeks. RAS was blocked using angiotensin-converting enzyme inhibitor (ACEi, trandolapril, 6 mg/L) and sEH with an sEH inhibitor (sEHi, *c*-AUCB, 3 mg/L).
Results: All the sham-operated HanSD survived until the end of the experiment. The untreated ACF TGR started to die in the 10th week and after the 29th week none had survived. ACEi treatment greatly improved the survival rate, to 86% at the end of study. Surprisingly, combined treatment with ACEi and sEHi worsened the rate (53 % vs. 86 %, $p<0.05$).
Conclusion: An addition of sEHi to ACEi treatment does not provide better protection against CHF progression and does not increase the survival rate in ACF TGR; indeed, the rate decreases significantly. Thus, combined treatment with sEHi and ACEi seems not to be a promising approach to retard progression of CHF.

All experimental procedures are conformed to the European Guidelines for the Care and Use of Laboratory Animals. They were approved by Ethical Committee for Animal Research. The research project was also approved by the Ministry of Health Committee (on 16.03.2016 according to the law 246/1992 Sb. under the register number 19/2016 and official full name: "Farmakologické zásahy do metabolické cesty cytochromu P-450 jako nový přístup pro léčbu chronického srdečního selhání: preklinické studie na potkaním modelu.").

Prevention of the development of HFpEF by the PDE-5A inhibitor Vardenafil in rats with type 2 diabetes

Bálint András Barta[1], Alex A. Sayour[1], Klára A. Stark[1], Csaba Mátyás[1], Balázs T. Németh[1], Attila Oláh[1], Marianna Török[1], Mihály Ruppert[1], Dalma Kellermayer[1], Gábor Szabó[2], Gábor Kökény[3], Eszter M. Horváth[4], Beáta Bódi[5], Zoltán Papp[5], Béla Merkely[1], Tamás Radovits[1]

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[2]Department of Cardiac Surgery, University of Heidelberg, Heidelberg, Germany
[3]Institute of Pathophysiology, Semmelweis University, Budapest, Hungary
[4]Department of Physiology, Semmelweis University, Budapest, Hungary
[5]Division of Clinical Physiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Corresponding author: Bálint András Barta (barta.balint@gmail.com), 5th year student at Faculty of Medicine

Keywords: Vardenafil, cGMP, HFpEF, Diastolic dysfunction, LV stiffness

Introduction: Heart failure with preserved ejection fraction (HFpEF) shows an increasing prevalence. Specific co-morbidities play a crucial pathophysiological role in the development of HFpEF. Despite its epidemiological relevance, currently available therapies have not proven to be successful in decreasing the mortality of the disease. The contribution of deteriorated cyclic guanosine monophosphate (cGMP) signalling to the progression of HFpEF has been intensively investigated, while elevated levels of cGMP have been shown to exert cardioprotective effects in cardiovascular diseases. We investigated the effect of long-term preventive application of the phosphodiesterase-5A (PDE-5A) (the enzyme responsible for breaking down cGMP) inhibitor vardenafil in diabetic cardiomyopathy-associated HFpEF.
Methods: Zucker Diabetic Fatty (ZDF) rats were used as a model of HFpEF. ZDFLean rats served as controls. Animals received vehicle (ZDFLean; ZDF) or 10mg/kgBW vardenafil (ZDFLean+Vard; ZDF+Vard) per os from the 7th to 32nd weeks of age. Cardiac function, morphology were assessed by left ventricular (LV) pressure-volume (P-V) analysis and echocardiography at the 32nd week. Key markers of cGMP signalling, nitro-oxidative stress, apoptosis, myocardial hypertrophy and fibrosis were examined.
Results: ZDF animals showed increased LV stiffness (slope of the LV end-diastolic P-V relationship (EDPVR) and prolonged time constant of LV relaxation, markers of diastolic dysfunction, while systolic performance was preserved. Decreased myocardial cGMP level coupled with impaired protein kinase G (PKG) activity, increased nitro-oxidative stress, enhanced cardiomyocyte apoptosis, hypertrophic and fibrotic remodelling of the myocardium were observed. Vardenafil effectively prevented the development of HFpEF by maintaining diastolic function (decreased LV stiffness and improved LV active relaxation), by restoring cGMP levels and PKG activation, by lowering apoptosis and by alleviating nitro-oxidative stress, myocardial hypertrophy and fibrotic remodelling.
Conclusion: We reported that vardenafil successfully prevented the development of diabetes mellitus associated HFpEF. Thus, PDE-5A inhibition in a preventive manner might be a promising option in the management of HFpEF patients with diabetes mellitus.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
PEI/001/2374-4/2015, 30th July 2015, Pest County Government Office
Grants and financial support: This work was supported by the Hungarian Scientific Research Fund (OTKA-PD100245 (TR), OTKA-K 109083 (ZP)) and by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences (T.R.)

Assessment of left ventricular reverse remodeling by cardiac CT after transcatheter aortic valve implantation

Borbála Vattay, Judit Simon, Bálint Szilveszter, Daniel Oren, Márton Kolossváry, Júlia Karády, Ferenc Suhai, Béla Merkely, Astrid Apor, Pál Maurovich-Horvat

MTA-SE Lendület Cardiovascular Imaging Research Group, Heart and Vascular Center, Semmelweis University, Budapest, Hungary

Corresponding author: Borbála Vattay (bori.vattay@gmail.com), 5th year student at Faculty of Medicine

Keywords: CT angiography, TAVI, reverse remodeling

Introduction: Aortic stenosis (AS) provokes pressure overload in the left ventricle (LV), resulting in LV hypertrophy and remodeling. Transcatheter aortic valve implantation (TAVI) is a safe and effective treatment alternative for high-risk patients with severe AS. We aimed to assess the impact of the TAVI procedure on LV remodeling manifested by measurable CT-based LV mass changes.
Methods: We retrospectively performed gated CT angiography for the planning and follow-up of the TAVI procedure in 81 patients. We measured LV mass on serial CT images. We defined LV hypertrophy (LVH) as >115 g/m^2 for male, >95 g/m^2 for female. Reverse remodeling was defined as a reduction in LV mass below the LVH threshold. Hypo-attenuated leaflet thickening (HALT) was evaluated using the consensus of reads by 3 experienced radiologists. Pre- and post-procedural left ventricular mass was compared using the Wilcoxon signed rank test. Multivariate logistic regression analysis was performed to identify the independent predictors of reverse remodeling.
Results: The average time since implant was 1.7 ± 1.1 years for the follow-up scans. We found significant reduction in LV mass after the TAVI procedure: 177.8 [138.1-231.9] grams for pre- and 137.0 [105.5-154.5] grams for post-TAVI, respectively, $p<0.001$. Reverse remodeling was observed in 31 patients (38.3%). Average HALT score was 2.5 ± 2.1. From multivariate logistic regression analysis we found that the HALT score was an independent factor of LV remodeling over age, gender and traditional risk factors: Odds ratio 0.735 (95% C.I. 0.560-0.964), $p = 0.026$.
Discussion: CT provides reliable and reproducible assessment of anatomical data and remodeling processes. In our study we observed substantial reduction in LV mass, leading to a reverse remodeling in 38% of patients.
Conclusion: Significant regression of LV mass was observed after the TAVI procedure on CT images. HALT severity was an independent predictor of reverse remodeling and showed an inverse association with the beneficial structural changes.

Ethical Committee Approval: SE TUKEB 173/2016, 2016.10.10.

Sustained potentiation of α1-adrenergic vasoconstriction by sphingosine-1-phosphate

Rita C. Panta[1], Éva Ruisanchez[1], Dorottya Móré[1], Péter Tibor Dancs[1], Andrea Balogh[1], Stefan Offermanns[2], Gabor Tigyi[3], Zoltán Benyó[1]

[1] Institute of Clinical Experimental Research, Semmelweis University, Budapest, Hungary
[2] Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany
[3] Department of Physiology, University of Tennessee Health Science Center, Memphis, Tennessee, USA

Corresponding author: Rita C. Panta (sokszem@gmail.com), 6th year student at Faculty of Medicine

Keywords: sphingosine-1-phosphate; adrenergic vasoconstriction; potentiating effect, signal transduction

Introduction: In our present project we aimed to examine the vasoactive effects of sphingosine-1- phosphate (S1P) by evaluating its capability to alter the basal vascular tone and to influence vasoconstriction mediated by α1-adrenoreceptors. Our results were obtained using pharmacological tools and gene knockout (KO) animal models.
Methods: Effects of S1P and the α1–adrenoreceptor agonist phenylephrine (PE) on the tone of the mouse thoracic aorta were determined under isometric conditions with myography. Responses of vessels isolated from wild type (WT), $S1P_1$, $S1P_2$, and $S1P_3$ receptor-, or $G\alpha_{12/13}$-KO mice were measured and tone changes were normalized to vasoconstriction induced by 124 mM K^+.
Results: Addition of 5 µM S1P, which is in the concentration range reported in human serum, did not cause a significant change in the resting vascular tone. In contrast, EC50 of the vasoconstrictor effect of PE decreased, whereas Emax increased following 20 min incubation with 5 µM S1P in WT vessels, indicating a marked potentiating effect. Similar enhancement of the vascular reactivity was detected in $S1P_1$- and $S1P_3$-KO segments. In $S1P_2$-KO vessels, however, this phenomenon was absent. In addition, the potentiating effect of S1P was also lacking in vessels of $G\alpha_{12/13}$-KO mice and after inhibition of Rho-kinase by Y27632 in WT vessels. In further experiments we aimed to evaluate the duration of the S1P-induced enhancement of α1-adrenoreceptor-mediated vasoconstriction. Therefore, contractions evoked by 100 nM PE were determined every 20 minutes, repeatedly, following a 20-min incubation with S1P. Reactivity remained enhanced for 3 hours in WT segments, whereas this increase could not be detected in $S1P_2$-KO vessels.
Discussion and Conclusions: Although S1P does not directly modify the resting vascular tone by itself, it significantly enhances α1-adrenoreceptor-mediated vasoconstriction even at three hours after exposure. The $S1P_2$ receptor / $G_{12/13}$ / Rho-kinase pathway appears to be responsible for this potentiating effect of S1P. The sustained enhancement of vascular reactivity detected emphasizes the potential pathophysiological significance of this phenomenon in diseases associated with enhanced S1P production.

Ethical Committee or Institutional Animal Care and Use Committee Approval: All procedures were carried out according to the guidelines of the Hungarian Law of Animal Protection (28/1998) and were approved by the National Scientific Ethical Committee on Animal Experimentation (PEI/001/2706-13/2014).

Sexual differences in the biomechanics and vasoreactivity of coronary resistance arteries in exercise induced left ventricular hypertrophy

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Keywords: coronary adaptation, gender differences

Introduction: There is evidence that sustained, heavy physical exercise has effects on the heart, and it is also well known that the sex influences the cardiovascular risk. Most studies are based on the epicardial coronary arteries; common knowledge is still lacking about the functional adaptations of small arterioles. Synthesizing this evidence and changing the region of interest, in this study we observed the different ways of adaptations of resistance coronary arterioles in left ventricular (LV) hypertrophy depending on gender.
Methods: Young adult Wistar rats were distributed into four groups with 8 rats in each group. They were separated by the sex and half of the groups were trained by a 12 weeks long heavy swimming exercise program lasting 200 min/day. The controls also spent time in the water for 5 min/day. Ventricular adaptation was controlled by echocardiography at 11 weeks. At the termination of the study the intramural resistance coronary arteries (200 μm outer diameter) were removed to examine the biomechanical adaptation by pressure arteriography. Contractility, endothelium dependent dilation (EDD), tangential wall stress and elastic modulus were examined. Elastic remodeling was studied on resorcin-fuchsin stained histological sections.
Results: Relative heart mass increased in swimmers without arterial hypertension ($p<0.001$), ejection fraction ($p<0.001$), and fractional shortening were both elevated ($p<0.001$). Resistance arteries had thicker walls and reduced isobaric tangential wall stress ($p<0.05$). Elastic modulus at physiological pressures and density of inner elastic membrane increased in swimmers ($p<0.05$). Both spontaneous ($p<0.05$) and TxA_2 agonist induced tone ($p<0.001$) were elevated and endothelium dependent (bradykinin, $p<0.05$) and independent (adenosine, $p<0.001$) relaxations were more effective. Female swimmers had more vigorous contraction ($p<0.001$), while male swimmers had a more improved endothelial dependent vasodilation ($p<0.025$).
Discussion: Our results contribute to the understanding of sexualdifferences in coronary adaptation.
Conclusion: The range of vascular reactivity in coronary segments increased in both genders, but its mechanism was different between males and females.

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Effects of juvenile-onset depression on the preclinical signs of cardiovascular diseases – preliminary results

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Keywords: pediatric depression, cardiovascular risk

Introduction: It is still debated whether depression itself or the concomitant behavioral risk factors (smoking, lack of exercise, *etc.*) are the ones that increase the cardiovascular morbidity and mortality.
Methods: This examination is a substudy of a prospective longitudinal trial started in 1999. Patients who had juvenile-onset depression (JOD), their never depressed siblings, and age- and sex-matched control peers were enrolled. The plan is to evaluate 900 participants. Between 2016 and 2017, 283 participants [99 probands (54 women; median age: 25.7 (23.2-27.1) years, 113 never depressed siblings (58 women; median age: 23.8 (21.7-26.8) years, 71 unrelated normal peers (23 women; median age: 22.5 (21.5-22.9) years] were enrolled. Participants underwent psychosocial (psychiatric interview, self-report questionnaires), cardiovascular [biometric, peripheral and central blood pressure, and pulse wave velocity (PWV) measurements], and laboratory tests. Central arterial pressure and PWV were measured with a noninvasive technique: mechano-transducers were placed on the skin above the right carotid and femoral arteries [Complior (Colson, Garges les Gonesses, France)]. Mann-Whitney *U* test, Kruskal-Wallis probe, and ANOVA were used as statistical methods.
Results: No significant difference was found among the three groups regarding the central arterial pressure [probands: 83 (76-88) mmHg, never depressed siblings: 83 (76-88) mmHg, controls: 82 (76-86) mmHg; p=0.495]. However, the PWV, which provides information about the elasticity of the vessel wall, was significantly higher (p=0.028) both in probands [7.07 (6.33-7.53) m/s] and in their never depressed siblings [7.03 (6.57-7.47) m/s] compared to controls [6.73 (6-7.37) m/s].
Discussion: No prior study has employed a sibling-cohort design with a large, well-characterized sample of youth with JOD histories to predict early physiological markers of cardiovascular disease.
Conclusion: Besides depression, genetic and environmental factors also seem to have a role in the development of the preclinical signs of cardiovascular diseases.

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Reendothelisation and freezing methods aimed for vascular tissue engineering

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Keywords: reendothelisation; tissue engineering; cryopreservation

Introduction: Clinical use of vascular grafts is largely limited by the lack of functionally active endothelium. During freezing protocols, the endothelial layer on the luminal surface of cadaver grafts is often damaged. This may affect the feasibility of surgical use. Cryopreservation solution usually contains DMSO to prevent ice-crystal formation during freezing. However, it can be toxic to the cells. DMSO may also cause further side effects which can lead to enhanced thrombotic and infectious complications in the implanted grafts. Our aim was to compare DMSO containing (5-10%) and DMSO-free cryopreservation of vascular cells and grafts in order to improve cell survival and graft functionality.
Methods: We used 0.3% sodium-dodecyl-sulphate (SDS), 1% Triton X-100, and 0.1% peracetic acid solution for the decellularisation of human aortic segments. Aortic segments (60 μm thin and 3 mm long) were precoated with fibronectin to further improve the yield of cell seeding. Recellularisation of the aortic segments with HUVEC was evaluated at day 7. The viability of the reseeded cells was visualised by tetramethylrhodamine methyl ester (TMRM). Furthermore, the expressions of key endothelial markers were examined by polymerase chain reaction and immuncytochemistry before and after freezing.
Results: We have successfully decellularised and recellularised human vascular matrices. The reseeded endothelial cells maintained their phenotype and viability. We used the ImageJ post-processing program to quantify the extent of the surface covered by cells on the recellularised matrix. Thereby we proved that endothelial cells retained their levels of TMRM and endothelial marker expressions (CD31, VE-cadherin) during the recellularisation process on human aortic biomatrix segments.
Discussion: Of importance, synthetic grafts are not capable of key endothelial functions, e.g. vasoactive and inflammatory responses. Patients who suffer from recurrent vascular graft failures and infection, e.g. diabetic atherosclerosis obliterans, may benefit the most from a functionally active engineered graft.
Conclusion: This type of recellularisation of aortic segments can be appropriate for use in a future clinical trial. Furthermore, this model and method can be an option for examining the effect of different cryopreservation solutions on the grafts.

Ethical Committee or Institutional Animal Care and Use Committee Approval: SE TUKEB 164/2017 issued in July 31, 2017.

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Importance of cardiac screening for master athletes over age 35 due to cardiovascular risk

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Keywords: Athletes; Prevention; Sports; Risk; Middle aged

Introduction: It is known that regular screening of professional athletes is important for preventing sudden cardiac death. The number of master athletes is increasing and even though this population's cardiovascular (CV) risk is rising, not enough attention is being paid to their cardiac examination.
Aim: At the Semmelweis University Heart and Vascular Center during 2015-16 we examined the occurrence frequency of the CV risk factors and diseases in 50 master athletes who participated in our screening.
Methods: The athletes underwent an extended cardiac screening. The obligatory parts were detailed questionnaire, physical examination, 12-lead resting ECG, laboratory examination, blood pressure measurement, echocardiography and cardiopulmonary exercise testing (CPET).
Results: We summarized the results of 50, age 35+ athletes (male: 40, age 43.8±7.0 y). In terms of the CV risk factors 16 athletes had a positive personal and 33 athletes had a positive family history. The average BMI was 26.4±4.2 kg/m2, 44% (n=22) were overweight, 14% (n=7) were obese. 44% (n=22) had elevated serum cholesterol levels, and serum LDL-cholesterol levels were above normal in 44% (n=22) of the athletes. 14% (n=7) of the CPET ended with a positive result because of ST-T abnormalities or multiple ventricular extrasystoles, and in 18% (n=9) we found elevated stress blood pressure values. Holter ECG examination was performed based on these results and proved significant ventricular extrasystoles in 3 cases. During the coronary angiography we found 3 LAD-bridges, 3 coronary plaques which were at the significance limit and 1 significant coronary plaque. Coronarography done in 3 athletes showed non-significant coronary plaques. According to the SCORE points 8% (n=4) of the athletes had >5% CV mortality risk. We suggested lifestyle changes for 21 (42%), start/modification of medication therapy for 10 (20%) athletes and in 5 cases the limitation of sport activities.
Conclusion: Intensephysical activity can be a trigger of a possibly fatal CV event, the probability of which is significantly increased due to existing CV risk factors. Our study emphasizes the importance of the cardiac screening of middle aged athletes due to the frequent risk factors that often presentin this population.

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Improved cognitive performance following transcatheter aortic valve implantation

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Keywords: cognition, intracranial embolism, transcatheter aortic valve replacement

Introduction: Data available regarding the cognitive trajectory of patients undergoing transcatheter aortic valve implantation (TAVI) are scarce and contradictory. With the increasing use and the indication area expanding towards younger generations, understanding the effects on cognitive function is important.
Methods: Patients were included from the prospective arm of the RETORIC (NCT02826200) study, ongoing in the Heart and Vascular Center. Global cognitive function and specific cognitive domains were investigated using Mini Mental State Examination (MMSE) and Addenbrooke's Cognitive Examination (ACE). Tests were performed within 24 hours before and within 3 days after the procedure. After the intervention all patients underwent brain DTI MRI to identify potential new ischemic lesions.
Results: As of November 2017, 51 people with full datasets of pre-, postoperative cognitive tests and brain MRI have been included. Novel lacunar cerebral lesions were detected in 89% of the subjects. Global cognitive performance confirmed significant improvement in the postoperative results (mean MMSE score: 25.47± 3.1 vs. 26.03±3, p=0.03, mean ACE score: 71.41±12.8 vs. 74.12±13.41, p=0.01). When the analysis was confined to those patients whose baseline score was under the study population's average a more outstanding improvement was observed (MMSE: n=22, 22.55±2.6 vs. 24.5±3, p= 0.0001, ACE: n=33, 61.73±8.3 vs. 66.12±9.8, p=0.0004). Regarding specific cognitive domains, anterograde memory improved significantly following intervention (12.45 ±5.19 vs 14.06±4.97, p=0.007
Discussion: Although few and limited-size studies have investigated the cognitive trajectory of patients undergoing TAVI, and even fewer have applied advanced brain imaging, according to current literature, there is a clear discrepancy between the incidence of novel ischaemic lesions and cognitive impairment following implantation, which is in accordance with our results.
Conclusion: Our initial results indicate no short term cognitive decline, in fact, patients showed significant improvement in cognitive function following TAVI, despite the high incidence of peri-operatively developed novel lacunar cerebral lesions.

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The effect of myocardial bridge on the presence of coronary artery disease: case-control study

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Keywords: Myocardial bridging, Coronary artery disease, Computed tomography angiography

Introduction: Previous studies have suggested that the presence of myocardial bridge (MB) increases the risk of atherosclerosis proximal to the MB. Our aim was to assess the effect of MB on the quantity and composition of plaques proximal to MBs.
Methods: Patients who underwent coronary CT-angiography due to suspected coronary artery disease were enrolled into this retrospective case-control study. We selected patients with MB on their left anterior descending (LAD) coronary artery and a control group matched for age, sex, BMI and cardiovascular risk factors such as smoking, hypertension, diabetes mellitus and dyslipidaemia. Using semiautomated plaque quantification software we segmented the LAD segment proximal to the MB in the cases and an identical length segment of the LAD in each control patient. We compared the plaque volume and plaque composition between the case and control groups (Wilcoxon signed-rank test).
Results: In total, we assessed 50 case-control pairs (mean age: 59.5±12,1 versus 59.2±11.9 years, p=0.162; males: 78%). The average lengths of the analysed segments proximal to MBs were 46.5±9.3 mm in the case and 46.4±9.3 mm in the control group (p=0.144). The total plaque volume did not differ between the groups (154 mm^2 [IQR:110.8-201.9] versus 142 mm^2 [IQR:100.4-196.9], p=0.904). We found no significant difference in the calcified plaque volume (21 mm^2 [IQR:9.6-39.3] versus 13 mm^2 [IQR:8.6-28.3], p=0.322), and in the non-calcified plaque volume (79 mm^2 [IQR: 53.0-103.8] versus 85 mm^2 [IQR:62.4-114.5], p=0.415) between the groups.
Discussion: Our results suggest that the MB has no effect on plaque volume and composition in the coronary segment proximal to the MB.
Conclusion: Presumably, MBs do not increase the risk of coronary artery disease.

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Increased visceral arterial tortuosity in Marfan syndrome: a possible new approach to predict aortic dissection.

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Keywords: Marfan syndrome, arterial tortuosity, visceral arteries, risk stratification

Introduction. In Marfan syndrome (MFS) it is of utmost importance to create a model that could precisely determine the risk and also the probable onset of aortic dissection to choose the optimal timing of preventive surgical interventions. For this purpose currently aortic growth rate and family history are evaluated, however clinical evidence suggests that this approach is not reliable enough. According to our previous findings plasma levels of transforming growth factor-β (TGF-β) could help to predict the aortic risk, while other studies highlighted the role of aortic and carotid artery tortuosity in the risk stratification. The aim of this study was to investigate the tortuosity of renal and splenic arteries that are not influenced by the skeletal features of MFS.
Methods. We performed a retrospective analysis on 29 MFS patients and 23 control subjects using helical thoracic and abdominal CT angiography imaging. The centerline of splenic and renal arteries was exported with the use of dedicated software. To measure tortuosity, distance metric (DM) and the 3D versions of sum of angles metric (SOAM) and inflection count metric (ICM) were calculated. In the case of 15 MFS patients, plasma TGF-β level was measured by ELISA. Mann-Whitney U-test and Spearman rank correlation were used for statistical analysis.
Results. DM of the right and left renal, and splenic artery was significantly higher in MFS patients than in controls (1.30±0.27 vs. 1.10±0.07 p=0.0096; 1.63±0.38 vs. 1.21±0.15 p<0.001; 2.39±0.51 vs. 2.04±0.60 p=0.008 respectively). SOAM of the left renal artery was significantly lower compared to controls (0.46±0.15 vs. 0.57±0.10 p=0.037). We observed a tendency of positive correlations between TGF-β levels and the DM and ICM values calculated for the left renal and the splenic artery.
Discussion. To our knowledge this is the first demonstration that visceral arterial tortuosity, which is independent of the skeletal deformities characteristic of the syndrome, is increased in MFS. The observed opposite tendency between the DM and SOAM metrics suggests that arterial tortuosity in MFS is dominated by curves of lower frequency but higher amplitude.
Conclusion. Visceral arterial tortuosity could be a possible new predictor of adverse cardiovascular outcomes.

Ethical Committee: Medical Research Council ETT-TUKEB 12751-3/2017/EKU (31/03/2017).
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Heritability of left ventricular morphology: A classical twin study

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Keywords: twin study; coronary ct angiography; left ventricular mass; heritability

Introduction: Increased left ventricular (LV) mass is one of the strongest independent predictors of adverse cardiovascular outcomes. The extent of genetic and environmental influences over LV mass has been evaluated predominantly by echocardiography with controversial results. However, CT angiography (CTA) provides a more reproducible, 3D volumetric assessment of LV dimensions. Therefore, our aim was to estimate the inheritance of the LV mass within same sex twins using CTA.
Methods: We measured LV mass of 190 healthy monozygotic (MZ) and dizygotic (DZ) twins who underwent 256-slice CTA. We measured LV mass and end-diastolic volumes (EDV) using a semi-automated software based on epi- and endocardial contour detection. All continuous variables were expressed as mean±SD. Concordance between MZ and DZ pairs were assessed by Pearson correlations. Heritability - an estimate of genetic determination - was calculated for LV mass and EDV using the Falconer method. Data analysis was performed using SPSS (v24).
Results: Mean age of MZ and DZ twins was 54.8 ± 9.6 and 58.5 ± 9.5 years, respectively. Mean BMI was 27.8±5.3 kg/m2 for MZ and 27.7±5.3 kg/m2 for DZ twins. The measured mean LV mass and EDV was 84.8±24.7 grams and 122.3±30.3 ml for MZ, 83.1±24.7 grams and 117.7±30.3 ml for DZ twins, respectively. A broad heritability estimate of 0.48 was found for LV mass and 0.45 for EDV.
Discussion: We found moderate heritability in LV mass using highly reproducible CTA based measurements. Increased LV mass has been linked to several cardiovascular risk factors and adverse cardiac outcomes. Our study provides important insights into genetic and environmental influences on LV structure.
Conclusion: In this classical twin study we have demonstrated that LV mass and EDV have a moderate heritability as assessed by coronary CTA. This provides opportunity for pharmacological modification of LV mass and volume.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The national ethics committee approved the BUDAPEST-GLOBAL study (institutional review board number: 58401/2012/EKU [828/PI/12]; Amendment: 12292/2013/EKU), and all patients provided written, informed consent.
Grants and financial support: The initial part of the study was supported by a grant from the EFSD New Horizons Program to György Jermendy, MD. Szilárd Vörös, MD, is a shareholder in Global Genomics Group, LLC, and receives salary from Global Genomics Group, LLC. The authors have no other funding, financial relationships, or conflicts of interest to disclose.

New possibilities in aortic valve surgery

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Keywords: Intervention, Cardiology, aortic stenosis, TAVI, Perceval

Introduction: Severe aortic stenosis causes serious morbidity and mortality in the elderly population. It cannot be treated by medication. The survival of symptomatic aortic stenosis is 2-3 years with risk of sudden death. The mortality of severe aortic stenosis is 30-50%. The only therapeutic treatment is surgery. However, in cases of certain age and diseases the majority of the patients are rejected due to comorbidities. The new therapeutic possibilities include the sutureless minimal invasive technique and the transcatheter aortic valve implantation.
Methods: Since 2014 Perceval sutureless valve has been implanted in 8 cases in aortic position and Lotus transcatheter valve implantation has been carried out in 10 cases in the Heart Clinic. In the Perceval group the mean age was 74,6 and the percentage of men was 50%, in the Lotus group: the mean age was 80,4 and the percentage of men was 30%. The two groups were compared regarding the success of the surgery, the complications and the long term outcomes.
Results: In both groups the aortic gradient was significantly decreased. There was one exit in the Perceval group due to stroke. The ejection fraction did not change significantly. During the long term follow up both groups reported significant improvement regarding physical capacity and quality of life.
Discussion: In spite of the low number of cases, TAVI is an evident alternative of the heart surgery intervention.
Conclusion: In case of severe aortic stenosis both techniques are surgically sufficient and convenient alternatives. The rate of surgical complications is lower than in the surgical aortic valve replacement in cases of well-chosen patient groups.

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Drug Development and Effects (DDE)

Modeling doxorubicin-induced endothelial toxicity *in vitro;* endogenous and exogenous preventive and protective mechanisms

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Keywords: drug-induced vascular injury, doxorubicin, prevention, neuregulin-1

Introduction: Myocardial toxicity of doxorubicin (DOX) is well described, however, little is known about its direct cellular effects on endothelial cells (ECs). Intact cardiac and vascular ECs have pivotal roles in the regulation of cardiovascular physiology. Preventing DOX-induced endothelial toxicity may attenuate its myocardial toxicity. EC-derived neuregulin-1 (NRG-1) is a prerequisite in the cardiomyocyte-endothelial interaction, and its involvement in DOX-induced cardiotoxicity has been suggested. This study aims to examine the effects of DOX on ECs and the production of NRG-1, as well as the putative preventive and reversal effects of the frequently used lipid-lowering drug rosuvastatin (RO).
Methods: Human induced pluripotent stem cells-derived EC (hiPSC-EC), human umbilical vein EC (HUVEC) and human microvascular EC (HMVEC) were treated with increasing doses of DOX (0.1-15μM, 24h). To assess toxicity, ECs were labeled with caspase-3/7 (apoptosis, FAM FLICA assay), propidium iodide (necrosis, PI) and Hoechst33342 fluorescent stains and quantified with ImageJ. Preventive RO was given prior to DOX treatment (0.5-5μM, 6h). NRG-1 levels were measured from cell culture media using sandwich ELISA.
Results: DOX increased the apoptosis and necrosis of hiPSC-EC, HUVEC and HMVEC cells in a dose-dependent manner. DOX (5-15μM) showed significant increases in caspase-3/7 (5μM 37.8%±3.4; 10μM 52.8%±5.8; 15μM 53.0%±4.2 vs. control DMSO 5.6%±0.76; $p<0.05$) and in PI (5μM 59.4%±10.8; 10μM 81.0%±13.3; 15μM 84.8%±8.1 vs. DMSO 3.6%±1.8; $p<0.05$) activities. NRG-1 production increased parallel with the dose of DOX. High-dose RO (5μM) decreased the DOX-induced necrosis.
Discussion: In this study, a dose-dependent endothelial toxicity of DOX was modeled. High-dose RO attenuated, but did not fully revert endothelial damage. Endothelial NRG-1 secretion showed increasing tendency with higher doses of DOX, suggesting a protective role for NRG-1. Identification of protective treatment strategies and better understanding of the role of NRG-1 in DOX-induced vascular toxicity are warranted.
Conclusion: The effects of anti-tumor drugs on ECs can be examined in our in vitro model. Human iPSC derivatives may provide a platform for patient-specific assessment of therapeutic and adverse effects of drugs.

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Rofecoxib is cardioprotective but paradoxically shows hidden cardiotoxic properties

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Keywords: cardiotoxicity; ischemia-reperfusion injury; rofecoxib

Introduction: Unexpected ischemia-related cardiac adverse effects are the leading causes of discontinuation of clinical trials and withdrawal of drugs from the market. Drugs may exert unwanted effects by interfering with ischemic tolerance and inhibiting endogenous cardioprotective pathways which led to the novel concept of "hidden cardiotoxicity". Hidden cardiotoxicity cannot be revealed by toxicity screening methods currently available. Therefore, we aimed to explore the effect of drugs withdrawn from the market due to unexpected myocardial ischemic events on ischemia-reperfusion (I/R) injury and cardioprotection by ischemic preconditioning (IPC). In the present study rofecoxib, the use of which was associated with increased risk of myocardial infarction, was investigated in a rat model of I/R injury and IPC.
Methods: Male Wistar rats were treated with rofecoxib (5.12 mg/kg/day) and vehicle (control group) for 28 days. Both groups of animals were subjected to a 30-min left anterior descending (LAD) occlusion followed by 120 min of I/R or IPC elicited by 3 cycles of 5-min LAD occlusion and 5-min reperfusion before the 30-min LAD occlusion/reperfusion. After the 120-min reperfusion, area at risk and infarct size were measured by Evans blue and triphenyltetrazolium chloride staining. An electrocardiogram was recorded throughout the entire experiment.
Results: Chronic rofecoxib treatment increased acute mortality (I/R + rofecoxib: 44% vs I/R + vehicle: 11%) and tended to increase the occurrence of ventricular fibrillations compared to the vehicle treated group. The treatment reduced infarct size per se (I/R + rofecoxib: 29.89±5.4% vs I/R + vehicle: 47.1±3.8%) and did not affect the cardioprotective effect of ischemic preconditioning (IPC + rofecoxib: 12.43±1% vs IPC + vehicle: 20.30±3.7%).
Conclusion: We were able to show that rofecoxib has hidden cardiotoxic properties as it increased acute mortality due to arrhythmic complications during ischemia/reperfusion. Rofecoxib decreased infarct size but did not interfere with the cardioprotective effect of ischemic preconditioning. This is the first demonstration that rofecoxib exerts a cardioprotective effect but shows hidden cardiotoxic properties.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
Pest County State Bureau PE/EA/1784-7/2017

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Nimodipine treatment preserves the efficacy of neurovascular coupling in the ischemic rat cerebral cortex

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Keywords: neurovascular coupling, cortical spreading depression; nimodipine; hemodynamic brain response

Introduction: In ischemic brain injury, neurovascular coupling is impaired, which causes inadequate perfusion response to neuronal activity. Nimodipine is a clinically used neuroprotective agent, but its effect on neurovascular coupling is unknown. Furthermore, nimodipine administered systematically to patients causes hypotension, an unfavourable side effect. All considered, we aimed to assess the protective effect of nimodipine on neurovascular coupling, and to develop a method for drug delivery that targets ischemic tissue zones exclusively.
Methods: Two open cranial windows were prepared over the parietal cortex of isoflurane-anesthetized, male Sprague-Dawley rats (n=28). The right femoral artery was cannulated for blood pressure (BP) monitoring. In half of the animals, the common carotid arteries were occluded (2VO). Local field potential and cerebral blood flow (CBF) were recorded from the rostral craniotomy, in which nimodipine (100 µM) or its vehicle were administered topically. CBF was assessed in response to repeated whisker stimulation and subsequent spreading depolarization (SD) events triggered by 1 M KCl applied in the caudal craniotomy.
Results: Nimodipine significantly increased the magnitude of hyperemia in response to whisker stimulation (15.4±6.6 vs. 6.2±2.9%, 2VO nimodipine vs. vehicle), decreased the amplitude of SD (13.0±3.0 vs. 15.5±2.6 mV, 2VO nimodipine vs. vehicle), and increased the amplitude of hyperpolarization subsequent to SD (3.7±0.6 vs. 2.2±0.9 mV, 2VO nimodipine vs. vehicle). At the same time, nimodipine excerted no impact on BP (107.5±18.9 vs. 111.3±13.7%, intact nimodipine vs. vehicle), but elevated baseline CBF gradually (120.1±16.8 vs. 102.4±1.9%, intact nimodipine vs. vehicle).
Discussion and conclusion: Nimodipine effectively protects neurovascular coupling in the ischemic brain, and inhibits the evolution of injurious SD. Since local nimodipine administration did not alter BP in our experiments, our next project will focus on intravenous targeted drug delivery with nanoparticles that have been designed to release nimodipine in response to ischemic tissue acidosis.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The experimental procedures were approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (Licence number: XXXII./878/2015). The procedures conformed to the guidelines of the Scientific Committee of Animal Experimentation of the Hungarian Academy of Sciences (updated Law and Regulations on Animal Protection: 40/2013. (II. 14.) Gov. of Hungary), following the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.
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Selected concepts and investigations among 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones as biologically active compounds

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Key words: thiazoles, anticancer activity, anti-inflammatory activity.

Introduction. The non-condensed heterocyclic systems with thiazole/thiazolidinone moieties and their structure-related analogs are important structural components of bioactive molecules, and, as a result, they serve as attractive targets for rational design of "drug-like" compounds. Hence, among substituted thiazole derivatives agents have been identified with antibacterial, anti-inflammatory, anticancer, antifungal and antifilarial activities. Moreover, substituted thiazole derivatives are well known as COX-2 inhibitors, serine protease urokinase (uPa) inhibitors, adenosine A1 receptor antagonists, and metabotropic glutamate receptor 5 (mGluR5) antagonists.
Methods. The corresponding research studies in the literature regarding synthesis have been analysed. The synthetic procedure of obtaining appropriate biologically active 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones in the Claisen–Schmidt condensation was adopted. The synthesized compounds were evaluated for anticancer activity in NCI60 cancer cell lines and for antimicrobial activity on the method of diffusion into agar.
Results. The novel 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones were synthesized from 1-[2-amino-4-methylthiazol-5-yl]ethanones and various aromatic aldehydes in the Claisen–Schmidt condensation. As a result, a library of new substituted thiazole derivatives for searches of new anticancer and antimicrobial agents has been designed and synthesized.
Discussion. The tested 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones demonstrated promising activity in the *in vitro* screen on the tested cell lines, as well as some distinctive patterns of selectivity. The synthesized compounds were highly active on a colon cancer HCT-117 cell line and leukemia CCRF-CEM cell line. Antimicrobial screening led to the identification of active compounds against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Interestingly, that tested compounds showed better antifungal activities than amphotericin-B.
Conclusion. The preliminary results allowed identification of active compounds with promising anticancer and antimicrobial activities.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Protocol № 3 from 18/03/2013 of the BioEthics committee of the Danylo Halytsky Lviv National Medical University.
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Impact of acquired drug resistance on the anticancer activity of the experimental anticancer lanthanum complex KP772

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Keywords: drug resistance, ribonucleotide reductase, metal drug

Introduction: Drug resistance is still a huge problem in cancer treatment, making the search for new antineoplastic drugs necessary. KP772 is a ribonucleotide reductase (RR) inhibitor with promising anticancer activity *in vitro* and *in vivo*. Noteworthy, recent research has shown that ABCB1-overexpressing cancer cells remain sensitive to KP772, which demonstrates that this metal drug might be of interest for patients not responsive to standard chemotherapy.
The aim of this work was to explore the influence of KP772 on different cancer cell lines resistant to the clinically investigated RR inhibitors.
Materials and methods: Several RR inhibitor-resistant cell lines were generated by continuous exposure of the respective chemo-naive parental cells to increasing concentrations of triapine (Wild types of cell lines were obtained from ATCC). Cell viability was evaluated by MTT assay. Proteinlevels were measured by Western-blot analysis.
Results: Viability assays revealed that the anticancer activity of KP772 was not hampered by resistance against hydroxyurea (KB-HU) or gemcitabine (Capan/Gem). In contrast, both triapine-resistant models had altered sensitivities towards KP772: The ovarian carcinoma model A2780/Tria was significantly less sensitive to KP772 than its parental line. In contrast, SW480/tria cells exhibited an increased sensitivity towards KP772. This finding is very likely associated with strong ABCB1 overexpression by this cell line, as shown by Western blotting, and resulted in enhanced senescence levels in SW480/tria cells upon KP772 treatment. In contrast, enhanced RR expression (a common resistance factor e.g. to hydroxyurea and gemcitabine) had no impact on KP772 efficacy.
Conclusion: KP772 is a promising new anticancer drug, which is active even against cells resistant to other RR inhibitors.

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Neurodegenerative Diseases (NDD)

Isolating Neural Stem Cells of Subependymal Zone from Cerebrospinal Fluid

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Keywords: neural stem cells, subependymal zone, cerebrospinal fluid, neuroaminidase and fibroblast growth factor 2

Introduction: Cell replacement therapy has emerged as a potent therapeutic tool for central nervous system diseases involving cell type-specific defects, such as untreatable hypomyelinating disorders called leukodystrophies. We have developed a novel method that enables the release of neural stem and progenitor cells from the subventricular zone (SVZ) neurogenic niche into the cerebrospinal fluid (CSF), making them easily accessible for collection. Postnatal brain neural stem cells (pbNSCs) can be subsequently collected from the CSF and then expanded in vitro before being used for autologous transplantations.
Methods: Neuraminidase was injected or infused into the ventricles of young rats combined with an integrin-β1 or a dystroglycan blocking antibody and fibroblast growth factor 2 (FGF-2). Cells were collected from CSF 3 days post-surgery and immunocytochemistry was performed to assess their profile. Coronal brain sections and SVZ wholemount preparations of treated and untreated control animals were immunostained to assess the damage.
Results: There was a higher number of cells collected from CSF after injecting drugs as compared to intact subependymal zone (SEZ) control. However, there were no differences in the total number of collected cells between different drugs. Co-injection of FGF-2 increased the proliferation of cells in the SEZ and led to the release of a higher proportion of Sox2+ cells. Immunostaining of coronal brain sections revealed the existence of reactive astrocytes.
Discussion: The cell-type profile of collected cells from CSF matched that of the SEZ niche, as co-injection of FGF-2 resulted in increased numbers of Sox2+ cells. Histological analyses revealed that the drugs specifically targeted the wall of the lateral ventricles at the site of the SEZ.
Conclusion: This is a new method for collection of pbNSCs aimed at cell-based therapies and a useful tool for the performance of longitudinal studies of pbNSCs, for example in animal models of neurodegeneration or ageing.

IACUC Approval: The Animal Welfare and Ethical Review Body (AWERB) at Cambridge approved this study (Award RG75148 RRZA/047) on 0025072014.
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E-cadherin as potential biomarker for progression of intracranial meningioma

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Keywords: E-cadherin, loss of heterozygosity, microsatellite instability, meningioma

Introduction: Recent studies have shown that the epithelial-mesenchymal transition (EMT), a biological process necessary for embryogenesis, could also be involved in invasiveness of different tumor types. One of the prominent features of EMT is loss of expression of E-cadherin. In our research we wanted to verify if loss of E-cadherin could be a potential marker of the molecular changes responsible for the control of cellular mobility and therefore for progression of intracranial meningioma.
Methods: We analysed genetic changes of the E-cadherin gene (CDH1) and its protein expression in 45 samples of human meningioma with different grades of malignancy. Genetic alternations of CDH1 gene (loss of heterozygosity [LOH], microsatellite instability [MSI]) were tested by polymerase chain reaction (PCR) using microsatellite marker D16S3025 and analysed by electrophoresis on Spreadex gels. To assess and localize E-cadherin expression, we used DAB-labeled immunohistochemical reaction using streptavidin horseradish peroxidase/DAB (EnVisionTM, Dako REALTM) and specific monoclonal antibody against E-cadherin.
Results: The results for CDH1 changes showed that genetic changes of E-cadherin were present in a portion of meningioma (9% of samples showed LOH, 13% of samples showed MSI- and 4% of samples showed LOH and MSI). Protein expression was still high or moderate in samples demonstrating genetic changes but varied in samples with different grades of malignancy. Two atypical meningiomas lacked E-cadherin expression.
Discussion: Samples with genetic changes did not demonstrate low expression of E-cadherin. This finding could be explained by a second functional allele in the genome. Some samples with higher grades of malignancy did not express E-cadherin, partially confirming our hypothesis of E-cadherin involvement in meningioma progression.
Conclusion: To confirm that our findings can indicate the involvement of reduced E-cadherin expression in meningioma progression, experiments and statistical analysis should be performed on a higher number of meningioma with grades II and III. After additional future analyses our findings could be useful as potential biomarkers for cellular mobility of invasive intracranial meningiomas.

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Study of the Phylogenetic Background of Neurotransmitters in the Unicellular Organism *Tetrahymena Pyriformis*

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Keywords: phylogeny, neurotransmitter, Tetrahymena, chemotaxis

Introduction: The key function of the brain is communication. Neurons form networks, governed mainly by complex neurotransmitter interactions, that connect with each other allowing a delicate interplay between the different regions of the brain. The objective of the present experiment investigates the phylogenetic background of the human central nervous system (CNS) by creating a comparable binary and ternary interplay of interactions between different neurotransmitters (norepinephrine, histamine, serotonin, acetylcholine, glutamate, dopamine) in the unicellular eukaryote *Tetrahymena pyriformis*. Tetrahymena – as a protozoon - has no known nervous system; however, it has been shown to store, synthesize and secrete biogenic amines as well as to have binding sites for the corresponding G-protein-coupled receptors of some of these molecules.
Methods: The chemotactic responsiveness of the neurotransmitters was examined in Tetrahymena cells, using a modified version of Leick's two-chamber capillary chemotaxis assay with 20-minute incubation times. The concentration course of each molecule was determined and the concentration eliciting the strongest effect (chemoattractive or chemorepellent) was further used to examine the chemotactic response of the neurotransmitters when used in pairs and in groups of three.
Result and Discussion: Adequate cellular responses (chemoattractant or chemorepellent) were detected in both cases when the neurotransmitters were used alone or in combinations and more importantly, a pattern was observed between these responses. This proves that the chief regulatory molecules of the CNS can be identified even in lower, unicellular levels of phylogeny.
Conclusion: In summary, our results support the theory that any evolved nervous system could stem from a common origin. Therefore, identifying the "ancient" function of a molecule or its receptor effect can open new windows in the advancement of therapeutic interventions.

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Multifractal properties of dynamic functional brain connectivity in the resting state

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Keywords: Electroencephalograpy, Brain, Fractals, Neural net

Introduction: Network theory has proven to be an effective tool in describing functional brain connectivity. Although most of these studies consider functional connections static, the connection strength is constantly changing according to neural activity. Recent studies revealed that multifractal (MF) dynamics is an inherent property of the brain. The aim of our study was to investigate the MF properties of dynamic functional connectivity (DFC) based on human electroencephalography (EEG) measurements.
Methods: 24 adults (12 ♀) participated in this study. 5-5 minute EEG recordings were performed in eyes open (EO) and eyes closed (EC) states. Artifact removal was done by independent component analysis, and synchronization likelihood was used for pairwise DFC estimation for the δ, θ, α, β and γ frequency bands. Three different network metrics - density (D), clustering coefficient (C) and efficiency (E) - each capturing separate aspects of the network - were computed for every time point, resulting in network metric time series (NMTS). Subsequently, these NMTSs were made subject to focus-based MF time series analysis, yielding the following MF parameters: Hurst exponent ($H(2)$) capturing the long-term memory, $\Delta H15$ representing the degree of multifractality and focus (F) associated with the total variance in the signal. Significant differences were identified with two-way repeated measures ANOVA and Bonferroni post hoc tests ($p<0.05$).
Results: Significant differences in $H(2)$, $\Delta H15$ and F were found between the network measures in every frequency band. Furthermore, prominent gender-related differences appeared in all MF measures at multiple bands.
Discussion: Separate aspects of the topology of functional brain networks captured in the different network metrics express different dynamics, suggesting an underlying system with complex spatio-temporal characteristics.
Conclusion: Gender-related differences in our results suggest that gender should be considered as a factor when evaluating connectivity dynamics based on EEG measurements.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The study was approved by the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (April 24th, 2017. Approval number: 94/2017). All subjects provided written consent prior to measurement.
Acknowledgements: Adam Kiraly, Zhuo Duan

The rapid effect of 17-β-estradiol on diffusion dynamics of p75 receptor in live neurons

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Keywords: estradiol, p75 neurotrophin receptor, single molecule imaging, non-classical action

Introduction: In addition to classical genomic action, gonadal steroid 17-β-estradiol (E2) is known to exert rapid non-classical effects on membrane receptors and signaling molecules. The changes in surface movement of the receptors are essential to their function. These changes form membrane protein complexes that activate signalling molecules. Neurotrophin receptor p75 (NTR) is a regulator of neuronal survival. The key mechanism in NTR activation is the change of surface movement of the receptor. However, the effect of E2 on NTR surface trafficking is completely unknown.
Methods: We applied a unique total internal reflection microscopy system to allow super-resolution imaging of membrane receptor molecule surface trafficking. Trajectories of NTR molecules on live neurons were individually tracked and analysed. Mean square displacement (MSD) and the diffusion coefficient (D: μm2/sec) were determined both on the soma and neurites. Movement parameters were calculated using at least 200 trajectories and compared statistically to the corresponding vehicle control.
Results: The MSD function of NTR molecules in the soma and neurites saturates, suggesting restricted motion of NTR molecules in control conditions. After 100nM E2 application the MSD curve of NTR molecules changes to a straight line in soma and neurites demonstrating unrestricted motion of NTR molecules after E2 application. Furthermore, our results showed that administration of 100 nM E2 rapidly increases the D of NTR molecules both on the soma and neurites.
Discussion: The MSD function and D of NTR molecules showed that surface movement of NTR molecules changed to unrestricted after 100 nM E2 application and E2 rapidly increased the surface diffusion of NTR molecules. These effects were irrespective of the location of NTR in the neuronal membrane.
Conclusion: Our findings demonstrated that E2 rapidly alters the surface diffusion of NTR molecules. The effect of E2 on molecule movement of NTR may be implicated as a neuroprotective mechanism of E2.

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Neuroprotective effect of estradiol in basal forebrain cholinergic neurons in vivo: results and perspectives

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Keywords: estradiol, Alzheimer's disease, basal forebrain cholinergic neurons

Introduction: A major neuropathological hallmark of Alzheimer's disease (AD) is widespread neuronal death in basal forebrain cholinergic (BFC) neurons, which is thought to result from the accumulation of soluble aggregates of neurotoxic amyloid-β peptides, such as $A\beta_{1\text{-}42}$. The gonadal steroid 17β-estradiol (E2) exerts non-classical actions on intracellular signaling that is important for mediating the cytoprotective effect of E2. Here we examined the neuroprotective effect of E2 on BFC neurons in an AD animal model.
Methods: Female mice were ovariectomized under Avertin anaesthesia. Fourteen days after ovariectomy $A\beta_{1\text{-}42}$ were stereotaxically microinjected into the nucleus basalis magnocellularis under isoflurane anaesthesia. Mice were administered 33ng/g of E2 or vehicle subcutaneously 1h after $A\beta_{1\text{-}42}$ microinjection. Animals were killed 14 days after injections by an overdose of anaesthetic (Avertin) and perfused through the heart with 4% paraformaldehyde. Brains were removed and 30μm thick coronal sections were cut on a freezing microtome. The cholinergic fibre loss in the somatosensory cortex (SC) was visualized by AChE histochemistry.
Results: All animals that received unilateral injection of $A\beta_{1\text{-}42}$ showed a profound decrease in AChE-stained fibers in layers IV and Vof the SC from the lesioned hemisphere. Administration of E2 significantly attenuated the $A\beta_{1\text{-}42}$ -induced AChE fiber loss in the SC.
Discussion: We report that a single dose of E2 administered 1 h after intracerebral injection of Aβ effectively reduced the fiber loss of BFC neurons. However, E2 activates both the classical and non-classical pathways inducing uteotropic effects. One strategy to overcome this shortcoming has been to develop synthetic compounds, activators of non-classical estrogen like signaling (ANGELS), that do not exhibit side-effects.
Conclusion: Our findings suggest that E2 treatment has neuroprotective potential in AD. Based on our results we plan to test novel ANGELS compounds designed by computer modelling that provide protective action against AD without E2-induced side-effects.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
Accreditation number: AA3.0/2016; Certificate number: 19/A2/2016; Animal Welfare Committee, University of Pécs

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Hypoperfusion in response to anoxic depolarization is caused by the failure of autoregulation in the cerebral cortex

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Keywords: spreading depolarization, cerebral autoregulation, neurovascular coupling, ischemic stroke

Introduction: Recurrent ischemic spreading depolarizations (iSD) occur in the cerebral cortex from minutes up to weeks following acute brain injury, and contribute to lesion progression possibly by the associated reduction of cerebral blood flow (CBF). Recent evidence proves the role of high extracellular potassium concentration ($[K^+]_e$) in the mediation of iSD related vasoconstriction. Yet, it is uncertain whether CBF reduction is actively regulated under severe anoxic/ischemic conditions in response to spreading anoxic depolarization (AD).
Methods: An open cranial window was created over the parietal cortex of isoflurane-anesthetized, male Sprague-Dawley rats (n=5) for the recording of tissue $[K^+]_e$, local field potential (LFP) and CBF. The right femoral artery was cannulated for blood pressure (BP) monitoring. Incomplete global forebrain ischemia was achieved by the bilateral occlusion of the common carotid arteries, followed 30 min later by the withdrawal of oxygen from the anesthetic gas mixture for 5 min. Hypoxia was confirmed by arterial blood gas analysis.
Results: The negative deflection of LFP indicated the occurrence of iSD minutes after ischemia onset, and AD upon oxygen withdrawal. AD was associated with higher peak $[K^+]_e$ (50.14±2.2 vs. 43.54±6.66 mM) and longer $[K^+]_e$ shift duration (689.4±263.83 vs. 236.85±205.44 s) than iSD. At the same time, hypoperfusion in response to AD and iSD varied in the same range (6.65±4.21 and 10.66±2.2 %, respectively). Pearson correlation analysis revealed that CBF reduction with iSD developed independently of BP ($r<0.300$), while hypoperfusion with AD was significantly related to decreasing BP ($r>0.670$).
Discussion and Conclusion: In contrast to the high $[K^+]_e$-mediated vasoconstriction in response to iSD, hypoperfusion in response to AD is the result of a passive loss of arterial tone due to failing autoregulation. These findings give new insight into the mechanisms that shape the CBF response to injurious spreading depolarization events, and set AD apart from iSD.

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Ethical Committee or Institutional Animal Care and Use Committee Approval: The experimental procedures were approved by the National Food Chain Safety and Animal Health Directorate of Csongrád County, Hungary (License number: XXXII./878/2015). The procedures conformed to the guidelines of the Scientific Committee of Animal Experimentation of the Hungarian Academy of Sciences (updated Law and Regulations on Animal Protection: 40/2013. (II. 14.) Gov. of Hungary), following the EU Directive 2010/63/EU on the protection of animals used for scientific purposes.

Translational Medical Research (TMR)

Aging-related changes in staining optical density of Insulin Receptor α, Insulin-like Growth Factor 1b Receptor and Leptin Receptor in male murine brain

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Key words: aging, insulin, leptin, hypothalamus, hippocampus

Introduction: Hypothalamic nuclei and the hippocampus are shown to be implicated in feeding behaviour and energy homeostasis regulation. Aging seems to influence the metabolic status by inducing changes in those brain areas. Therefore, it is of crucial importance to understand the different patterns of changes that take place in these regions, in order to better understand the impact of aging on energy homeostasis.
Materials & Methods: Male C57BL/6 mice (n=5) were divided into five groups based on their age at the time of sacrifice: 3 months old (3MO), 6 months old (6MO), 12 months old (12MO), 16 months old (16MO) and 20 months old (20MO).The animals were anesthetized with isoflurane, and prefunded ("perfused with") 4% paraformaldehyde in phosphate buffer saline (pH = 7,4). Brains were isolated, perfunded, cryoprotected ("fixed and cryoprotected") using 30% saccharose, and stored at -80 °C. The specimens were later cut on a cryostat (Leica CM3050S) into 35 micronthick slices. The following regions of interest were subsequently identified: arcuate hypothalamic nucleus (Arc), periventricular hypothalamic nucleus (PeV) and dentate gyrus of the hippocampus (DG). The regions were immunohistochemically stained for Insulin Receptor α (IRa), Insulin-like Growth Factor 1b Receptor (IGF1-Rb) and Leptin Receptor (ObR). Slices were photographed using a Zeiss Axioskop 2 MOT microscope, and quantification of staining optical density was performed using ImageJ software.
Results: Immunohistochemical analysis shows that IRa tends to yield a denser signal in older animals compared with younger ones in Arc and PeV, but not in DG, where its optical density decreases as the animal ages (3MO 6,15%, 6MO 6,71%, 12MO 2,56%, 16MO 2,37% and 20MO 1,04%). The ObR signal does not seem to change significantly with aging, with the exception of marked depletion in Arc of 20MO mice when compared to the younger animals (16MO 46,2% vs. 20MO 4,19%). IGF1-Rb showed a steady trend of increase in signal density as the mice age, except for PeV where optical density does not change much until an animal reaches 20 months (average optical density for 3MO-16MO being 3,36% vs. 20MO reaching 18,41%).
Conclusion: Results have shown that Arc, PeV and DG in male mice might undergo different patterns of aging-related changes in IRa, ObR and IGF1-Rb expression. Further study should involve reward regions due to close connections with satiety regions and cerebellum as the regions least affected by aging.

Ethical Committee Approval: Ministry for Agriculture, Committee for Animal Research: registration number HR-POK-005.
Funding: The study has been funded by Croatian Science Foundation under project number IP-09-2014-2324.

The Expression of Inflammation Markers in Fatty Tissue of Female Sprague Dawley Rats Following Chronic Stress.

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Key words: adipocytes, chronic stress, inflammation, macrophages, aging

Introduction: The aim of this study was to show differences in the size of adipocytes and the appearance of inflammation in the adipose tissue of young and old female rats after the exposure to sham or chronic stress.
Materials and Methods: The study included 40 female Sprague Dawley rats (10 per group) of age 3,5 or 14,5 months that were exposed to either sham or stress for 3 cycles per 10 days. Fixed tissue was cryoprotected or embedded in paraffin. Paraffin blocks of adipose tissue were hemalaun/eosin stained to determine the surface area of adipocytes. The degree of inflammation was determined immunohistochemically using markers CD68 and CD163. Reticulin staining was done according to Gomori. Images were taken under a microscope, and quantification was made using ImageJ.
Results: Older stressed female rats had significantly larger adipocytes and a higher degree of inflammation than younger rats. The number of M1 macrophages decreased in younger animals after chronic stress, and the opposite was observed in older animals. The number of M2 macrophages decreased after chronic stress in both groups of animals.
Conclusion: Chronic stress had the opposite effect on young and old animals. While in younger animals it reduced the low-grade inflammation in adipose tissue, in older animals the inflammation was increased.

Ethical Committee Approval: Experiments were carriedout at the Animal Facility of Faculty of Medicine, Osijek, approval number: HR-POK-005 and the Animal Facility of Faculty of Pharmacy, Szeged, approval number: CSI/01/3796-7/2015.
Acknowledgments: This study was funded by Croatian Science Foundation project IP-09-2014-2324 and Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program and the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association). Part of this study was performed at the Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, under the supervision of Prof. Robert Gaspar.

Changes of inflammation markers in visceral adipose tissue of Sprague Dawley rats fed with HFHS diet after antidiabetic therapy

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Key words: visceral adipose tissue, inflammation, Sprague Dawley rats, antidiabetics

Introduction: High fat high sugar (HFHS) diet is considered a risk factor for developing diabetes type 2 in research animals. In clinical practice, early therapy for prevention of diabetes includes the antidiabetics metformine and liraglutide. We tested these treatments in an animal model.
Methods: We studied 64 animals divided into 8 groups, with 4 female groups and 4 male groups. The first group in each sex received standard chow (SD); the second group was given high fat high sugar diet (HFHSD); the third was on HFHSD and given metformin 50 mg/kg/day s.c, and fourth group had HFHSD and given liraglutide 0.3 mg/kg/day, s.c.. The standard histological staining of visceral adipose tissue was used to measure the size of the lipid vacuole. The degree of inflammation was estimated imunohistochemically by using markers CD68 and CD163. Results were quantified and compared.
Results: The size of lipid vacuoles increased in the second group ($p<.001$) and stayed the same in the fourth compared to the control group ($p<.001$). The third group had also increased size of the lipid vacuole, but less than the second group. Inflammation marker CD68 increased in the second ($p<.001$) and fourth ($p<.001$) groups compared to the first group but decreased in the third group ($p<.001$) in comparison to the second group. CD163 decreased in the second ($p<.001$) and third ($p<.001$) groups compared to the first group but increased in the fourth group ($p<.03$) compared to second group.
Discussion: HFHSD increased the visceral adipocyte size, while HFHSD combined with an antidiabetic tended to normalise adiposity. Inflammatory markers changed with the HFHS diet, with mostly CD68 increasing and CD163 decreasing compared to the control group. Metformin decreases and liraglutide increases CD163 and CD68 markers.
Conclusion: HFHSD increases the size of adipocytes, but antidiabetic therapy can modulate the changes in vacuole size and inflammation in the tissue.

Ethical Committee Approval: Hungarian Ethical Committee for Animal Research, University of Szeged, registration number IV/3796/2015.
Funding: The study has been funded by RECOOP HST Association, and in part by the Croatian Science Foundation under project number IP-09-2014-2324.
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Biocompatibility of D-mannose-coated maghemite nanoparticles tested on neural stem cells

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Keywords: biocompatibility; neural stem cells; iron nanoparticles; D-mannose; nanomedicine

Introduction: Coating of nanoparticles changes their biological and chemical features, which influences their biocompatibility. The hypothesis of this research study was that D-mannose-coated γ-Fe_2O_3 nanoparticles had increased biocompatibility compared to uncoated γ-Fe_2O_3 nanoparticles in vitro.
Methods: Both D-mannose(γ-Fe_2O_3) and uncoated(γ-Fe_2O_3) nanoparticles were used (courtesy of Dr. Horak from Academy of Sciences, Prague). Neural stem cells (NSC) were isolated from the telencephalic wall of E14.5 fetuses by microdissection and dissociation with StemPro Accutase. Nanoparticle biocompatibility was tested in vitro using MTT, CalceinAM/PI and neurosphere assays.
Results: The MTT assay showed that both nanoparticle types decreased the number of active/viable NSC in a dose-dependent manner. The CalceinAM/PI assay showed the mean number of living NSC for both nanoparticle types in all tested concentrations was higher than 90%. The neurosphere assay showed that although all spheres showed round an oval morphology, their growth was better in D-mannose(γ-Fe_2O_3) treated culture at the highest concentrations.
Discussion: All assays showed similar biocompatibility features of D-mannose(γ-Fe_2O_3) and uncoated nanoparticles. However, D-mannose(γ-Fe_2O_3) treatment slightly increased the potential of NSC to form neurospheres.
Conclusion: D-mannose(γ-Fe_2O_3) had increased biocompatibility features compared to uncoated(γ-Fe_2O_3) for NSC growth and differentiation.

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Research support: This study was supported by Cedars-Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association), by Croatian Science Foundation under the project IP-06-2016-1892, and by EU FP7 grant GlowBrain (REGPOT–2012–CT2012–316120)

Neural stem cell culture on bacterial nanocellulose and polylactic acid scaffolds

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Keywords: neural stem cells; bacterial nanocellulose; polylactic acid; bionanocomposite

Introduction: Designing an optimal nerve conduit for joining injured sites of peripheral nerve has been a focus in nerve tissue engineering for the last few decades. Desired properties of the material used as a nerve conduit are to mimic extracellular matrix morphology, be biocompatible and biodegradable and support neuronal attachment and proliferation. These requirements could be achieved in the milieu of a nanofibrous network of bacterial nanocellulose. So far, only four studies have investigated neural cells and nanocellulose interaction. In this work, we intend to study and compare biocompatibility of neural stem cells on bacterial nanocellulose, polylactic acid, and composite nanocellulose-polylactic acid scaffolds.
Methods: Scaffolds would be prepared by commercially available materials and undergo swelling test, dynamic mechanical analysis and scanning electron microscopy. Cell proliferation and viability would be assessed by MTT and calceinAM assays.
Results: Findings existing in the literature indicate that both materials promote cell proliferation. We expect improvement of those results on composite scaffolds. Further evaluation may reveal possible applications of the scaffolds.
Discussion: Examined materials could verify if the tested support is adequate for the cells. Therefore, advanced modifications such as surface modification, growth factor incorporation and enhancement of electrical conductivity followed by electrical stimulation could modify the scaffold to be used for peripheral nerve regeneration.
Conclusion: Bacterial nanocellulose could provide a basic 3D matrix convenient for additional morphological processing and composite synthesis with potential applications in regenerative neuroscience.

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Inhibition of the tyrosine-kinase c-Src has no effect on TASK-1 K^+ channel

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Keywords: Potassium channels; Pulmonary hypertension; Protein-Tyrosine Kinases

Introduction: Background K^+ channels determine the resting membrane potential and regulate cellular excitability. In pulmonary artery smooth muscle cells, (PASMC) TASK-1 is the major background potassium channel. Experimental results suggest that phosphorylation by c-Src tyrosine kinase activatesTASK-1 in human PASMC. It has also been reported that treatment of patients with the c-Src inhibitor dasatinib often causes pulmonary hypertension and the involvement of TASK-1 in this side effect was suggested. However, the data on the mechanism of dasatinib-induced pulmonary hypertension and the role of c-Src in the process remain contradictory. Our aim was to investigate the direct effect of dasatinib on TASK-1 in a heterologous expression system.
Methods: All three potential c-Src phosphorylation sites in TASK-1 were mutated to phenylalanine with site directed mutagenesis ("triple mutant channel"). Linearized plasmids were used as templates for *in vitro* cRNA synthesis. *Xenopus laevis* oocytes were injected with channel cRNA (*Xenopus* oocytes endogenously express c-Src, phosphorylation by c-Src plays an important role in fertilization) and currents were measured by the two-electrode voltage-clamp technique.
Results: The amplitudes of the expressed K^+ currents of TASK-1 and the triple mutant channel were identical when the oocytes were injected with equal amounts of cRNA. Oocytes expressing either wild type or mutant TASK-1 were pretreated with dasatinib (5 µM) for 45 minutes. The pretreatment failed to influence the current of the triple mutant channel (as expected), however, similar results were obtained with the wild type channel. The efficiency of our dasatinib stock to inhibit c-Src dependent processes was verified in another experimental system, excluding the possibility that it had degraded.
Discussion: Dasatinib pretreatment had no effect on the current of the TASK-1 channel expressed in *Xenopus* oocytes. Thus, the hypothesis that dasatinib causes pulmonary hypertension through blocking c-Src, and thereby leads to reduced TASK-1 activity, PASMC depolarization, calcium signal and increased tone is not supported by our results.
Conclusion: Mechanisms other than direct inhibition of TASK-1 are responsible for dasatinib-induced pulmonary hypertension.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Experimental procedures involving animals were approved by the Animal Care and Ethics Committee of Semmelweis University (approval ID: XIV-I-001/2154-4/2012).
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Analysis of Immunoglobulin Heavy Chain Variable Region Mutational Status in Chronic Lymphocytic Leukemia

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Keywords: Chronic lymphocytic leukemia, chain variable region genes

Introduction: Chronic lymphocytic leukemia (CLL) is a heterogenous disease with variable clinical outcome. CLL is characterized by several genetic lesions capable of predicting the disease course. Mutational status of the immunoglobulin heavy chain variable region genes (IGHV) has been proven to be a strong prognostic factor, with patients harbouring mutated (M) IGHV characterized by longer survival than patients with unmutated (U) IGHV genes. Furthermore IGHV-M cases respond better to conventional therapy, while current guidelines classify patients with IGHV-U as a high-risk CLL, demonstrating better outcomes with novel targeted therapies.
Methods: Genomic DNA samples were collected and analyzed by conventional PCR for 6 different HV gene families according to the European Research Initiative on CLL (ERIC) guidelines. Amplified PCR products were sequenced by a Sanger sequencer with the sequences analyzed using the IMGT/V-Quest tool for determining the closest germline gene and %identity. Interpretation of results according to %identity to the closest germline gene were as follows: <97%= IGHV-M, >98%= IGHV-U and 97%-97.9% was categorized as borderline (IGHV-B). Arrest tool was used to determine the IGHV subset.
Results: Analyzing 224 CLL cases, IGHV-U genotype was observed in 58% (130/224) of cases, 37% (82/224) of the cases presented with IGHV-M, and 5% (12/224) characterized by IGHV-B genotype. Cases with IGHV-U displayed significantly worse overall survival ($p<0.0001$) and shorter time to first treatment ($p=0.0473$) compared to IGHV-M cases. In the subgroup of TP53 disrupted cases (TP53 status was available as part of the diagnostic workup), an even more remarkable difference in survival between IGHV-U and IGHV-M was observed.
Conclusion: Our study demonstrates that IGHV mutation status is a strong prognostic factor and important biomarker for the risk stratification of CLL. The strong survival difference between IGHV-U and IGHV-M in TP53 disrupted cases indicates the strength of IGHV status as an independent prognostic factor.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
Institution: ETT-TUKEB, Date: 2016-08-31, ID: 45371-2/2016/EKU
Acknowledgements: Dr. Csaba Bödör

Characterization of lymphedema in a transgenic experimental mouse model

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Keywords: lymphedema; lymphatic system; transgenic; mouse model

Introduction: Loss or dysfunction of lymphatics leads to the development of primary or secondary lymphedema. In addition to accumulation of extracellular fluids, chronic lymphedema may result in fibrosis, swelling and recurrent infections in the affected organs. These complications seriously reduce the function of the affected organs and lead to the degradation of the quality of life for patients. Development of novel therapeutic approaches requires the understanding of the organ specific functions of the lymphatic system and the pathophysiology of lymphedema.
Aims: We aimed to characterize the pathophysiology of lymphedema in a novel transgenic experimental mouse model.
Methods: To induce the organ specific elimination of lymphatics the *Flt4-Cre^{ERT2}; iDTR$^{flox/flox}$* mouse strain was used. Tamoxifen was administrated to induce the expression of the diphtheria toxin receptor (DTR) followed by the injection of diphtheria toxin into the paws, ears and peritoneum to eliminate the lymphatic endothelial cells. The development of the lymphedema was assessed by daily measurement of paw and ear thickness and monitoring of clinical disease signs. Paraffin based histology followed by routine histopatholgy and immunostainings were performed.
Results: We found that *Flt4-Cre^{ERT2}; iDTR$^{flox/flox}$* mice develop significant swelling of the ear and paw within one week after the diphtheria toxin injections. We demonstrated that this genetic system allows us to eliminate the lymphatic endothelial cells in the affected organs. The edema reaches its peak 7 days after the diphtheria toxin treatment (acute phase), and after a transient decrease it develops again during the chronic phase. In our experiments we performed the detailed characterization of the histological changes in the acute and chronic phases of lymphedema. We also successfully eliminated the lacteals by intraperitoneal injection of the toxin.
Conclusions: We have successfully applied and characterized a genetic system, which allows us to induce the organ specific elimination of lymphatic endothelial cells. The transgenic experimental disease model provides an excellent tool for studying the molecular mechanisms that are involved in the development of lymphedema, which may serve as novel therapeutic targets in the future.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Animal protocol: PEI/001/404--8/2015
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Relevance of endothelial and neuronal NOS in the recovery of the cerebrocortical blood flow after unilateral carotid artery occlusion in mice

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Keywords: NOS1, L-NAME, cerebrocortical microcirculation, carotid artery occlusion, cerebrovascular regulation

Introduction: The understanding of cerebral autoregulation has become of notable importance due to the increased incidence of carotid artery stenosis in the elderly population which entails an increased risk of stroke, the 2nd most common cause of mortality worldwide. Multiple mechanisms are debated in the literature, including the role of nitric oxide synthases (NOS). As we found earlier endothelial NOS (eNOS), surprisingly, does not seem to play an important role in the adaptation of the cerebrocortical microcirculation to unilateral common carotid artery occlusion (CAO). Now we aimed to determine the relevance of the neuronal nitric oxide synthase (nNOS), as well as to investigate the effects of general NOS blockade using L-NAME.
Methods: Experiments were performed on anesthetized male nNOS deficient (nNOS-KO) mice, as well as on their wild type littermates and C57Bl6 mice as controls. Cerebrocortical blood flow (CoBF) was determined in the parietal and temporal regions using the laser-speckle method immediately ("acute phase") as well as 5 minutes ("subacute phase") after CAO. Simultaneously, arterial blood pressure changes were recorded through a canula placed in the femoral artery. Arterial blood gas and acid-base parameters were determined before terminating the experiments.
Results: There were no significant differences in the CoBF changes either in the acute or in the subacute phase between control and nNOS-KO mice in any of the investigated regions. In addition, L-NAME treatment failed to alter the CoBF changes in the acute phase of CAO. Interestingly, however, L-NAME resulted in altered recovery of CoBF in the parietal region indicated by the more severe remaining CoBF reduction in L-NAME treated as compared to control animals.
Discussion and conclusions: Our results indicate that neither eNOS nor nNOS are involved in the acute adaptation of the cerebrocortical circulation to CAO. In the subacute phase, however, although the different isoforms of NOS can compensate the loss of each other, NO has an important role in cerebral autoregulation, since simultaneous inhibition all NOS isoforms with L-NAME worsens the recovery of CoBF after CAO.

Institutional Animal Care and Use Committee Approval: National Scientific Ethical Committee on Animal Experimentation (PEI/001/2706-13/2014, 17.12.2014).
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High-efficiency isolation of extracellular vesicles from blood plasma using iodixanol density gradient ultracentrifugation

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Keywords: extracellular vesicles; exosomes; density gradient centrifugation; iodixanol; plasma

Introduction: Extracellular vesicles (EVs) derived from cells play a role in cell-to-cell communication both in physiological and pathological processes. Circulating EVs are extensively investigated both as biomarkers and therapeutics; however, EV isolation from blood plasma is a great challenge. For functional analysis, it is required to isolate the majority of plasma EVs, and also eliminate impurities such as lipoproteins and soluble proteins. The aim of our study was to optimize an iodixanol density gradient ultracentrifugation (DGUC) based isolation method.
Methods: Rat blood samples underwent differential centrifugation to remove cells and debris. Density gradient was prepared by layering 1.33 mL of 50, 30 and 10% iodixanol solutions, then 0.5 mL plasma was loaded on top and centrifuged at 120,000×g for 24 hours. Ten fractions (F1-10) were collected from top to bottom. The efficiency and purity were assessed by Western blot (WB); equal volume or protein from samples were loaded, then EV and non-vesicular impurity markers were detected. Morphology and size distribution of particles were examined by dynamic light scattering (DLS) and electron microscopy (EM).
Results and discussion: EV markers Alix and Tsg101 were detected in F6-7 (density: 1.13-1.17g/mL) by WB; relative band intensity of both markers was over 60%, showing higher efficiency compared to currently described methods. Presence of membrane-bound vesicles in F6 was confirmed by EM and also with DLS, showing particles with a mean diameter of 38-40 nm. Approximately 90% of lipoprotein and 80% of albumin contamination were separated from exosome-containing fractions. However, 60% of fibrinogen was present in F6, highlighting the fact that non-vesicular plasma components are also present in the EV-rich F6.
Conclusion: Iodixanol DGUC is a highly efficient isolation method to separate a heterogeneous population of EVs from vesicle-like lipoproteins and is also able to reduce the amount of contaminating soluble proteins. DGUC-purified EV-rich fractions may be suitable for in-vivo studies on EVs. However, for analytical studies further purification of DGUC-purified EV-rich fractions will be necessary.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Pest County Government Bureau, PE/EA/1784-7/2017, Oct 31, 2017
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Inhibition of Mitochondrial Substrate-Level Phosphorylation Kills Glutaminolytic Cancer Cells

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Keywords: cancer; substrate-level phosphorylation; oncometabolism

Introduction: In a dynamic environment where oxygen and nutrients are frequently limiting, 'rewiring' of metabolism for supporting growth has been recognized as a hallmark of cancer. Furthermore, many tumors exhibit mutations leading to enzyme defects and perturbed redox homeostasis. Under such circumstances, oxidative phosphorylation may be impaired, and the risk of mitochondria switching from energy producers to consumers is heightened. Yet, cancer mitochondria manage to employ alternative energy-harnessing mechanisms, playing a major role in tumor progression. An extensive body of work in our laboratory has shown that mitochondrial substrate-level phosphorylation (mSLP) substantiated by succinate-coA ligase produces high-energy phosphates in the absence of oxidative phosphorylation, protecting against ATP depletion in conditions involving impaired respiration, as may occur in cancer.
Methods: In this study we used VM-M3, VM-M2, BV-2 and NIH-3T3 cells, lines that exhibit variabilities in glutaminolytic capacity.
Results: VM-M3 and VM-M2 cells show robust mSLP, little glycolytic dependence as deduced by oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) measurements, and a requirement for glutamine in the media in viability assays involving targeted inhibition of respiratory chain components. On the other hand, BV-2 and NIH-3T3 cells exhibited little glycolytic dependence. Knocking down of Suclg1 -a subunit of succinate-CoA ligase- by siRNA killed all transfected VM-M3 cells within 48 hours, but not NIH-3T3 cells.
Discussion: The purpose of glutaminolysis in cancer cells is probably to serve the provision of high energy-phosphates through substrate-level phosphorylation, substantiated by succinate-CoA ligase.
Conclusion: This work pinpoints mSLP as a potential new chemotherapeutic target for glutaminolytic tumors.

Ethical Committee or Institutional Animal Care and Use Committee Approval: No ethical approval required; the study was performed on cancer cell lines.
Grants and financial support: -
Acknowledgements: -

Phylogenetical Molecular Evolution of CCL2 Chemokines – Investigations Of Chemotactic Ability of 47R, 72K AND 89H in Tetrahymena Model-Cell.

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Keywords: chemokine phylogeny, Tetrahymena, chemotaxis, CCL2

Introduction; Coevolution of chemokines and their receptors is a significant characteristic of signaling networks in chemotaxis. Our previous work proved fMLF and FPR have an adaptive evolution in mammals, based on positively selected amino acids. In this study, we focused on human CCR2-CCL2 interaction. In order to investigate the CCL2 evolution, we evaluated (i) the contribution and their chemotactic ability of phylogenetically conserved domains of CCL2 (47R, 72K, 89H); (ii) the GAG-binding domain (89H) chemotactic property; and (iii) the comparision of their effects to the intact CCL2 chemokine (MCP-1).
Methods; The well conserved amino acid residues of CCL2 were investigated by Codeml – PAML package from 34 vertebrate species (47R, 72K, 89H). Each library was synthesized as sets of overlapping pentapeptide motives (scanned length 9 amino acids) and the nonapeptides of the whole scanned length. Chemotactic responses of *Tetrahymena pyriformis (chemotactic model cells)* were measured with capillary assay/ impedimetry.
Result and Discussion; CC2-receptors were detected in Tetrahymena. The pentapeptides reading-frame scanning of CCL2 domains resulted only in partial activation of CCR2 (chemoattractant sequences: 47R – VQRLA; 72K – VAKEI, AKEIC, KEICA). The nonapeptide, showed the significant activity, and was only the 47R nonapeptide. There was no significant activation by the GAG-binding domain in both penta- and nonapeptides. Compared with the intact CCL2 activity, the most chemoattractant material was the intact CCL2. (CCL2-195% vs. KEICA-160%).
Conclusion; The reduced activity of pentapeptides is supposed as a result of a longer or more adequate 3D structure (47R nonapeptide); however, in the other domain the elongation resulted in loss of activity (72K penta- and nonapeptides). Neutral moeity of the GAG-binding 89H domain in the penta- and nonapeptides points to the significance of this domain only as the ligand attachment promoting part of CCL2. The intact chemokine represents the optimal structure of conserved and non-conserved domains with a surpassing chemotactic activity.

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Identification of potentially cardioprotective microRNAs in porcine acute myocardial infarction model after ischemic conditioning

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Keywords: microRNA; cardioprotection; ischemia-reperfusion injury

Introduction: Ischemic conditioning of the heart in preclinical animal models of myocardial infarction is proven to be cardioprotective, however currently there are no efficient pharmacological agents available that can successfully reduce ischemia-reperfusion injury. MicroRNAs may play a pivotal role in the development of cardioprotection; the therapeutic and diagnostic relevance of microRNAs could be significant in the future. Thus, we aimed to identify microRNAs that play a potential role in ischemic conditioning induced cardioprotection in porcine acute myocardial infarction models.
Methods: Domestic pigs were divided into control (Con), ischemia (Isch), precondition (IPreC), postcondition (IPostC) and remote precondition (RIPerC) groups. Myocardial ischemia was induced by left anterior descending (LAD) coronary artery occlusion for 90 minutes, and followed by 3 hours of reperfusion. In the Isch group no conditioning methods were performed. Ischemic conditioning cycles were performed before the LAD occlusion in the IPreC group, and after the LAD occlusion in the IPostC group. In the RIPerC group, ischemic conditioning of an extremity was performed during the LAD occlusion. This was followed by the collection of tissue samples from the infarcted region of the left ventricle of every group, from which total RNA isolation and microRNA profile examination were performed using high throughput qRT-PCR technique.
Results: Expressions of 19 microRNAs were increased and 18 were decreased in Isch compared to Con (min. $1\times\log_2$ expression change, $-\log_{10}p>1$ vs. Con). Expressions of another 43 microRNAs were changed in the IPreC, 34 in the IPostC and 39 in the RIPerC group compared to Isch (min. $1\times\log_2$ expression change, $-\log_{10}p>1$ vs. Isch). Expression of 8 microRNAs changed at the same time in IPreC, IPostC and RIPerC compared to Isch.
Discussion: We successfully demonstrated the changing of expression profile of microRNAs due to different conditioning interventions of the heart compared to the Con and Isch group. To prove the cardioprotective effect of the identified microRNAs further investigations are planned in cultured cardiomyocytes in a simulated ischemia-reperfusion model.

Ethical Committee or Institutional Animal Care and Use Committee Approval: This investigation was carried out according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) and according to the ARRIVE guidelines, to the EU Directive (2010/63/EU) and was approved by the animal ethics committee of Hungarian National Food Chain Safety Office (SOI/31/26-11/2014).

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MicroRNA-mRNA network alterations under hypercholesterolemia in rat myocardium

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Keywords: hypercholesterolemia; network analysis

Introduction: Cardiovascular diseases are still the major cause of death in the industrialized world. Hypercholesterolemia as a risk factor possibly leads to coronary heart disease and impairs systolic and diastolic cardiac dysfunction as an independent factor. One of the pathophysiological changes in hypercholesterolemia is the alteration in the microRNA (miRNA; non-coding molecules that play a role in the regulation of gene expression) profile of the myocardium.
Aim: We aimed to explore novel hypercholesterolemia-induced pathway alterations in the heart with microRNA target prediction and validation.
Methods: Male Wistar rats were fed either with or without cholesterol supplemented chow (2% cholesterol and 0.25% cholate, ad libitum) for 12 weeks. Then, hearts were isolated and perfused with a Langendorff system. Then miRNA was extracted from the myocardium and a microRNA microarray analysis was performed to analyse changes in the miRNA expression profile in the hypercholesterolemic group vs. the control group. Using an online mRNA database, mRNAs with at least 4 interacting upregulated miRNAs were determined by a network theoretical approach. Then, two selected mRNA coded proteins were validated by Western Blot analysis. Selected mRNA-miRNA interactions were further validated by luciferase-assay in cell culture experiments.
Results: We have shown that 47 miRNAs were up- and 10 miRNAs were downregulated in the hypercholesterolemic rat hearts as compared to the normocholesterolemic control hearts. To find miRNA target hubs, a classical miRNA-target network was constructed. There were 11 hub mRNAs, from which putative targets of miRNA were further selected with systematic review of the literature. Adrb2 (adrenoceptor beta 2) and Ppp3r1 (calcineurin B type 1, CNB1) showed downregulation at the protein level due to hypercholesterolemia.
Conclusion: We assume that the downregulation of Adrb2 and the decreased production of the CNB1 protein (a product of Ppp3r1) are involved in the process leading to impaired cardiac function. This approach is suitable for identification of new targets involved in the detrimental effects of hypercholesterolemia on the myocardium.

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Renal effects of acute postnatal hyperglycaemia in rats – preliminary study

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Key words: neonatal, hyperglycaemia, renal, hypoxia, rat

Introduction: Neonatal hyperglycaemia is common in preterm infants. Little is known about how neonatal hyperglycaemia influences the ongoing nephrogenesis in preterms. The aim of this study was to reveal the possible morphologic alterations in the kidneys of newborn rats exposed to hyperglycaemia and hyperoxia.
Methods: Newborn Sprague-Dawley rats were cross-fostered and divided into two groups after birth. They were maintained in either normoxic (Cont) or a daily alternating hypo/hyperoxic (HO) environment from postnatal day (PD)1 to 14. Both groups were further subdivided into two. The pups were injected intraperitoneally with either 100 mg/kg streptozotocin (STZ) to induce hyperglycaemia (HG) or citrate buffer as a control (Cont). On PD17 kidneys were removed after euthanasia and proceeded to routine HE and PAS-H histology procedures.
Results: STZ treatment resulted in hyperglycaemia by PD2 and lasted until PD7 in the HO group and PD4 in the controls. The thickness of the Bowman's space was significantly smaller in the HG-Ox group than in the other 3 groups. We also observed a difference in the HG-Cont group compared to controls. The diameters of the proximal tubules were significantly larger in both of the hyperglycaemic groups compared to controls. Cortical thickness was significantly less in both Ox groups than in the normoxic controls. The width of the nephrogenic zone was extended in the HG-Ox group compared to the other three groups.
Discussion and conclusion: Hyperglycaemia in the early postnatal period causes detectable morphological changes in the developing kidneys. We emphasize the importance of management of hyperglycamia and monitoring kidney function in premature infants.

Ethical Committee or Institutional Animal Care and Use Committee Approval
Animal housing, care and application of experimental procedures were in accordance with institutional guidelines under approved protocols (No: BA02/2000- 15024/2011, University of Pecs following the European Community Council directive).

Role of Tocopherol Prooxidant-Antioxidant Balance in Liver of Rats Under Water-Immobilization Stress

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Key words: stress, α-tocopherol, prooxidant-antioxidant balance.

Introduction. Stress is a risk factor for development and exacerbation of many diseases. Excessive neurohumoral activation under stress leads to intensification of active forms of oxygen (AFO) production and development of cell structure damage chain reactions.
As α-tocopherol has antioxidant activity, it is presumed it would have an opportunity for corrective effects on the disruption of the prooxidant-antioxidant balance that occurs under stress.
Methods. The research was carried out on 30 male rats weighing 180-220g, which were divided into 3 groups (G): IG – control, IIG – WIS (water-immobilization stress) 5h, IIIG – α-tocopherol 150mg/kg administration for 3 days and WIS 5h. Determined in homogenate of liver were the activities of superoxide dismutase (SOD) and catalase, the levels of SH-groups, lipid oxidation products (LOPs), malondialdehyde (MDA), oxidative modification proteins (OMP), and mean molecular mass (MMM). Statistical analysis of the results was carried out according to criteria of the Student's t-test.
Results. In IIG relative to IG there were decreases of: SOD by 42,7%, catalase by 14,2%, SH-groups by 48%; increases of: LOPs by 245%, MDA by 197,2%, OMP by 452,8%, MMM by 169,9%; $p<0,05$.
In IIIG relative to IIG there were increases of: SOD by 53,3%, catalase by 18,1%, SH-groups by 72%; decreasesof: LOPs by 50,5%, MDA by 59,2%, OMP by 18,5%, MMM by 86,4%; $p<0,05$.
Discussion. In IIIG there was increasing of antioxidant protection (AOP) – SOD, catalase and SH-groups – and near normal, decreasing of LOPs, MDA, OMP, and MMM relative to IIG. These findings points to decreasing of the free radical processes (FRP) level under WIS with the background of α-tocopherol administration, possibly, because α-tocopherol as a hydrogen donor can react with free radicals to create non-radical substances.
Conclusion. Therefore, influence of WIS leads to decreasing of AOP activity and FRP intensification. However, WIS with the background of α-tocopherol administration leads to increasing of AOP activityand FRP decreasing, that serves as evidence for a positive α-tocopherol impact on the redox processes under WIS.
Ethical Committee or Institutional Animal Care and Use Committee Approval: Protocol № 3 from 16/03/2015 of the BioEthics committee of the Danylo Halytsky Lviv National Medical University.
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Chronic stress and ovariectomy affect the liver antioxidative status of adult rats

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Key words: antioxidative enzymes, chronic stress, oxidative stress, ovariectomy, rat liver

Introduction: Prolonged exposure of the body to different stress conditions can cause an increase in reactive oxygen species (ROS) production. At low concentrations, ROS are useful in some physiological processes, whereas higher concentrations can cause oxidative cell damage and thus play a major role in the pathogenesis of various human diseases. The aim of this study was to elucidate the impact of chronic stress on the oxidative stress development and antioxidative response in the liver of non-ovariectomized (NE-OVX) and ovariectomized (OVX) adult female rats.

Methods: As indicators of oxidative stress and antioxidative response, the activities of catalase (CAT), glutathione-reductase (GR) and superoxide-dismutase (SOD) were measured.

Results: Results showed that chronic stress caused a significant increase in CAT activity in the OVX group of animals. The ovariectomy significantly reduced GR activity while SOD activity remained unchanged due to the ovariectomy and chronic stress exposure.

Discussion: Obtained results showed that both chronic stress and estrogen deficiency, either alone or in combination, could affect antioxidative liver status of adult female rats. Comparing these results with the results of antioxidative enzyme activities obtained in a previous study conducted on younger rats exposed to the same chronic stress protocol, the younger rats showed a different liver antioxidative response.

Conclusion: Changes in antioxidative response and increased production of reactive oxygen species cause cell damage and could be associated with numerous pathological conditions of the liver.

Ethical approval: This study was performed at the Animal Facility of the Faculty of Medicine Osijek and was approved by the Ethics Committee of the Croatian Ministry of Agriculture, approval number: 2158-61-07-11-51.

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Clinical Research: Oncology, Infections, Immunology, Quality of Life and et al. (CLR)

Relationship between blood eosinophil level and early re-exacerbations in COPD

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Keywords: Chronic Obstructive Pulmonary Disease (COPD); Eosinophilia

Introduction: Patients with COPD exacerbation (COPDE) and elevated blood eosinophil count can respond more favorably to systemic corticosteroid treatment. However, it is not well studied whether increased blood eosinophil count at COPDE is related to re-exacerbations. We aimed to evaluate the relationship between blood eosinophil count measured in patients hospitalized with COPDE and early disease re-exacerbations.
Methods: Patients treated with COPDE between 25 February and 14 August 2017 at the Dept. of Pulmonology, Semmelweis University, Budapest, Hungary were enrolled. Blood eosinophil cell counts at hospital admission were registered. All patients received systemic corticosteroid treatment during hospitalization. After hospital discharge patients were interviewed on the phone every 3 months or at the1st moderate/severe COPDE to assess future exacerbations. Results were analyzed by Kaplan-Meier survival curve, log-rank test, Cox regression and Fisher exact test.
Results: Out of the 72 enrolled patients, 58 could be followed for at least 3 months (died: n=2, lost to follow-up: n=12). Patients were divided into eosinophil (Eos+, >2% or >150 cells/µl; n=25; age: 65±9 yrs) and non-eosinophil (Eos-, n=33; 64±9 yrs) groups according to admission blood test results. 52% Eos+ patients and 39% Eos- patients exacerbated after 3 months (RR=1.32; p=0.34; Fisher test: p=0.25). Similarly, 64% Eos+ patients and 52% Eos- patients exacerbated after 6 months (RR=1.24; p=0.33; Fisher test: p=0.24). There was no difference in time to first exacerbation between Eos+ and Eos- patients (median: Eos+: 11 weeks, Eos-: 14 weeks; hazard ratio: 1.40; log-rank: p=0.30).
Discussion: Based on our short-term follow-up data (3-6 months) increased blood eosinophil cell count during COPDE might not affect the frequency of re-exacerbations.
Conclusion: For more accurate analysis of the relationship between blood eosinophil level and re-exacerbations in COPD, patient enrollment and the follow-up period should be extended.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Ethical approval (No. *34/2015* (9 March 2015) and *191/2017* (4 October 2017)) was issued by Semmelweis University Regional and Institutional Committee of Science and Research Ethics.
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In silico cell model of Chronic Lymphocytic Leukaemia

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Keywords: Chronic Lymphocytic Leukemia, CLL, B-cell, Computer simulation

Introduction: Chronic Lymphocytic Leukaemia (CLL) is the most common leukaemia type in the Western world, yet the clinical outcome is highly varied due to inter-patient heterogeneity. This variability causes difficulties in selecting the optimal treatment, however, decision support algorithms could help us to find the best options.
Methods: Computational models can be customized to replicate the behavior of the patient's tumor, supporting researchers and clinicians to gain insight into the molecular mechanisms influencing response to therapy. Our goal was to build a simulated, in silico CLL cellular model, in order to select the best molecular hypotheses differentiating the treatment response for further experimental validation.
Results: As the basis of the model, the protein network provided by Turbine contains ~1400 proteins, and their ~4000 interactions, allowing comprehensive characterisation of cellular signaling pathways. However, CLL specific signalling mechanisms needed to be expanded. With extensive literature mining we are working on the following CLL specific pathways to be curated into the network for better modelling: BCR-signalling, MYD88 pathway, Notch1 pathway, and ZAP70 pathway. Henceforth the hypotheses described in previous studies can be used for model validation, such as prognostic differences of IGHV mutated- and unmutated patients; effect of driver mutations (e.g. TP53, BIRC3); and efficacy of different drugs (e.g. Ibrutinib, Venetoclax) in tumor cells with diverse mutational profiles.
Discussion: In order to customize the signalling network for CLL, a reference transcriptomic profile was generated providing protein concentration estimations, using RNA-sequencing data from 98 patients. Based on the extensive manual curation of 939 patients' mutational profiles, individual simulated cells were constructed with patient-specific activation landscape of the proteins originating from the genomic dataset.
Conclusion: Our current work is focusing on the validation of the constructed models using the above mentioned hypotheses. With the comparative analysis between patient-specific models combined with clinical outcome we intend to expand the knowledge of cellular behavior in CLL, especially related to therapy response, and make new predictions of possible outcomes in prognosis.

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In vitro model of the functional alterations of human neutrophil granulocytes in severe sepsis

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Keywords: Sepsis; Neutrophils; Superoxides; In Vitro Techniques

Introduction: Neutrophil granulocytes are essential in eliminating invading bacteria, however, in severe sepsis their physiological functions become substantially altered. This results in impaired elimination of bacteria, and elevated extracellular superoxide production. Our group had previously found that these functional defects correlate well with severity, and can be transferred to naive neutrophils with septic blood plasma. The transfer agents are heat-stable, protease-sensitive and 3-12 kDa. Comparison of healthy and septic plasma samples showed differences mainly in cytoskeletal peptides, suggesting the idea of the intracellular origin of these transfer factors.
Methods: For our experiments, we used blood samples of healthy donors and isolated all the different cell fractions (RBC, PMN, lymphocytes, monocytes). The cells underwent freezing and ultrasound sonication, in order to prepare a lysate. *Methicillin-sensitive Staphylococcus aureus* and *Escherichia coli* received the same treatment. Naive neutrophils of healthy donors were treated with the prepared lysates, and we measured their viability (cell count, apoptosis and necrosis factors), and functional properties (superoxide production, phagocytotic capacity, elimination of bacteria). The samples, in which elevated superoxide production and reduced ability to kill bacteria were present, also received heat denaturation and protease digestion (as the septic plasma did previously), and underwent dialysation, using membrane filters with a pore size of 12 kDa.
Results and Discussion: Treatment with neutrophil- and bacteria lysates both induced elevated superoxide production and impaired killing in the naive neutrophils, especially with the less than 12 kDa fraction of the neutrophil dialysatum, but the functional alterations previously examined with septic plasma were not present when PMN were treated with RBC, monocyte or lymphocyte lysates.
Conclusion: Sepsis-related alterations were observable in cases of PMN- and bacteria lysatum treated PMN, suggesting a unique role of bacteria and PMN in sepsis related PMN dysfunction.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Number of certificate: 1563/2015. Issuing agency: The Public Health Division of the Hungarian Government Regional Office in Budapest (Főváros Kormányhivatala Népegészségügyi Szakigazgatási Szerve).

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Enhanced cell killing in B16F10 melanoma cell line by combining chemotherapy with conventional or modulated electro-hyperthermia

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Keywords: melanoma; hyperthermia; modulated electro-hyperthermia; apoptosis

Introduction: Our aim was to examine if hyperthermia can augment the cell killing effect of various chemotherapeutic agents on the B16F10 mouse melanoma cell line. We compared the effect of two types of hyperthermia: modulated electro-hyperthermia (mEHT) and conventional hyperthermia (convHT).
Methods: B16F10 melanoma cells were treated with mEHT using LabEHY100 (Oncotherm™) or convHT in a water bath at 42°C for 1 hour. For analyzing gene expression with qPCR, cells were harvested 1, 3, 9 and 24 hours after treatment. In combined protocols, after hyperthermia induced by convHT or mEHT cells were treated with paclitaxel (40 nM), dacarbazine (40 mM) or nutlin-3 (10 μM). Apoptosis and cell viability were measured 24 hours post-treatment. We used cleaved caspase-3 to detect apoptosis by flow cytometry and cell titer blue to quantify cell viability.
Results: Similar changes in the expression of HSPA2 were induced by mEHT and convHT: three hours after treatment the peak levels of HSPA2 were 3 times higher as compared to the untreated control in a time-course experiment. Next, we showed that in parallel to the upregulation of pro-apoptotic genes Puma, Bak-1, and Bax, downregulation of pro-survival genes XIAP, Bcl-2, Bcl-XL were induced both by convHT and mEHT with similar kinetics and magnitude.
The most effective combination resulting in the highest level of cleaved caspase-3 and decreased viability to 40% was hyperthermia followed by nutlin-3 treatment.
Discussion and Conclusions: Hyperthermia disrupts tumor membranes allowing greater permeability of chemotherapeutics to access the cell resulting in greater tumor damage. As opposed to convHT, where the heat dissipates into the surrounding tissues, mEHT is a well-focused, tumor-selective treatment. Our results demonstrate that mEHT and convHT efficiently augment the cell killing effect of chemotherapeutics. Induction of pro-apoptotic genes and the concomitant upregulation of cleaved caspase-3 is reflected in low cell viability.

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Nanofabricated poly(vinyl alcohol) scaffolds for abdominal hernia repair

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Keywords: Abdominal Hernia, Surgical Mesh, Tissue Engineering, Electrospinning

Introduction: Abdominal hernia is defined as any organ or tissue protrusion through a defect in the abdominal muscular wall, therefore, the only definitive treatment is surgery. Widely popular is the mesh repair involving implantation of a surgical prosthetic mesh to repair the defect, strengthen the abdominal wall and prevent recurrence. It is observed however, that currently applied dry, woven, non-absorbable meshes are far from perfect. In this regard our objective was the production of a biocompatible mesh as a possible alternative to the presently utilized ones.
Methods: Nanofabricated poly(vinyl alcohol) (PVA) meshes were electrospun with a homemade instrument (15 kV potential, 15 cm target distance, 1 mm/h flow rate). Post-electrospinning processing was implemented to strengthen the scaffolds and subsequent sterilization was performed using ClO_2. *In vivo* studies began with a preliminary biocompatibility study on Wistar rats (Group I, n: 15) where scaffolds were implanted intraperitoneally; then continued with two more groups (Group II, Group III, n: 30), where artificial defects (D: 2 cm) were created and thereupon repaired with PVA scaffolds (D: 2.5 cm). A fourth group (Group IV) served as control where the defects were repaired conventionally. A preliminary study with larger animals was then performed on pigs (n: 2). PVA and polypropylene meshes (D: 8 cm) were implanted intraperitoneally and fixed on each side of the anterior abdominal wall. Rats from each group were terminated on the 7th, 14th, 28th, 90th and 180th postoperative days while the pigs during the 5th week. Scaffolds were evaluated macroscopically and microscopically.
Results: No complications were observed in any of the animals. All animals survived until their termination date while histological studies revealed physiological wound healing and scaffold integration.
Discussion: The results are positive but further long-term studies are required.
Conclusion: PVA meshes indeed have potential future prospects as alternative surgical meshes.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The experimental protocol adhered to rules laid down by the Directive of the European Parliament and of the Council on the protection of animals used for scientific purposes, and was approved by the Semmelweis University's Institutional Animal Care and Use Committee. The accreditation number of the laboratory is 22.1/1244/3/2011.
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The effects of music therapy and Kangaroo Mother Care (KMC) method on preterm babies

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Keywords: preterm, music, kangaroo mother care, NIRS

Introduction: Developments in neonatology over recent years lead to improved survival rates among preterm infants. Patient and family centered care has resulted in improving quality of life outcomes of babies on NICU (Neonatal Intensive Care Unit). The primary aim of our research was to detect the changes in brain tissue oxygenation using near infrared spectroscopy (NIRS) and other physiological parameters in response to skin-to-skin contact (KMC) and music therapy with the mother's voice. We compared these data with the baseline parameters measured in the incubator.
Methods: Our research was conducted on the NICU of 1st Department of Paediatrics, Semmelweis University, between March 2017 and November 2017. During our prospective crossover study, we monitored the oxygen saturation, heart rate, brain tissue oxygenation over the temporal lobe (NoninSenSmart x-100) under four different conditions.
We compared the physiological data recorded in the incubator with those during KMC, music therapy and the post intervention period. Mothers were aided in their singing by specialist music therapists, accompanied by live guitar music.
ANOVA repeated measures method was used for statistical analysis.
Results: 14 patients have been recruited in the research up to date. Their average gestational age was 29.1±2.7 weeks, their birth weight was 1319±498 grams. The study was timed after their early stabilization, on the average corrected gestational age of 34±3 weeks.
We have found that the brain tissue oxygenation levels (measured using NIRS) during KMC and music therapy were significantly increased (77.9, CI:76.214-79.510) when compared with the baseline levels measured in the incubator (76.2, CI:74.206-78.115) p=0.036. Oxygen saturation levels and heart rates changes were not found to be significant.
Discussion: Our study seems to suggest that the combination of KMC and music therapy has favorable effects on the cerebral haemodynamics in preterm infants. These effects may result from stimulation of the central nervous system and the auditory cortex. Further research is necessary to establish the long-term effects of maternal singing on neurobehavioural development of preterm neonates.

Ethical Committee or Institutional Animal Care and Use Committee Approval:
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Protocol for studying the effect of modulated electrohyperthermia on melanoma in a mouse model

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Keywords: melanoma; modulated electrohypertermia; immune recognition

Introduction: Conventional hyperthermia is used to accentuate many of the current modalities of tumor treatment, but one cannot exceed a certain temperature range as doing so leads to significant damage to surrounding healthy tissue. Herein lies the benefit of modulated electro-hyperthermia (mEHT) which delivers high temperatures with increased tumor specificity. Since tumors have a decreased capacitance they are inherently prone to act as capacitors for the current produced by the mEHT. We look at the effect of mEHT on melanoma in a mouse model.
Methods: B16-F10 cells were injected subcutaneously into C57Bl/6 mice and the tumors were exposed to 42 °C for 30 minutes with mEHT using two protocols. In the first protocol we treated mice for three consecutive days, in the second protocol the treatment was administered every second day for three times. Tumors were removed after 24 hours in the first protocol or 48 hours in the second then weighed and digested to single cell suspension. The supernatant of tumor cells and the plasma were used to screen a membrane-based cytokine array. The tumor cells were stained for flow cytometry to determine the changes induced on tumor cells.
Results and Discussion: Both mEHT protocols resulted in significant reduction in size of the primary melanoma tumors. Flow cytometry analysis revealed the increase of MHC class I on the surface of melanoma cells and concomitant activation of CD8+ cytotoxic T lymphocytes in response to treatment. Increased numbers of CD4+ T cells and Gr1+ granulocytes were detected, although changes in the various subpopulations need further analysis. F4/80+CD11b+ macrophages and mature CDs were slightly elevated in the treated tumors. Cytokine array analysis showed that several cytokines responsible for leukocyte recruitment were enriched in the treated tumor. Complement activation was induced by mEHT both in the tumor microenvironment and plasma, indicating an acute inflammatory response to treatment. Furthermore, the long pentraxin PTX3 was elevated in the tumor and plasma of the treated mice, suggesting that mEHT induced the tumor clearance.
Conclusion: Taken together, our results demonstrate that the mEHT treatment was effective in reducing the tumor size by enhancing immune recognition of B16-F10.

Ethical Committee Approval: All procedures were carried out according to the guidelines of the Hungarian Law of Animal Protection (28/1998) and were approved by the National Scientific Ethical Committee on Animal Experimentation (PEI/001/2706-13/2014).
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Exhaled carbon monoxide in obstructive sleep apnea

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Keywords: obstructive sleep apnea; airway inflammation; sleep disorders

Introduction: Obstructive sleep apnea (OSA) is a common disorder which is characterized by recurrent collapse of the upper airways with repetitive episodes of intermittent hypoxia. Intermittent hypoxia may trigger airway inflammation and oxidative stress. Exhaled carbon monoxide (eCO) is a non-invasive marker of airway inflammation and oxidative stress, nevertheless its role in OSA has not been discovered yet.
Methods: We involved 35 non-smoker volunteers (54±16 years, 16 men) who participated in an overnight polysomnography (Somnoscreen Plus, Somnomedics GmBH, Germany). eCO measurements were performed before and after sleep (Smokelyzer, Bedfront, UK). eCO levels were compared between groups, before and after sleep and to clinical variables.
Results: 22 volunteers were diagnosed with OSA based on polysomnography. Higher eCO levels were detected in OSA (1.86±1.67 vs. 1.08±0.64 ppm, p=0.04), which were correlated significantly with the apnea hypopnea index (p=0.03, r=0.46). Morning eCO levels were not different between the two groups (p=0.29). No significant change was observed between the evening and morning eCO values in OSA (p=0,16) or in the control group (p=0,85).
Discusson: Elevated eCO levels suggest accelerated airway inflammation and oxidative stress in OSA.
Conclusions: Increased airway inflammation may play a role in the pathophysiology of OSA which can be monitored via eCO measurements.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The study was approved by the local Ethics Committee (Semmelweis University, TUKEB 30/2014) and informed consent was obtained from all participating volunteers.
Grants and financial support: The study was supported by the Hungarian Respiratory Society (grant to Andras Bikov) and Semmelweis University (grant to Laszlo Kunos).
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Genetic variants of the Krebs cycle enzyme encoding genes in pheochromocytoma and paraganglioma

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Keywords: pheochromocytoma, Krebs cycle, mutation

Introduction: Pheochromocytomas and paragangliomas (Pheo/PGL) are rare neuroendocrine tumours arising from the adrenal medulla or the symphathetic paraganglia respectively. Mutations of the Krebs cycle enzyme encoding genes could result in the development of Pheo/PGL.
Methods: We examined 131 patients with the diagnosis of Pheo/PGL who received care in the 2nd Department of Internal Medicine, Semmelweis University. The prevalence of the germline mutations of Pheo/PGL genes was determined. An *MDH2* variant was further investigated for functional consequences.
Results: 68,7% of the patients had Pheo, 29% PGL and 2,3% carried both tumours. Malignancy was present in 28%, and bilateral Pheo in 10% of all cases. Germline mutations were present in at least one of the Pheo/PGL genes in 34% of the patients: 10 (7,6%) *SDHB*, 9 (6,9%) *RET*, 5 (3,8%) *VHL*, *TMEM127*, *MDH2*, 4 (3%) *NF1*, 3 (2,3%) *SDHD*, 2 (1,5%) *SDHC* and *KIF1B*. 5 of 10 *SDHB* mutation carriers developed malignant disease. Both *SDHC* mutation carriers developed PGL with multiplex localisation in one case. 2 *SDHD* mutation carriers developed Pheo, 1 malignant PGL. 5 patients carried gene variant in *MDH2*, 3 had Pheo, 2 PGL, and malignancy was observed in one of them. Among the 10 patients with bilateral adrenal Pheo 4 *RET*, 2 *TMEM127* and 1 *VHL* mutation were observed. Germline mutation was identified in 17 of the 80 unilateral Pheos (21.2%; 4 *VHL*, 3 *NF1*, 2 *SDHB*, *SDHD*, *RET*, *TMEM127)*, whereas 16 of the 38 patients with PGL carried mutation in Pheo/PGL genes (41%; 8 *SDHB, 2 SDHC, KIF1BRET, 1 MDH2, SDHD)*. Malignancy was associated with the homozygous form of an *MDH2* variant. Cells transfected with the plasmid containing the *MDH2* variant showed decreased enzymatic activity compared to the wild enzyme. The prevalence of the *MDH2* variant did not differ between the Pheo/PGL and the control group.
Discussion: Our findings regarding the role of germline mutations in the development of Pheo/PGL are in accordance with the literature. The decreased activity of the MDH2 variant may result in the build-up of the Krebs cycle metabolites which, according to the literature, play a key role in Pheo/PGL pathogenesis.
Conclusion: Our study supports the diversity of the pathogenic background of Pheo/PGL and we aim to continue our research to prove our assumptions.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The study was approved by the Scientific and Research Committee of the Medical Research Council of Hungary (ETT-TUKEB 4457/2012/EKU).
Grants and financial support: Attila Patocs is a recipient of Lendulet grant from the Hungarian Academy of Sciences. This work was also supported by a Hungarian Scientific Research Grant (OTKA K125231 to Attila Patocs)

Examination of genotypic-phenotypic correlations in Marfan-syndrome in order to predict the severity of cardiovascular manifestations

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Keywords: Marfan-syndrome, *FBN1*, genetic-screening, cardiovascular

Introduction: Marfan syndrome (MFS) is an autosomal dominant, systemic connective tissue disorder. The revised Ghent nosology identifies major and minor manifestations, of which the dissection of the aorta is life threatening, so monitoring of its dilatation is crucial. In most cases a mutation of the *FBN1* gene is responsible for the disease, leading to the reduction (haploinsufficiency=HI) or to abnormal structure (dominant negative type mutation=DN) of the fibrillin-1 molecule.
We aimed to examine the correlations between the severity of the cardiovascular (CV) involvement and the genetic variations.
Methods: 35 patients with clinically diagnosed MFS were examined. Phenotypic evaluation was carried out in The Heart and Vascular Center of Semmelweis University and the genetic screening of the *FBN1* gene took place in the Molecular Diagnostic Laboratory of The Hungarian National Blood Transfusion Service using Next Generation Sequencing and Sanger sequencing techniques.
Results: In 20 out of 35 cases the pathogenic mutations were identified. Comparing the patients with detected mutations to the group with no mutations showed no difference (p=0,13) in terms of the major CV manifestations (dilatation and/or dissection of the aorta). 9 of the identified mutations are DN-type (missense) and 11 are HI-type (5 nonsense, 2 frameshift, 3 splice and 1 large deletion).
Discussion: There was no difference between the effect of DN and HI mutations on the major CV traits (p=0,07). 5 of the DN-type mutations affect cysteine leading to severe CV manifestations in all 5 cases. Cysteine is essential in the structure of the fibrillin-1 molecule. In patients with DN-type genetic variations with no cysteine involvement the CV manifestations were significantly less likely, than in patients with HI-type and cysteine involving DN-type mutations (p=0,0035).
Conclusion: Identifying the pathogenic mutations in MFS can help us estimate the severity of CV manifestations and plan the prophylactic aortic root surgery.

Ethical approval and consent to participate: Ethical approval was obtained for this study from the Scientific and Research Ethical Committee of the Medical Research Council of Hungary (ETT-TUKEB, 12751-3/2017-EKU). Prior to the investigation all participants were given written information about the details of the study and protocols were followed to ensure security of personal data. After reading and understanding the above informational statements all participants provided written informed consent to participate in the study.

Frequent subclonal *TP53* mutations in chronic lymphocytic leukemia detected by next generation sequencing

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Keywords: chronic lymphocytic leukemia, *TP53* mutation, subclonal architecture, next generation sequencing, prognosis

Introduction: In chronic lymphocytic leukemia (CLL), the worst prognosis is associated with *TP53* defects, that are, *TP53* mutation and/or deletion of the 17p region, with the affected patients being potentially directed to targeted therapy. Therefore, it is essential to detect all *TP53* defected patients including those with only minor-proportion (i.e. subclonal) mutations with variant allele frequencies (VAF) below the detection limit of Sanger sequencing.
Methods: For highly sensitive detection of small *TP53* mutant subclones on a MiSeq instrument, we applied a next-generation sequencing (NGS) strategy using the Illumina TruSeq custom amplicon approach in a historical, heterogeneous cohort of 276 CLL patients representing the general CLL population.
Results *TP53* mutations were detected in 25.7% (71/276) of the patients. In total, NGS identified 43 clonal and 102 subclonal *TP53* mutations in 71 patients (25.7%) with an average VAF of 13.5% (1.0%-81.5%). In total, 49.3% of all *TP53* mutant patients carried only subclonal mutations. Patients with *TP53* disruption had a significantly reduced time to first treatment (TTFT) compared to patients with wild type *TP53*. Unexpectedly, we identified 12 *TP53* mutant patients with an indolent phenotype, 2 of them showing an ultra-stable clinical course that never required treatment.
Discussion: Our data confirmed the high frequency of subclonal *TP53* mutations representing a significant proportion of *TP53* mutated (49.3%) and also of *TP53* defected (39.7%) patients, observed by recent NGS studies. An unexpected finding was the significant proportion (28%) of indolent *TP53* mutant patients suggesting that not all *TP53* mutations are associated with poor outcome.
Conclusion: Our data demonstrate that it is essential to apply a highly sensitive sequencing method such as NGS to completely identify all *TP53* mutant patients since nearly half of them would be considered wild type using *TP53* gene Sanger sequencing.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Institution: ETT-TUKEB, Date: 2016-08-31, ID: 45371-2/2016/EKU
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Cell surface receptor SLAMF5 enhances DC-autophagy via IRF8

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Keywords: Signaling Lymphocytic Activation Molecule Family, Autophagy, Dendritic Cells, Interferon regulatory factor-8, Inflammatory cytokines

Introduction: Recent studies have identified autophagy as both an evolutionary conserved, critical process for maintenance of cellular homeostasis and an integral part of the immune response. By controlling dendritic cell (DC) functions it facilitates immune-surveillance as well as guards against excessive inflammation. In light of the recently established link between autophagy and SLAMF receptors, we decided to examine the role of SLAMF5 in DC autophagy and in the cytokine production of DCs responding to inflammatory signals.
Methods: We performed RNA-interference targeting SLAMF5, IRF8 or SLAMF5 in combination with TRIM21 on freshly isolated monocytes, then differentiated them into DCs in the presence of GM-CSF and IL-4. To mimic infection with Gram-negative bacteria, cells were treated with LPS and IFNγ and then changes in DC functions were monitored in the absence of the above proteins.
Results: In DCs lacking SLAMF5 we found decreased autophagic flux both in the presence and absence of inflammatory stimuli. LPS/IFNγ treatment of SLAMF5-deficient DCs significantly increased production of IL-1β and IL-23, while IL-12 secretion was diminished. Strikingly, we detected greatly reduced protein levels of the autophagy regulator IRF8, which could be reversed in the presence of the proteasome inhibitor MG132. Furthermore, silencing of TRIM21, an E3 ubiquitin ligase, completely restored the level of IRF8 in SLAMF5 knock down cells. In addition, DCs made IRF8-deficient by RNA interference showed similar characteristics to those lacking SLAMF5.
Discussion and Conclusion: Taken together, data presented in this work indicate that SLAMF5 enhances autophagy and controls pro-inflammatory cytokine production of human DCs, presumably by inhibiting TRIM21 dependent proteasomal degradation of the autophagy master regulator transcription factor IRF8. As autophagy plays a key role in the pathogenesis of several human diseases including infections, cancer, autoimmune and neurodegenerative disorders, identification of cell surface receptors regulating autophagy may improve the clinical management of these conditions.

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Rheumatoid Arthritis – a Mathematical Model

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Keywords: rheumatoid arthritis, mathematical model, inflammation, autoimmune diseases.

Introduction: Rheumatoid arthritis (RA) is a common autoimmune disease that mainly affects the joints. Its pathogenesis is based on an abnormal immune response, followed by chronic articular inflammation. The inflammatory process is concentrated in the synovial membrane, and consists of macrophages, T-helper 17 (Th17) cells, and a pro-inflammatory phenotype of fibroblasts, as well as the cytokines these cells secrete. The effect of this sustained inflammation is destruction of the underlying cartilage, leading ultimately to loss of articular function. Here, we develop a mathematical model that aims to describe the network of interacting cells and cytokines, as well as their effect on the cartilage.
Methods: The model consists of a system of partial differential equations. It has three compartments: the synovial fluid, the synovial membrane and the cartilage and includes fibroblasts, macrophages, Th17 cells, and chondrocytes, interleukins IL-6, IL-23, IL-17, TNF-a, as well as GM-CSF, MCP-1, FGF, MMP and TIMP.
Results: We obtain, by running the model, similar concentrations of cytokines and densities of cells to those reported in the literature. The model also describes the thinning of the articular cartilage at rates that correspond to those found in patients.
Discussion: The results show that the model approximates the disease state. Therefore it could be used to determine the relative importance of the inflammatory factors described above, as well as to explore potential treatments.
Conclusion: Our model brings together different studies and data, giving us a more complete view of the pathological processes underlying RA.

Ethical Committee or Institutional Animal Care and Use Committee Approval: *not relevant*
Grants and financial support: *not relevant*

Viral Infections and Pregnancy

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Key words: virus, pregnancy, fetus

Introduction: Microbial infections are associated with fever, disease manifestations and activated immune defense. Pregnancy may influence the severity of the infection which may further influence maternal and fetal health. Asymptomatic infections may also be associated with severe consequences to the fetus.
The aim of our work is to compile literature information on viruses and their influence on the course of pregnancy and the effects on fetal development.
Method: A literature search was carried out to note records of viral infections which showed a potential of having adverse consequences on maternal or fetal health. The viruses were divided depending on the viral genome (DNA and RNA viruses) belonging to different families and genera. Only human pathogens were included.
Results: Some viral infections (e.g. herpes viruses) are known to have consequences for maternal and/or fetal health (embryo/fetopathy, abortion, etc.) or may be associated with acute or long-term neonatal disease. There are other viral infections that are suspected and not documented well such as those from enteroviruses. In recent years virus infections such as Zikaand Ebola have emerged, which affect the fetus and pregnancy outcome. These infections are concentrated in specific geographic areas and are a big concern to the different Health Care Institutions and Public Health officials and Ministries of Health especially in ~~to~~ the relevant countries.
Conclusion: We have assimilated information about all viruses recorded to have a potential to initiate adverse effects on the gravidity and maternal or fetal health. We show that the range of viruses considered for screening has increased in recent times, and varies rom those presented in the classical followed criteria.

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Pathomorphological Review and Differential Diagnostics of Osteogenesis Imperfecta in Maroteaux-Lamy syndrome

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Key words: Maroteaux-Lamy syndrome, orphan genetic disorders.

Introduction: Maroteaux-Lamy syndrome (MLS) is also known as mucopolysaccharidosis (MPS) type VI – a rare multisystem autosomal recessive lysosomal disorder caused by different mutations in N-acetylgalactosamine-4-sulphatase (arylsulfatase) gene. As a result, there are ARSB deficiency and accumulation of glycosaminoglycans in tissues.
The aim of our study was to investigate MPS type VI using autopsy material and clinical data to make a general diagnostic algorithm.
Materials and methods: For macroscopic and microscopic investigation, autopsy material was used. Clinical data about patients (sisters 7 and 12 y.o) was received from the Lviv Regional Children's Clinical Hospital "OKHMATDYT."
Results: There is a large number of MPS type VI manifestations, but they can be classified according to statistics into: described in 80-99% of patients; described in 30-79% of patients; described in 5-29 % of patients.
Pathomorphological changes in the 12 y.o. child with genetically verified MLS were: mucopolysaccharide accumulation in the lysosomes of interstitial cells in the valves and interstitium of myocardium; vacuolar degeneration and disruption and fragmentation of elastic fibers in systemic vessels; myxomatous change of the valves; vacuolar degeneration in the liver, kidneys, suprarenal glands, heart, and brain. Osteogenesis imperfecta (OI) had been diagnosed in the 7 y.o child. Pathomorphological changes were: scoliosis; fragile bone; congenital femoral deformation; upper and lower extremities deformations; brachycephalic skull with big frontal and parietal humps; and draft height. A differential diagnostic algorithm between MPS type VI and OI was made.
Discussion: Interestingly, mutation in the COL1A2 gene can cause both disorders simultaneously (MLS and OI) without any additional mutations in other genes. According to data from the Human Ontology Center, MLS is characterized by a wide spectrum of clinical and genetic heterogeneity. Only molecular genetic methods can prove the OI connected with MLS.
Conclusion: Described cases present typical pathomorphological and biochemical changes in patients with MLS and OI. Differential diagnostics between MLS and OI can be complicated.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Protocol № 3 from 19/10/2015 of the BioEthics committee of the Danylo Halytsky Lviv National Medical University.
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Medical Imaging (MI)

Modeling of DTI parameters for optimal image quality and downstream processing

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Keywords: Diffusion Tensor Imaging, Anisotropy, Sequence, MRI, Image Quality

Introduction: Since Basser's famous paper from the mid 1990s, there has been tremendous methodological advance in diffusion tensor imaging (DTI). Besides the recommendations based mainly on empirical knowledge and theory, there is no consensus or strict guidelines to follow. So far, there have been studies investigating the impact of sequence parameters on image quality, such as number of diffusion directions, gradient strength, scan repetitions etc. However, most of these studies have investigated impact of one or two parameters without any restriction in scanning time. To our knowledge none of them investigated influence of several parameters at once on image quality in a time-restricted environment.
Materials & Methods: One C57BL/6 mouse was sacrificed, and the brain was extracted, placed in PBS, and subsequently imaged with 7T Bruker BioSpec using SE-DTI sequence. In total, 24 acquisitions were done with iterations in scan parameters, such as number of directions, b-values, resolutions, and a number of averages while controlling for scan time (up to 3.5h). Selected region-of-interest was the anterior part of corpus callosum due to its high anisotropy. Fractional anisotropy (FA) index is therefore close to 1, and this index was used as a measure of quality of the sequence.
Results: A multiple regression model was used to predict FA in the anterior portion of the corpus callosum from number of directions, b-value, resolution, number of averages and time. These dependent variables statistically significantly predicted FA ($p<0.001$, $R^2=0.752$). All predictors except the number of averages added statistically significantly to the prediction $p<0.05$.
Conclusion: Most of the predictors influenced the FA. Higher FA can be achieved with anisotropic protocols, but these protocols are known to lead to downstream biases and should be avoided. Thus, optimization of isotropic protocols promises better image quality and optimal downstream processing.

Ethical Committee Approval was obtained from University of Zagreb School of Medicine, 380-59-10106-16-20/218 on Oct 20, 2016.
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Mouse bioluminescent neurons for in vivo imaging

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Mentor: Gajović Srećko

Keywords: transgenic mouse; bioluminescence; neurons; luciferase

Introduction: Gene activity can be visualised by firefly luciferase bioluminescence in living animals carrying the luciferase reporter. If neurons would be labelled by bioluminescence it would allow studies of brain repair after damage. Our hypothesis was that labelling the neuron-specific intermediate filaments of the cytoskeleton, neurofilaments, would enable visalising neurons in the mouse.
Methods: TurboLuc, fusion reporter generated by the fusion of the firefly luciferase Luc2 to the far-red fluorescent protein TurboFP635 by a 14-amino acid linker peptide, was engineered to be under control of the neurofilament heavy polypeptide promoter. For the better expression of the reporter, an intron was cloned in between the promotor and reporter and a mammalian poly(A) tail was placed as an extension of the TurboLuc reporter.
Results: The starting plasmids were characterized by the sequencing and restriction sites determined. This allowed the design of a cloning strategy. The created construct was used to create a transgenic mouse.
Discussion: One of the advantages of the luciferase-bearing neurons is they allow cell-type specific analysis of the mouse brain after ischemic injury as a model of stroke.
Conclusion: The generated transgenic mouse would carry both bioluminescent and fluorescent reporter and as such could be used for in vivo preclinical optical recording of tissues and organs, as well as in histological analysis.

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In vivo localization of deep brain electrodes in mouse neurophysiology experiments

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Keywords: Preclinical imaging, CT, MRI, Mouse brain atlas, in vivo electrode localization

Introduction: Optogenetics and extracellular recording allow researchers to investigate the activity of genetically defined neurons at the time resolution characteristic of action potentials. Using these techniques, we can investigate how the different neuromodulatory systems of subcortical areas take part in the formation of cognitive functions. To study optogenetically identified neurons, it is essential to precisely target the nuclei housing of these cell types. As these are typically small, sparsely populated structures, located deep in the brain tissue, such targeting represents a significant technical challenge. To confirm the success of these implantation surgeries, we developed a protocol which makes it possible to determine the exact position of electrodes in the brain in vivo, providing an alternative to the standard post mortem histological methods.
Methods: With this method, we investigated electrode implantations in mice (n=6). Under isoflurane anesthesia, we took CT and MRI images before the surgery, which provided anatomical information about the skull and the brain, and CT images also after the surgery, which provided information about the position of the electrodes. The images were then co-registered with mouse brain atlases. Later, confocal and fluorescent microscopy was used to investigate histological slices for comparison.
Results: Based on the co-registered images, we developed two localization procedures. The first one provides qualitative, three-dimensional visual information, while the second provides quantitative information in the form of atlas coordinates relative to bregma, showing the location of the electrodes.
Discussion: The high-resolution CT images provided coordinates with accuracy within 0.1 mm. The correctness of our method was verified by comparison with the findings of histology.
Conclusion: This method opens new avenues for verifying the success of the implantation shortly after surgery, using in vivo, non-invasive techniques, which allows corrections or facilitates early termination, thus saving valuable experiment time that could extend to months in the case of each implanted animal.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Animal experiments were carried out at the Nanobiotechnology and In Vivo Imaging Center, Semmelweis University, with permission from the local institutional animal ethics committee no. XIV-I-001/29-7/2012 and in compliance with the relevant European Union and Hungarian regulations.

Artificial Intelligence in the Diagnosis of Cancer

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Keywords: machine learning, bioinformatics, CT-scan, radiology, diagnostics

Introduction: Artificial Intelligence (AI) based diagnostic solutions in radiology are gaining ground rapidly. We have conducted our study on the diagnosis of early-stage pulmonary carcinoma with the help of AI since January 2017. A reliable solution would allow the introduction of annual screening for high-risk groups such as smokers over the age of 50, which, in turn, would allow the early diagnosis of lung cancer and radically increasethe odds for curative therapy. We have trained such a diagnostic AI and while some challenges, outlined below, prevent using it in clinical practice as of yet it is nevertheless a very promising direction we plan to pursue further.
Methods: Approximately 1 500 chest CT-records were acquired and annotated by radiology specialists. Another 1 500 cancer-free CT images were added to the "training set". This training set was then fed to the AI. The enrollment of other pulmonary diseases, eg. aspergillomas was present as well. Once the system was trained, we diagnosed previously unseen CT-records to determine the real-life accuracy.
Results: We determined that to achieve usable accuracy, the voxel-level segmentation of the cancerous tissue present in the images was required. Our experiments with approximate segmentation did not yield acceptable results. With the fully segmented set, we achieved a cross-validation accuracy of 96%, and an accuracy of 80.6% on other records.
Discussion: Our self-learning system seems to be possible to develop into an effective diagnostic tool in the future. According to our predictions, if we can use 10 000 more records to train the network, an accuracy of more than 95% could be reached.
Conclusion: Voxel-based segmentation and a larger training set seem to be key success factors in reaching a high enough level of accuracy to introduce the AI into clinical practice.

Ethical Committee or Institutional Animal Care and Use Committee Approval: The images were provided by the National Cancer Institute of United States and comprise CT images of US patients. The CT Images analysis was part of the National Lung Screening Trial in the USA https://biometry.nci.nih.gov/cdas/approved-projects/?study.raw=NLST&p=4
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The effect of segmentation experience on the values of cardiovascular radiomic features of coronary atherosclerotic lesions

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Keywords: radiomics; atherosclerosis; coronary artery disease

Introduction: Evaluation of radiological images has a subjective component attributable to the reporting physician. Due to this subjectivity, the ability to reproduce certain imaging biomarkers is relatively low. Radiomics is the process of calculating hundreds of different quantitative features from a region of interest allowing to objectively describe lesion characteristics. However, precise segmentation is needed for the calculation of these metrics. We wished to assess the effect of the segmentation experience on the reproducibility of radiomic parameters.
Methods: Two physicians with several years of experience, and three medical students, each with a single month of experience segmented 80 plaques. We calculated all together 5066 radiomic parameters for each plaque. Among the parameters there were 44 first-order metrics, 1440 grey level co-occurrence matrix (GLCM) statistics, 66 grey level run length matrix (GLRLM) parameters and 3516 geometric statistics. We characterized reproducibility using the intraclass correlation coefficient (ICC), as follows: weak ICC 0.00-0.39; moderate 0.40–0.59; good 0.60-0.75; and excellent 0.76-1.00.
Results: For first order parameters, the ICC for the physicians was 0.89±0.13; and 0.71±0.17 for the medical students. The combined ICC was 0.77±0.16. For GLCM metrics, the corresponding results were 0.81±0.20; 0.59±0.21; and 0.64±0.20, respectively. For GLRLM metrics: 0.87±0.14; 0.70±0.13; and 0.74±0.13, respectively. For the geometric metrics, these were 0.68±0.23; 0.52±0.20; and 0.56±0.21, respectively.
Discussion: We saw greater similarities in radiomic values between physitians and medical students for the first order and GLRLM parameters, while there was a greater discrepancy for GLCM and geometric metrics.
Conclusion: The first order and GLRLM statistics are more robust with respect to segmentation errors, therefore their potential predictive qualities are expected to be more easily generalized to other patient populations.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Institutional review board approved the study (SE TUKEB 1/2017) and because of the retrospective study design informed consent was waived.
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Assessment of the small intestinal blood flow by indocyanine green fluorescence using color-fluorescence laparoscope

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Keywords: indocyanine green, fluorescence, ischemia, blood flow, intestine

Introduction: Indocyanine green (ICG) is a sterile, water-soluble compound that can be administered intravenously or intra-arterially. ICG has a fluorescence property, emitting near-infrared (NIR) fluorescence with 800-850nm wavelength. We can assess blood and lymphatic flow by using a fluorescence camera. ICG is rapidly and extensively bound to protein (mainly albumin) and is confined to the intravascular compartment with minimal leakage into interstitial tissues. Almost 100% of ICG is cleared by liver, and then excreted into bile.
We assessed the presence of reperfusion of ischemic small bowels with ICG. In general, reperfusion of ischemic small bowel is ceased in about 6 hours and its irreversibility determines the surgical indication. Therefore, the aim of the present study is to compare the difference of ICG fluorescence (i.e. blood perfusion) between 4 and 7 hours after ischemia has been induced.
Methods: Four swine were anesthetized and underwent laparoscopy. Two parts of small intestine were used on each swine (i.e. 8 parts were used in total). The blood flow of each part of small intestine was clamped to induce ischemia, one part for 4 hours and the other part for 7 hours. ICG (1mg/kg) was injected intravenously into each of them before de-clamping and blood flow was observed. Prototype of color-fluorescent laparoscope equipped with Hyper-Eye CCD was used for detecting ICG fluorescence.
Results: Fluorescence of ICG along with reperfusion blood flow after 4 hours of clamping were identified in all swine. On the other hand, the ones after 7 hours of clamping did not show reperfusion.
Discussion and Conclusion: Those results were consistent with the general fact that ischemic necrosis starts around 6 hours after the bowel ischemia has been induced. The difference of the blood flow between clamping for 4 hours and 7 hours was shown accordingly by the ICG method using color-fluorescence laparoscope.

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Ethics committee, International University of Health and Welfare Mita Hospital. Date: May 19th, 2010, Approval number: H20-22

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3D Computer Tomography is a Predictor of Osteopenia caused by Maxillary Alveolar Bone Functional Asymmetry in Young People?

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Key words: three-dimensional imaging, computer tomography, osteopenia; maxilla; alveolar process.

Introduction: Functional asymmetry of the bone remodeling level leads to increased variability of degree of bone density (DBD). The aim of this study was to determine the changes of the maxillary alveolar trabecular and cortical DBD during edentia using clinical three-dimensional (3D) computer tomography (CT) imaging in young people.
Methods: 3D CT images were taken from 40 patients who were 22–35 years old and without any metabolic diseases related to bone disorders. Randomization of patients was carried out for the control group (patients without edentia) and group of patients with edentia. The edentia group was subdivided into two subgroups: the 1st subgroup had patients who were missing 1-2 teeth; the 2nd subgroup had patients who were missing 3 or more teeth. The state of bone density was defined by investigation of compact and spongy layers in oral and basal parts (CO, SL, CB, respectively) of the alveolar bone of the maxilla at the segments of incisive small and large molar teeth on the left and the right of all persons as observed by digital cone-beam tomography Point 3D Combi (PointNix, South Korea). The regional variations within each image, as well as percentage (%) differences of the bone density by CT attenuation parameters between target regions (with edentia) and related symmetric regions for each patient were computed and compared.
Results: CO, SL, and CB had higher variability in the 2nd edentia subgroup for both sexes than patients in the 1st subgroup. In females of the 1st subgroup with edentia CO thickness decreased by20% compared to the symmetric spaces while the thickness of the SL varied with the thickness of SL. The maximum thickness of the CO was at the left incisive segments, and the minimum at the level of large molar segments symmetrically on both sides.
Conclusions: Modern 3D radiological visualization allows the definition of early signs of structural changes of the maxillary alveolar bone that already begins after edentia of one tooth in both sexes. Functional asymmetry of maxillary alveolar bone is associated with osteopenia.

Ethical Committee or Institutional Animal Care and Use Committee Approval: Protocol № 3 from 16/03/2015 of the BioEthics committee of the Danylo Halytsky Lviv National Medical University.
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