

5th INTERNATIONAL CHOLNOKY SYMPOSIUM

ABSTRACT BOOK



25TH APRIL 2019

**DEAN'S CONFERENCE ROOM,
UNIVERSITY OF PÉCS MEDICAL SCHOOL,
12 SZIGETI STREET, PÉCS**



5th International Cholnoky Symposium

25 April, 2019, Pécs



ORGANIZERS:

Dr. Krisztian Kvell

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Dr. Timea Bencsik

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Kitti Garai

Krisztina Ferenczi

Fanni Krausz

Zoltan Adam



5th International Cholnoky Symposium

25 April, 2019, Pécs

Program

9:00-9:50:	Registration/ Information Desk
9:50-10:00:	Symposium opening
10:00-11:40:	I. Session
11:40-12:00:	Coffee Break
12:00-14:10:	II. Session
14:10-14:30:	Coffee Break
14:30-16:30:	III. Session
19:00-	: Dinner at Kalamáris Restaurant*

*Registration required

Address: 30 Rákóczi Street, Pécs

Maximum time of presentations is 20 minutes + 5 minutes of discussion for keynote speakers and 10 minutes + one question for all lecturers.



5th International Cholnoky Symposium

25 April, 2019, Pécs

Program and Presentations

9:00-9:50: Registration/ Information Desk

9:50-10:00: Symposium opening

10:00-11:40: I. Session

Keynote speaker:

Miklos Poor: Pharmacological/toxicological importance of albumin-ligand interactions

1. **Violetta Mohos:** Interaction of human and microbial metabolites of quercetin with serum albumin and biotransformation enzymes
2. **Eszter Fliszar-Nyul:** Interactions of 7,8-dihydroxyflavone with human serum albumin as well as with CYP2C9, CYP2C19, CYP3A4, and xanthine oxidase biotransformation enzymes.
3. **Zelma Faisal:** Interactions of zearalenone and its reduced metabolites with serum albumins and cyclodextrins
4. **Anett Szabo:** In vitro investigation of the role of non-enzyme catalyzed oxidation of ibuprofen under
5. **Gergely Buki:** Investigation of copy number variations in the development of rare diseases

11:40-12:00: Coffee Break

12:00-14:10: II. Session

Keynote speaker:

Emese Sipos: The situation of biosimilar medicines on the romanian pharmaceutical market

1. **Ferenc Boda:** Drug development: from snake venom toxins to therapeutic agents.
2. **Leidy Jaqueline Angel Ferro:** Cytotoxic and anti-inflammatory effect of thyme oil and thymol: evaluation in human macrophage cells.
3. **Krisztina Banfai:** Beige Hints of Thymic Adipose Involution.
4. **Kitti Garai:** The potential of exercise-derived exosomes to treat chronic diseases
5. **Zoltan Adam:** The changes of HRV due to regular physical activity in healthy young women.



6. **Nikolett Dosa:** Screening for Dysphagia in Multiple Sclerosis Patients
7. **Daniel Schranz:** Neuroprotective effect of estradiol in basal forebrain cholinergic neurons in vivo

14:10-14:30: Coffee Break

14:30-16:45: III. Session

1. **Davor Ćosić:** Immunophenotyping of CD3+CD4+ and CD3+CD8+ peripheral blood lymphocytes in psoriasis vulgaris
2. **Bruno Rugani:** Effect of hybrids quinoline molecules on cell cycle of tumour cells in vitro
3. **Marko Markulinčić:** Application of green chemistry methods in synthesis of biologically active heterocyclic compounds
4. **Anita Potočar:** Biotransformation of barley husk by *Trametes gibbosa* and *Ceriporiopsis subvermispora*
5. **Balint Kovacs:** Tabletop linear accelerator based on THZ pulses
6. **Szabolcs Turnar:** Possibilities of electron acceleration based on THZ pulses
7. **Gergő Krizsan:** High energy terahertz sources for particle acceleration
8. **Andras Gergo Gardos:** The effects of clinically used X-ray contrast agents on the actin cytoskeleton
9. **Jonatan Pasitka:** Examination of biologically relevant electron transfer in photolyases with ultrafast laser spectroscopy

19:00- : Dinner at Kalamáris Restaurant*



Abstracts

Pharmacological/toxicological importance of albumin-ligand interactions

Miklos Poor¹, Eszter Fliszar-Nyul¹, Violetta Mohos¹, Zelma Faisal¹, Beata Lemli²

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Introduction: Human serum albumin (HSA) is the most abundant plasma protein in the circulation. HSA maintains the oncotic pressure of the blood, and it has buffer, antioxidant, and pseudo-enzymatic functions. Furthermore, albumin is able to form stable complexes with several endogenous compounds (e.g., ions, fatty acids, bilirubin, and proteins), drugs, and xenobiotics. As a result of the strong non-covalent interactions, these molecules circulate largely in albumin-bound form in the blood. Because HSA affects the tissue distribution and elimination of highly albumin-bound drugs, the displacement of drugs/xenobiotics from HSA can significantly modify their pharmacokinetic/toxicokinetic properties. Moreover, some reactive electrophilic compounds are able to form covalent complexes with HSA, which can be used as a biomarker of exposure. HSA is built up from three domains (I, II, and III), and each domain contains two subdomains (A and B). HSA has two major drug binding sites: Sudlow's Site I (in subdomain IIA) and Sudlow's Site II (in subdomain IIIA). Recent studies also highlighted the common involvement of a third binding region, the Heme site (located in subdomain IB).

Materials and Methods: Besides the physiological properties of HSA, we need to notice its importance in diagnostics (e.g., diabetes), medicinal therapy (e.g., treatment of shock or hypoalbuminemia), development of depot preparation of drugs (e.g., LEVEMIR insulin), and targeted drug therapy (e.g., "drug affinity complexes").

Results and Discussion: In this presentation, I will emphasize the most important properties and utilizations of HSA. Furthermore, using selected studies from the literature and examples from our investigations regarding mycotoxin-albumin and polyphenol-albumin interactions, I will demonstrate some interesting issues of albumin-ligand interactions, including covalent or non-covalent interactions, binding affinity vs. in vivo plasma protein binding (%), non-conventional binding sites, species-dependent alternations of albumin-binding, and difficulties in the identification of binding site(s).

Interaction of human and microbial metabolites of quercetin with serum albumin and biotransformation enzymes

Violetta Mohos, Attila Panovics, Eszter Fliszar-Nyul, Monika Moravcova, Premysl Mladenka, Paul W. Needs, Paul A. Kroon, Miklós Poór

Department of Pharmacology

Quercetin (Q) is contained by several fruits, vegetables, and dietary supplements. The oral bioavailability of Q is low, due to its poor aqueous solubility and significant presystemic elimination. Q is highly metabolized in the intestines and liver, during which methyl (isorhamnetin, IR), sulfate (quercetin-3'-sulfate, Q3'S), and glucuronide (quercetin-3-glucuronide, Q3G; isorhamnetin-3-glucuronide, I3G) conjugates are formed. After the oral administration of dietary supplements with high Q content (e.g., 1000 mg), micromolar plasma concentration of total Q (Q and its metabolites) appears in the circulation. Furthermore, Q can also be metabolized by the microflora in the colon through the degradation of the flavonoid ring(s) into smaller fragments.

Previous studies highlighted that Q and its conjugated metabolites can interact with several proteins. Therefore, the interactions of Q metabolites with human serum albumin (HSA) as well as with xanthine oxidase (XO) and cytochrome P450 (CYP2C9, CYP2C19, and CYP3A4) enzymes were examined.

Among the microbial metabolites, only 3-coumaric acid, 4-methoxysalicylic acid, 2,4-dihydroxyacetophenone, pyrogallol, and desmethylangolensin formed stable complexes with HSA. Q3G and I3G bind with significantly lower, while Q3'S and IR bind with even higher affinity to albumin than Q. Q3'S and IR displaced significantly the Sudlow's site I ligand warfarin from HSA; however, pyrogallol and desmethylangolensin showed significant but only slight effects. Q3'S, IR, and pyrogallol proved to be strong inhibitors of XO enzyme: pyrogallol was weaker while Q3'S and IR were stronger inhibitors of XO-catalyzed 6-mercaptopurine oxidation compared to Q or the positive control allopurinol. The CYP2C9 enzyme was only slightly affected by Q metabolites, however, statistically significant inhibition was observed regarding desmethylangolensin, Q3'S, and IR, for example. Furthermore, each conjugated metabolite proved to be a strong inhibitor of CYP2C19 and CYP3A4 enzymes.

Based on these observations, the consumption of dietary supplements with high Q content may influence the albumin-binding and/or the biotransformation of certain drugs. Therefore, the simultaneous administration of Q with drugs may lead to the development of pharmacokinetic interactions.

Interactions of 7,8-dihydroxyflavone with human serum albumin as well as with CYP2C9, CYP2C19, CYP3A4, and xanthine oxidase biotransformation enzymes.

Eszter Fliszar-Nyul, Violetta Mohos, Miklós Poór

University of Pécs, Medical School Department of Pharmacology

Introduction: 7,8-dihydroxyflavone (DHF) is a flavonoid, it can activate tyrosine kinase B receptors in the central nervous system. DHF is a promising candidate in the treatment of Alzheimer's disease, Parkinson's disease, and traumatic brain injuries due to its neuroprotective effects. Despite most of the pharmacokinetic parameters of DHF is known, only limited information is available regarding its interactions with human serum albumin (HSA) and biotransformation enzymes.

In this study, the interactions of DHF with HSA as well as with cytochrome P450 (CYP2C9, CYP2C19, and CYP3A4) and xanthine oxidase (XO) enzymes were investigated.

Materials and Methods: Binding constant of DHF-albumin complex was determined based on fluorescence quenching. The displacement of drugs from HSA by DHF was evaluated using ultrafiltration. Interactions of DHF with CYP and XO enzymes were tested using XO enzyme and CypExpress™ (2C9, 2C19, and 3A4) kits. Substrates and products were quantified by HPLC.

Results and Discussion: Fluorescence quenching studies suggest the formation of stable DHF-albumin complexes ($\log K_a = 5,7$). Based on ultrafiltration studies, DHF can displace both site I and site II markers from albumin. DHF proved to be a strong inhibitor of each biotransformation enzyme tested. It was a similarly strong inhibitor of CYP2C9 than the positive control warfarin. To investigate the inhibition of CYP2C19, CYP3A4, and XO enzymes, ticlopidine, naringenin, and allopurinol were used as positive controls, respectively. DHF significantly inhibited these enzymes showing 1.8-fold (CYP2C19), 1.4-fold (CYP3A4), and 1.7-fold (XO) weaker effects compared to the positive controls.

Our results demonstrate that DHF can displace drugs from HSA, and it is a strong inhibitor of each biotransformation enzyme tested. Considering the above-listed observations, co-administration of DHF with drugs may interfere with the drug therapy due to the development of pharmacokinetic interactions.

Interactions of zearalenone and its reduced metabolites with serum albumins and cyclodextrins

Zelma Faisal, Virag Vörös, Rita Csepregi, Tamas Koszegi, Beata Lemli, Sandor Kunsagi-Mate, Mónika Balint, Csaba Hetenyi, Lajos Szente, Miklos Poor

Department of Pharmacology

The mycotoxin zearalenone (ZEN) is produced by *Fusarium* species. ZEN frequently occurs in cereals and cereal-based products. As a xenoestrogen, ZEN causes reproductive disorders in animals and humans. Reduced metabolites of ZEN show similar or higher toxicity than the parent compound, while glucuronide and sulfate conjugations results in less toxic derivatives. Serum albumin is the most abundant plasma protein. Formation of stable albumin-ligand complexes can affect the distribution and elimination of xenobiotics. Cyclodextrins (CD) are ring-shaped molecules built up from glucose units. The hydrophilic external part of CDs leads to their excellent aqueous solubility, while their lipophilic internal cavity can accommodate apolar guest molecules (including mycotoxins). In this presentation, I will summarize our recent results regarding the interactions of ZEN and its metabolites with albumin and CDs, including the potential relevance and utilization of these interactions.

ZEN and its metabolites form stable complexes with albumin, showing large species-dependent alternations. Typically, mycotoxins formed the most stable complexes with rat albumin, while the lowest complex stability was observed with porcine and/or with bovine serum albumins. Modeling studies highlighted that ZEN and zearalenols occupy non-conventional binding site(s) in human serum albumin (HSA) between subdomains IIA and IIIA.

ZEN and zearalenols form host-guest type complexes with native and chemically-modified CDs. The complex formation results in strong increase in the fluorescence of these mycotoxins, therefore, the analytical utilization of CD technology seems promising. Beta CD bead polymer successfully eliminated ZEN and zearalenols from buffers and ZEN from spiked beer samples, showing the suitability of CDs as potential mycotoxin binders. Interestingly, CDs also interact with the masked mycotoxin zearalenone glycoside which is also a common contaminant in the food chain. Cell experiments showed that CDs can decrease the *in vitro* toxicity of ZEN, likely due to decreased cellular uptake of the mycotoxin. Based on this observation, the further investigations of CDs as *in vivo* mycotoxin binders seem reasonable.

In vitro investigation of the role of non-enzyme catalyzed oxidation of ibuprofen under hyperglycaemic conditions

Anett Szabo, Attila Almasi, Pal Perjesi

University of Pécs Medical School Institute of Pharmaceutical Chemistry

Introduction: It is well documented in the literature that several acute and chronic diseases are accompanied by an elevated level of reactive oxygen species. Among others, diabetes and chronic inflammations are those ones that can cause increased non-enzymatic oxidation of both endogenous compounds and xenobiotics. In our earlier investigations, we studied the intestinal absorption of the non-steroidal anti-inflammatory drug ibuprofen by an in vivo perfusion model. During the experiments, data were collected on the kinetics of elimination of the molecule and changes in the metabolite profile in experimental diabetic animals. In these latter case oxidative metabolites of ibuprofen (2-OH-ibuprofen and carboxyibuprofen) have been identified.

Materials and Methods: As a continuation of our earlier studies, we investigated the oxidation of ibuprofen by two different in vitro methods. In both methods (Udenfriend, Fenton) reactive oxygen species are considered to be the oxidizing agents. The incubation samples were extracted by liquid-liquid extraction and the extracts were analyzed HPLC-MS method.

Results and Discussion: The results of our study confirmed the non-enzyme-catalyzed formation of 2-OH-ibuprofen in the incubation mixtures. Hyperglycemia can result in oxidative stress and accompanied by the increased formation of reactive oxygen species (ROS). One of the major sources of ROS formation in diabetic patients is NADPH oxidases of the Nox family. Nox1 is particularly highly expressed in the epithelial cells of the gastrointestinal tract. Accordingly, the results provide further experimental evidence of possible non-enzyme-catalyzed oxidative metabolism of xenobiotics under hyperglycaemic conditions.

Investigation of copy number variations in the development of rare diseases

Gergely Buki, Bela Melegh, Judit Bene

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Introduction: Copy number variations (CNV) are large DNA segments (1kb or larger) present at different copy numbers with respect to a reference genome sequence. CNVs can be classified as deletions, duplications or amplifications. Since CNV can encompass part or all of a gene, or be a genomic segment containing several genes, some CNVs are likely to have a role in the alteration of human physiological functions. Moreover, CNVs can cause Mendelian or sporadic traits, or be as well associated with complex diseases. Mutation rates of CNVs can vary widely at different loci, likely reflecting the differences in CNV formation mechanism and local or regional genome architecture inciting genomic instability.

Aims: To study the possible role of copy number variations in the development of Marfan syndrome and Cystic fibrosis.

Materials and methods: After negative results of systematic sequencing of *FBN1*, *TGFBR1* and *TGFBR2* genes for Marfan syndrome and *CFTR* for Cystic fibrosis, MLPA (multiplex ligation-dependent probe amplification) analysis (P065-P066 MRC-Holland and P091 MRC-Holland) was performed in 43 patients suffering from Marfan syndrome and in 87 patients with Cystic fibrosis.

Results: In contrast to the generally detected large deletions, a novel two-exon deletion (exon 46-47) in *FBN1* gene has been observed in one patient in the Marfan patients group. This results in the loss of 31-32nd calcium binding EGF-like domain which is responsible for the development of classic Marfan phenotype. Two small and one large deletion (3.4 % of all cases) were identified in the *CFTR* gene and all of them are involved in the development of Cystic fibrosis phenotype.

Conclusions: Less than 10% of the disease causing mutations are copy number alterations, in which single or multiple exon deletions can be detected by MLPA in a cost-effective manner even in the NGS era.



The situation of biosimilar medicines on the romanian pharmaceutical market

Emese Sipos

University of Medicine, Pharmacy, Sciences and Technology of Targu Mures, Romania

Introduction: The biosimilar medicines are almost identical with the original biological products, whose similar effect with the reference product is proved through elaborate comparative studies, concerning the physical-chemical properties, efficacy and safety. The biosimilar products are a very important class of human medicines, because their price is much lower as of the originals, so they offer more possibilities of treatment for a wider level of population. Beside this, they represent a huge advantage for authorities and the state, by reducing significantly the gigantic costs of the biological products.

Materials and Methods: The authorization of biosimilar medicines is a very complex process, and it is controlled by the European Union with special guidelines. The authorisation is made by a centralised procedure, which means, that once on the market, these medicines can be commercialised in entire Europe.

Results and Discussion: In Romania there are only a few biosimilar products, compared to the European market, but by every year new medicines appear, and new national healthcare initiatives are started, which encourage the use of these products. The price of biosimilars is much lower than of the original products, moreover, they are refunded 100% by the Romanian authorities.

Drug development: from snake venom toxins to therapeutic agents.

Ferenc Boda, Berta Lavinia, Szabó Zoltan Istvan

*University of Medicine, Pharmacy, Sciences and Technology of Targu Mures, Faculty of Pharmacy,
Department of Fundamental Pharmaceutical Sciences, General and Inorganic Chemistry*

Introduction: Snake venoms are a complex mixture of proteins, peptides and small molecules. The proteinaceous components show various biological activities, targeting important physiological functions. Due to the vast number of bioactive molecules, snake venoms represent a promising source of lead molecules for the development of new therapeutic agents.

Materials and Methods: In this work, we present the methods used in the development of therapeutic agents from bioactive molecules isolated from snake venoms.

Results and Discussion: To identify possible lead molecules, toxins are isolated from snake venoms using chromatographic methods and their physicochemical properties are determined with a myriad of analytical tools, including but not limited to: Edman degradation, gel electrophoresis, mass spectrometry etc. Once the target toxins are characterized, they are tested in various in vitro and in vivo studies to determine their effect and mechanism of action. In some cases, the toxins isolated from snake venoms are used in their native form, e.g., batroxobin or ancrod. If the amount of the isolated toxin is insufficient, heterologous expression can be used to obtain a larger quantity, e.g., textilinin-1 or vicrostatin. More often, however the isolated toxins function as true lead molecules, and are subject to structural modifications in order to obtain a therapeutic agent with suitable biopharmaceutical properties. This approach led to the development of several important therapeutic agents, such as captopril, tirofiban or eptifibatide.

Cytotoxic and anti-inflammatory effect of thyme oil and thymol: evaluation in human macrophage cells.

Leidy Jaqueline Angel Ferro, Krisztina Banfai, Kitti Garai, Sophie Iglewski, Peter Bukovics, Krisztian Kvell, Györgyi Horvath

University of Pécs. Department of Pharmacognosy / Department of Pharmaceutical Biotechnology

Introduction: Essential oil of *Thymus vulgaris* L. (Lamiaceae) or thyme oil (TEO) shows great variability of its composition with six main chemotypes: geraniol, linalool, α -terpineol, carvacrol, thymol, and trans-thujan-4-ol/terpinen-4-ol types. Due to this large chemical diversity, the subject of several investigations was to identify and determine their properties, including their potential antibacterial, antifungal, antioxidant and anti-inflammatory effect.

Materials and Methods: Our aim was to determine the effect of TEO and thymol on the inflammatory cascade of U937 (human macrophage cell line) by means of the analysis of the gene expression using SYBRTM Green and TaqManTMPCR for specific cytokines with anti and pro-inflammatory character like; TNF α , IL1 α/β , IL-6, IL-10, IL12 α/β , among others, that were secreted due to the initial activation of the cascade through exposition of the cell line to LPS. To achieve that objective, previously it was necessary to standardize all the experimental conditions in order to identify the adequate concentrations of TEO and thymol on which there was an anti-inflammatory effect but not cytotoxicity greater than the inherent one due to over-saturation of the medium by determining cell survival after treatment by Automatic Image-Based Cytometer FASCOPETM B (Curiosis).

Results and Discussion: The two components showed concentration-dependent cytotoxicity but considerably insignificant or inconsiderable in low concentrations that turned out to be, on the other hand, effective in the up-regulation of the anti-inflammatory cytokine gene expression and without a significant increase or in some cases, down-regulation of the pro-inflammatory type as was expected.

Beige Hints of Thymic Adipose Involution.

Krisztina Banfai, David Ernszt, Attila Pap, Peter Bai, Kitti Garai , Djeda Belharazem, Judit E. Pongracz,
Krisztian Kvell

University of Pécs

Introduction: During aging of the thymus the epithelial network shrinks to be replaced by adipose tissue called thymic involution. Transcription factor TBX-1 controls thymus organogenesis during embryonic development, however it has also been reported to coordinate beige adipose tissue development. Given this dual role of TBX-1, we have assessed whether its thymic expression remains active during adulthood until adipose involution begins. We have also examined if thymic adipose involution could actually yield beige adipose tissue.

Materials and Methods: Mouse and human thymus tissue from various ages was used to evaluate the kinetics of TBX-1 expression, as well as mouse (TEP1) and human (1889c) thymic epithelial cells (TECs). Transmission electron microscopy was used to detect lipid deposits typical of beige adipose cells. Histology staining was carried out to show the accumulation of neutral lipid deposits. qPCR measurements were applied to display persistent and/or elevating levels of beige-specific and beige-indicative markers (TBX-1, EAR-2, UCP-1, PPAR-gamma). We have also performed miRNome profiling using qPCR-based QuantStudio platform and amplification-free NanoString platform. Finally, using the Seahorse metabolic platform we have recorded a metabolic profile (OCR/ECAR ratio) indicative of beige adipose tissue.

Results and Discussion: Our results support that thymic adipose tissue emerging with senescence is beige adipose tissue. Several beige adipose tissue regulatory miRNAs were identified. Further miRNAs showed significant changes affecting cellular / tissue level senescence with focus on the thymus. Our data show how the borders blur between a key immune tissue (the thymus) and a key metabolic tissue (beige adipose tissue) with senescence.

The potential of exercise-derived exosomes to treat chronic diseases

Kitti Garai, Krisztina Banfai, Robert Herczeg, Zoltan Adam, Adam Gyebrovski, Attila Gyenesei, Judit Pongracz, Marta Wilhelm, Krisztian Kvell

University of Pécs

Introduction: Exosomes are endocrine-like intercellular communication vesicles that carry proteins, lipids, sugars and nucleic acids between cells and tissues. Previous studies demonstrated that circulating miRNAs are rapidly upregulated during acute physiological stimulus, thus accurately reflect intracellular responses. To date none of the published articles have evaluated the effect of 6-month long moderate intensity combined endurance-resistance training on healthy, young sedentary individuals. However, characterization of exosome profile will likely lead to the development of interventions in several chronic diseases.

Materials and Methods: The lifestyle program included 60-minute personal-trainer guided exercise sessions, three times per week, for 6 months. At the end of the program 17 participants completed the six month-long lifestyle program. Blood samples were collected at baseline, after 3 months and 6 months. Serum samples were pooled (100µl/subject) for each time point assessed (0, 3 and 6 months). Total RNA was extracted from exosomes, analyzed using Agilent Bioanalyzer small RNA chips and miRNA profiling was performed using human NanoString nCounter miRNA microarray assay to evaluate 800 miRNAs. The 10 most abundant miRNAs contained within exosomes were analyzed using miRDIP to determine the target gene landscape and finally network analysis was conducted to identify the dominant biological processes and pathways modulated by exercise-derived exosomal miRNAs.

Results and Discussion: 67 miRNA species showed substantial transcript level change and 10 presented ± 2 -fold change after 6 months regular training (miR-142-3p, miR-518f-3p, $P < 0.01$). Targeted pathways were related to cancer development and metastasis formation, neurodegenerative disease, osteoporosis, inflammatory disease, immune-senescence and cardiovascular events.

The data presented help defining the miRNA landscape of regular exercise-derived exosomes and highlight new pathways in the molecular mechanisms mediating systemic adaptation.

Acknowledgment: The project was supported by THE ÚNKP-18-3-1 2018/2019 NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY OF HUMAN CAPACITIES TO KG.

The changes of HRV due to regular physical activity in healthy young women.

Zoltan Adam, Kitti Garai, Adam Gyebrovski, Marta Wilhelm

University of Pécs

Introduction: The effects of regular physical activity on the circulatory system is a well investigated area of sports biology, however there are some remarkable disagreements concerning heart rate variability. Physical inactivity plays a crucial role in the formation of cardiovascular diseases [1]. Heart rate variability (HRV) proved to be a good biomarker of overtraining and dysfunctions originating from low-level of physical activity [2]. Measuring HRV is used on a regular basis evaluating the fitness status of athletes, yet studies focusing on the changes of HRV in healthy young people due to regular exercise are lacking. The aim of the present study was to determine the effects of a 6-month long training program (involving both resistance and aerobic training) on the functions of the autonomic nervous system.

Methods: 16 untrained women (23.57 ± 2.41 y) participated in a six-month long training program (3x60 min/week). The level of physical activity was determined with the use of wrist worn accelerometer (Actigraph). Monitoring heart rate (HR) was conducted during pre-exercise phase (5 min sitting), exercise (walking and jogging at 65% $\text{VO}_{2\text{max}}$, for 30 min) and in the recovery phase (5 min sitting) as well. We performed time domain and frequency analysis on five-minute segments of the recorded data at baseline, 3-, and 6 months.

Results: Remarkable elevation occurred after the first 3 months in mean values of the resting time domain parameters (mean R-R: 724.65 ± 94.03 to 760.36 ± 97.62 ; RMSSD: 27.89 ± 11.50 to 33.16 ± 16.38 ; NN50: 36.85 ± 35.74 to 49.82 ± 45.98) and a detectable decrease in the aerobic segment of the training (mean R-R: 450.88 ± 30.31 to 429.72 ± 38.16 ; RMSSD: 6.28 ± 2.03 to 5.08 ± 1.33 ; NN50: 0.59 ± 1.13 to 0.142 ± 0.27) but no significant change was observed during the recovery periods. With the aid of frequency data analysis, we revealed a significant decrease of sympathetic activity measured during resting, and endurance periods (increased LF/HF, decreased LF Power, decreased HFnu), while the recovery period remained unchanged ($p < 0.05$).

Conclusion: Our results show that regular mixed moderate intensity physical activity has beneficial effects on the cardiovascular system through the modulation of the autonomic nervous system which caused an overall increase of HRV even in healthy young individuals due to 6-month of training. However, the variability originating from interindividual differences is high, we suggest the evaluation of HRV data individually in future studies.

Acknowledgement: The project was supported by THE ÚNKP-18-3 2018/2019 NEW NATIONAL EXCELLENCE PROGRAM OF THE MINISTRY OF HUMAN CAPACITIES TO ZA.

Screening for Dysphagia in Multiple Sclerosis Patients

Nikolett Dosa

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Introduction: Dysphagia is a common life-threatening symptom in the Multiple Sclerosis patients. According to recent studies at least one third of people with MS are suffering from swallowing difficulties. The dysphagia can potentially cause severe problems, reduce the quality of life and increase the risk of dehydration and aspiration pneumonia. The aim of the study is to identify the frequency of dysphagia and describe the potential “medical nutritional treatments” in Multiple Sclerosis.

Materials and Methods: According to some studies in our descriptive study we describe the characteristics of the dysphagia in MS. We also present the latest suggested nutritional treatments. We used to search the PubMed, Scopus, Springer Link. Our inclusion criterium were: DYMUS, EAT10, EDS.

Results and discussion: The appearance of dysphagia is related with EDS score and type of MS, but not related with age, gender and duration of disease. The DYMUS score was significantly higher in progressive MS. The DYMUS and the average score of EAT10 was correlated. The doctors suggest to MS patient the mediterranean diet, as a nutritional treatment, due to the omega 3 fatty acids content. The latest effective diet in the USA is the Wahl’s Protocol. DYMUS, EAT10 and the EDS score help us to diagnose the dysphagia among Multiple Sclerosis patients.

Neuroprotective effect of estradiol in basal forebrain cholinergic neurons in vivo

Daniel Schranz

University of Pécs Medical School Institute of Physiology

Introduction: A major neuropathological hallmarks of Alzheimer's disease (AD) is widespread neuronal death in the basal forebrain cholinergic (BFC) neurons, which is thought to result from the accumulation of soluble aggregates of neurotoxic amyloid- β peptides, such as A β 1-42. The gonadal steroid 17 β -estradiol (E2) exerts non-classical actions on intracellular signaling that is important for mediating cytoprotective effect of E2. Here we examined the neuroprotective effect of E2 on BFC neurons in AD animal model.

Materials and Methods: Female mice were ovariectomized in Avertin anaesthesia. Fourteen days after ovariectomy A β 1-42 were stereotactically microinjected into the substantia innominate-nucleus basalis magnocellularis(SI-NBM) under isoflurane anesthesia. Mice were administered 33ng/g of E2 or vehicle subcutaneously 1h after A β 1-42 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 30 μ m thick coronal sections were cut on a freezing microtome. The cholinergic fibre loss in the somatosensory cortex (SC) were visualized by acetylcholine esterase (AChE) histochemistry and cholinergic cell loss by choline-acetyl transferase immunohistochemistry(ChAT).

Results and Discussion: All animals that received unilateral injection of A β 1-42 showed a profound decrease in cholinergic cell number in the SI-NBM and AChE-stained fibers in the layer V, and IV. of SC from the lesioned hemisphere. Administration of E2 significantly attenuated the A β 1-42 -induced AChE fiber loss in the SC but had no effect on ChAT-positive cell number in the SI-NBM. We report that a single dose of E2 administered 1 h after intracerebral injection of the A β effectively reduced the fiber loss of BFC neurons. Our findings suggest that E2 treatment has neuroprotective potential in AD.

Immunophenotyping of CD3+CD4⁺ and CD3+CD8⁺ peripheral blood lymphocytes in psoriasis vulgaris

Davor Ćosić, Paula Herek, Zvonimir Grgić, Vera Plužarić, Martina Mihalj, Mario Štefanić, Stana Tokić

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Introduction: Vulgar psoriasis (PV) is a common, relapsing erythematous squamous dermatosis mediated by various inflammatory cells. Previous reports have demonstrated an important role for susceptible genetic background, impaired skin barrier and uncontrolled immune response, particularly T cells, in disease etiology. Accordingly, increased numbers of CD3+CD4⁺ T cell helper (Th) and CD3+CD8⁺ cytotoxic (Tc) lymphocytes, particularly Th1, Th17, Tc1 and Tc17 cells, have been reported in psoriatic skin lesions. However, the specific shares and migration patterns of peripheral CD3+CD4⁺ and CD3+CD8⁺ lymphocyte populations in PV are still insufficiently clarified. Thus, we aim to investigate whether CD3+CD4⁺ and CD3+CD8⁺ T cell frequency and phenotype are affected in PV patients.

Materials and Methods: To achieve this goal, peripheral blood mononuclear cells (PBMC) were isolated from 4 ml of heparinized blood samples collected from 14 healthy controls (CTRL) and 14 patients diagnosed with PV. In the following step, PBMCs were stained with the CD3 FITC, CD4 PE-Cy7 and CD8 PerCP monoclonal antibodies. Dead cells were excluded using LIVE/DEAD Fixable Near IR Kit. Flow cytometry was performed on BD FACS Canto II cytometer and data were analysed with the FloLogic software.

Results and Discussion: Compared to controls, psoriatic patients had significantly lower frequency of circulating CD3+CD8⁺ T cells [PV vs CTRL (mean±SD): 33.3±9.2 vs 41.8±7.4, P=0.0083 (Mann-Whitney test)], whereas peripheral CD3+CD4⁺ T cell count was significantly higher [PV vs CTRL: 59±9.9 vs 49.3±10.4, P=0.023]. Our preliminary findings support the role of peripheral T cell compartments in psoriasis and warrant functional characterisation of CD4⁺ and CD8⁺ T cell population in larger studies.

Effect of hybrids quinoline molecules on cell cycle of tumour cells in vitro

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Introduction: Quinoline is nitrogen containing heterocyclic aromatic compound with broad pharmacological activities. Hybrids molecules with quinoline structure showed high potential for DNA binding and subsequent inhibition of DNA-dependent enzymes, followed by inhibition of cell transcription. Based on previous knowledge, new quinoline hybrids are synthesized in direction to interact with DNA, inhibiting tumour cells proliferation, affecting cell cycle distribution.

Materials and Methods: The potential cytotoxic effect of quinoline hybrids was determined on colon adenocarcinoma (CaCo-2), breast adenocarcinoma (MDAMB231), acute lymphoblastic leukaemia (CCRF-CEM) cells, and on Madin-Darby canine kidney fibroblast like cells (MDCK I) as a normal cells. Cytotoxicity was tested by MTT assay after 72H incubation with new quinoline hybrids at concentrations of 1×10^{-4} M, 1×10^{-5} M and 1×10^{-6} M. Flow cytometry was used to determine changes in cell cycle distribution in CaCo-2, MDAMB231 and CCRF-CEM cells treated with 5×10^{-6} M of quinoline hybrids for 24H.

Results and Discussion: Tested quinoline hybrids showed dose and structure dependent cytotoxic effect on treated cells. Further biological evaluation is required.

Key words: quinoline molecules, tumour cells, cell cycle

Application of green chemistry methods in synthesis of biologically active heterocyclic compounds

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Introduction: A synthesis of biologically active compounds for potential application in medicine, pharmacy, cosmetic industry, have been intensified in the last few decades. Not only it became desirable to find a new synthetic paths for the synthesis of such heterocyclic compounds, the focus has been put on finding the synthetic routes with the minimal adverse impact on the environment. When utilizing a green chemistry procedures and methods in the synthesis, one can be guided by the green chemistry principles. Deep eutectic solvents (DES), as a green solvents, fit these green chemistry principles, since they are synthesized from non-toxic and cheap biodegradable components, with 100% atom economy. They are formed from the hydrogen bond acceptor (HBA) and hydrogen bond donor (HBD), mixed together in various ratios, to form a eutectic mixture. DES have been extensively used in a wide range of synthetic transformations, both as the solvents and catalysts. Our work is focus on choline chloride based DES which were successfully applied for the synthesis of different heterocyclic compounds.

Materials and Methods: DES synthesis: an equimolar amount of choline chloride and corresponding HBD is mixed together and heated to 80 °C until a clear liquid is formed. The synthesized DES is used in the synthesis of desired compounds without further purification. N-Acetylation of different amines: Amines and acetic anhydride are mixed together (1:1.2 molar ratio) and added to choline chloride:urea (1:2) DES. The reaction was monitored by TLC, yielding acetylated amines in good to excellent yields. Synthesis of mercapto quinazolinones: An equimolar amount of anthranilic acid and various isothiocyanates was added to corresponding DES and stirred until the completion of reaction, monitored on TLC. Synthesis of coumarinyl Schiff bases: an equimolar amount of coumarinyl hydrazide and substituted aldehydes was mixed together and stirred until all reactants were consumed. A solid product precipitated upon addition of water. One-pot synthesis of 1,2,4-triazoles: Coumarinyl hydrazides and substituted isothiocyanates were mixed and stirred in DES until the completion of reaction. Water was added and the solid product precipitated.

Results and Discussion: Since DES can be synthesized from different HBA and HBD, their physical and chemical properties vary greatly, depending on the components mixed together, so they can be referred to as tunable solvents. Their ability to be recycled for several times without adverse effect on the reaction products makes them even more desirable in synthetic chemistry. They have been successfully applied for the synthesis of different heterocyclic compounds with no need for catalysts. N-Acetylated amines were successfully synthesized in good to excellent yields in this improved reaction, since the amount of acetic anhydride, which



is often used in these kind of reactions, was drastically reduced. Mercapto quinazolinones were successfully synthesized in choline chloride:urea DES with no need for catalysts, which are usually utilized in this kind of reaction. Obviously, DES can serve as a solvent and as catalyst as well. Coumarinyl Schiff bases were also synthesized in good to excellent yields, with no need for addition of acetic acid as a catalyst. One-pot synthesis of 1,2,4-triazoles was also performed, with no catalysts needed, from corresponding hydrazide and isothiocyanates.

Deep eutectic solvents were proven to be an excellent reaction media for all these reactions, while the isolation of the products was very easy, and included only the addition of water and filtration.

Biotransformation of barley husk by *Trametes gibbosa* and *Ceriporiopsis subvermispota*

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Sustainable food production has become an imperative in modern food industry. It includes the valorisation of production process residues, which have been so far mainly treated as waste. The aim of this work was to investigate the possibility to use barley husk (BH) as a source of bioactive phenolic acids and to enhance the extractability of phenolic acids as well as the antioxidant activity by the implementation of biological treatment (SSF).

Two fungi (*Trametes gibbosa* and *Ceriporiopsis subvermispota*) were used for SSF of BH during 1-10 days. Ultrasound assisted extraction of bioactive phenolic compounds from untreated and treated BH was performed with eco-friendly solvent (50 % aqueous ethanol). Obtained extracts were analysed by gradient UHPLC method for quantification of 10 phenolic acids. Antioxidant activity was determined by DPPH method. Additionally, activity of enzyme laccase was measured spectrophotometrically, every day, in obtained water extracts, using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) as a substrate.

Among all tested phenolic acids in untreated BR extracts, syringic acid (77.00 $\mu\text{g/g}_{\text{db}}$) and ferulic acid (63.45 $\mu\text{g/g}_{\text{db}}$) were represented in the greatest, while ellagic acid was presented in the lowest amount (1.20 $\mu\text{g/g}_{\text{db}}$). SSF of BH with *T. gibbosa* enhanced the extractability of protocatechuic acid for 7.09-folds, while the SSF with *C. subvermispota* increased the extractability of *o*-coumeric acid for 6.84-folds. Laccase activities up to 270 U/dm³ were obtained after 10 days treatment with *T. gibbosa*, while *C. subvermispota* didn't produce laccase under investigated conditions.

Tabletop linear accelerator based on THz pulses

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Introduction: Investigations of miniature particle acceleration structures are important, due to their low fabrication costs and compact sizes. In recent years, the THz sources have been developed, approaching ~1 mJ THz pulse energy and opening new possibilities to laser-driven particle acceleration schemes such as Dielectric Laser Accelerators (DLA). Due to the benefits of mm wavelength the interaction length between the particles and the acceleration field is going to be longer, hence the energy gain is increased.

Materials and Methods: In our proposed structure we exploit the advantages of THz-driven DLA coupled by metallic horn. In our simulation the single-cycle THz pulse propagates through the waveguide structure while the peak electric field is increasing continuously until it reaches the DLA. Due to the dispersion, at the end of the structure a multi-cycle THz pulse evolved, which is more suitable to accelerate electrons by the multi-periodic DLA.

Results and Discussion: In our simulations the peak electric field increased from 1.5 MV/cm to 4 MV/cm until it reached the vacuum channel of the DLA, thus we achieved more than 600 keV kinetic energy gain. Using the proposed setup we will be able to produce high quality electron beams in the future, which can be useful to biological and chemical applications.

Possibilities of electron acceleration based on THz pulses

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Introduction: In case of particle accelerators, the size, cost and the achievable accelerating gradient are the most important properties. Applying longer driver wavelengths than far infrared, the interaction length between particles and the electric fields can be extended and opens new availabilities to realize ultrashort electron beams with very high peak brightness and quality. At radio-frequencies (RF), where conventional sources are working efficiently, there are rather complex and costly devices. Strong-field THz pulse sources have been developed and it has given a chance to generate GV m⁻¹ terahertz fields. In consequence, these lower cost devices have given a possibility to exploit their advantages such as high repetition rate, small size, and compactness.

Materials and Methods: We have numerically investigated electron accelerations using single-cycle THz pulses in vacuum and in waveguide structures. In our simulations we have demonstrated table top particle accelerator setups, whereby we can produce electron bunches with sub-100-femtosecond duration in vacuum and ~130 fs duration in waveguide. The predicted electron bunches with narrow energy distribution are well suited for electron diffraction studies.

Results and Discussion: In my presentation I give an overview of our proposed THz-based particle accelerator setups and I am going to show the possibilities and challenges of THz-pulse-driven electron accelerators.

High energy terahertz sources for particle acceleration

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Introduction: Hadron therapy can be a solution for cancer cell treatment. Nowadays it is only available for few, because for that large and expensive facilities are needed. With terahertz (THz)-driven particle accelerators a relatively cheap, table top solution could be achievable, and the hadron therapy would be available for many.

Materials and Methods: The low- (0.1-2THz) frequency band of the THz range is optimally fitting for acceleration of charged particle beams. Semiconductors can be used as highly efficient terahertz sources if the pumping wavelength sufficiently long to eliminate two- and three-photon pump absorption and the associated free-carrier absorption in the THz range. At such long infrared pump wavelengths tilted-pulse-front (TPF) pumping has to be used for phase matching. Comparing to lithium niobate this angle is advantageously small ($<30^\circ$), and contact-grating (CG) is feasible.

Results and Discussion: In our experiment we demonstrated more than two orders of magnitude higher pump-to-THz conversion efficiency than before, using a pump wavelength of $1.7 \mu\text{m}$. $14 \mu\text{J}$ THz pulse energy at 0.7% efficiency were measured with a prism shape ZnTe source. We also realized a monolithic ZnTe CG THz source and demonstrated the generation of THz pulses with up to $3.9 \mu\text{J}$ energy and 0.3% efficiency. Our calculations show that single-cycle THz pulses can be generated with 15 GW/cm^2 pump intensity, 150 fs pump pulse duration and 2 THz phase-matching frequency with more than 3% conversion efficiency from an 8 mm thick GaP source.

The effects of clinically used X-ray contrast agents on the actin cytoskeleton

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Introduction: We have examined the effects of two clinically used iodine-containing contrast materials, Iomeprol and Iobitridol on the polymerization of the actin using spectroscopy. Previous studies have shown that iodine-containing intravasal contrast materials alter the shape of erythrocytes and endothelial cells, partly by influencing the organization of the actin cytoskeleton. The changes in the actin cytoskeleton and its molecular mechanism are not fully understood. The goal of our research is to clarify these mechanisms.

Materials and Methods: We used fluorimetry to examine the dynamics of the actin polymerization in the presence of different concentrations of contrast materials. We used labelled actin, covalently binding a fluorescent marker (N-(1-pyrene) iodoacetamide) to the Cys-374 amino acid of actin. The fluorescence of the used marker increases many folds through the polymerization compared to the labelled G-actin monomers. From the increase of fluorescence, we can infer the current level of polymerization in real time.

Results and Discussion: Our results have unequivocally proved on a molecular level that the examined contrast materials in vitro (and probably in vivo as well) influences the behaviour of the actin cytoskeleton network by directly affecting the actin, not through regulating proteins or other indirect mechanisms of action. We have shown that the examined contrast materials have a concentration-dependent effect on actin, significantly reducing the most important property of actin: its capability for polymerization, as well as the extent of its polymerization. During both series of measurements with different iodine-containing contrast materials, we have observed a significant, highly similar concentration-dependent decrease of the velocity of the actin polymerization.



Examination of biologically relevant electron transfer in photolyases with ultrafast laser spectroscopy

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Introduction: In the last few decades an enormous technological expansion could have been seen in the field of lasers. This resulted in the application of basicly biophysical methods in the every day medicine, methods like super-resolution microscopy, CARS microscopy, Raman-spectroscopy and even ultrafast laserspectroscopy.

Materials and Methods: With the help of ultrafast laser spectroscopy the recording of intramolecular procedures became possible. Several, biologically relevant processes are known that take place within the femtosecond scale. Such an example is photoisomerisation. We have been focusing on the ultrafast laser spectroscopic features of these latter two proteins. They are phylogenetically connected, however photolyase is a DNA repair enzyme while cryptochrome is the member of the group of proteins composing the circadian clock. The explanation for such different functions so affar from each other lays in the understanding of the procedures happening intramolecularly on the scale of picoseconds. We made two photolyase mutants, in which we altered the N378 AA, of which supposed that it was to change the redox potential of FAD in the enzyme, thus changing its functionality to resemble to cryptochromes. First, we had to construct the mutant proteins, for which we used a pPROEX plasmid, overlap extension mutagenesis PCR technique, TOP10 and BL21 E.coli strains.

Results and Discussion: With our measurements we could prove our expectations being correct, which means that the redox state of the FAD altered due to the given mutations to a state that is characteristic to cryptochromes.

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- Dr. Violetta Mohos
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- Anita Potočar
- Balint Kovacs
- Szabolcs Turnar
- Gergo Krizsan
- Andras Gergo Gardos
- Jonatán Pasitka

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This image shows a full page of a handwriting practice worksheet. It consists of numerous horizontal dashed lines spaced evenly across the page, providing a guide for letter height and placement. The background is plain white, and there are no other markings or text present.

