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Rīga, Latvia, 19–20 October 2017

MEETING ABSTRACTS

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Correspondence

Intrinsic Activity
c/o Institute for Experimental and Clinical Pharmacology
Medical University of Graz
Universitätsplatz 4
8010 Graz, Austria
Tel.: +43 (316) 380-4305
Fax: +43 (316) 380-9645
E-mail: info@intrinsicactivity.org

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Austrian Pharmacological Society
c/o Institute of Pharmacology
Centre for Physiology and Pharmacology
Medical University of Vienna
Währinger Straße 13a
1090 Wien, Austria
E-mail: office@aphar.at

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INTRINSIC ACTIVITY

2nd International Conference in Pharmacology: From Cellular Processes to Drug Targets

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MEETING ABSTRACTS

Speakers

A1.1

Can we put brakes on aging?

Inga KADISH[1,*], Thomas VAN GROEN[1], Amit PATKI[2], Tim R. NAGY[3] and David B. ALLISON[3]

[1]Department of Cell, Developmental and Integrative Biology, [2]Department of Biostatistics, [3]Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL, USA
**Corresponding author: Inga Kadish, Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, MCLM 656, 1918 University Blvd., Birmingham, AL 35294, USA. E-mail: ikadisha@uab.edu*

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The dramatic increase in average life expectancy has led to a rapid rise in aging population that has created a substantial burden in disease incidence and health-care costs. Age is an independent risk factor for several chronic diseases including cancer, type II diabetes, atherosclerosis, hypertension, stroke and neurodegenerative diseases. Caloric restriction (CR) is a long-established paradigm which extends longevity and delays development of age-related chronic diseases. CR works through multiple signaling pathways that regulate growth, metabolism, oxidative stress responses, inflammation, autophagy and proteostasis to modulate the aging process. However, it is not likely that humans will have the willpower to strictly adhere to CR regiment. Therefore, research has focused on finding CR mimetics that mimic the biochemical and physiological effects of CR without significantly reducing food intake. Ghrelin is a hunger-inducing gut peptide, and the interoceptive cues caused by ghrelin are likely similar to those produced by CR. In this long-term treatment study, we tested the novel hypothesis that a ghrelin agonist increases longevity, attenuates age-related behavioral and cognitive decline, and also changes energy metabolism and glucose tolerance in aged C57BL/6J mice and that these changes involve interoceptive cues, rather than reduced energy intake *per se*. Lifetime ghrelin agonist treatment increased longevity in pair-fed male mice, improved cognition at 12 and 24 months of age and improved glucose tolerance, without changing body composition. In female mice ghrelin agonist treatment improved glucose tolerance in ghrelin agonist treatment groups to compare with the control groups. There was significant ($p < 0.0001$) increase in energy efficiency in ghrelin-treated animals. Cognition was improved ($p < 0.04$) in ovariectomized (OVX) ghrelin-treated group to compare with OVX control, but did not significantly influence the cognitive outcome of 12-month-old intact mice. Insulin signaling was improved in hippocampus, and inflammation was attenuated in white matter and hypothalamus in ghrelin agonist-treated mice. In conclusion, "hunger" without caloric restriction has cognitive and glucose-handling benefits similar to caloric restriction, without the potential problem of weight loss in aging individuals.

Acknowledgements: Grant support: R01AG043972, P30NS47466, P30DK056336, P30DK079626.
Keywords: longevity – cognition – metabolism – caloric mimetics

A1.2

Treatment of Alzheimer's disease using small D-peptides

Thomas VAN GROEN[1,*], Inga KADISH[1], Nan JIANG[2] and Dieter WILLBOLD[2]

[1]Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, USA; [2]ICS-6, Forschungszentrum Jülich, Germany
**Corresponding author: Thomas van Groen, Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, MCLM 656, 1918 University Blvd., Birmingham, AL 35294, USA. E-mail: vangroen@uab.edu*

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For our research on a treatment for Alzheimer's disease we use transgenic mice that express two AD mutations, they develop the first amyloid β deposits at about five months of age, and the females develop cognitive deficits around 8 months of age (males at 10 months of age). We hypothesized that long-term treatments that interact with amyloid β(1–42) (Aβ42) would result in changes in amyloid deposition, and, likely, in the inflammatory reaction together resulting in improved cognition. In these studies we investigated the effects of small (12 aa), Aβ42-binding D-enantiomeric peptides on amyloid deposition, inflammation and cognition.
We use groups of AD Tg mice; the animals receive treatment for 1 month using Alzet minipumps, at the time point when cognition is declining (*i.e.*, at 7–9 months of age). At the end of the treatment period animals are tested for behavioral and cognitive changes. In the first studies we treated intracerebral, currently we use i.p. infusion as the method of choice.
Following behavioral analysis, the animals are sacrificed, the brain is cut in half, one half brain is cut in 35-µm sections, and these were stained with (1) amyloid β, (2) GFAP and CD11b. The density and size of labeling in the stained sections is quantified with densitometric analysis.

Edited by: Baiba Jansone (Latvian Society of Pharmacology, and Department of Pharmacology, Faculty of Medicine, University of Latvia, Rīga, Latvia; *baiba.jansone@lu.lv*)

Four-week treatments with an Aβ42-binding peptide (*i.e.*, D3) significantly improved cognitive functioning, and significantly reduced deposition of amyloid β in Tg mouse models of AD, and inflammation was significantly decreased around the amyloid deposits in the D3-treated animals compared to the control mouse groups. However, it should be noted, that the first D-peptide, D1, negatively impacted both pathology and cognition. On the other hand, RD2, and combinations of our D-peptides (*e.g.*, D3-D3, RD2-D3), in general, did not change amyloid β pathology but did improve cognition significantly.

Taken together, this suggests that the properties of Aβ42-binding D-enantiomeric peptides influence the changes in amyloid β pathology. Fibril-binding peptides such as D1 have no positive effects on the development of the pathology and cognitive deficits of AD, while most Aβ oligomer-oriented peptides positively affect cognition, but not plaque pathology.

Keywords: Alzheimer's disease – amyloid β – oligomers – D-enantiomeric peptides

A1.3

RGD as the putative neuronal activity centre of oligopeptide lunasin

Zane DZIRKALE, Ruta MUCENIECE, Ilva NAKURTE, Kaspars JEKABSONS, Baiba JANSONE, Juris RUMAKS, Jolanta PUPURE, Karina NARBUTE and Vija KLUSA*

Department of Pharmacology, Faculty of Medicine, Riga, University of Latvia

**Corresponding author: Vija Klusa, Department of Pharmacology, Faculty of Medicine, University of Latvia, 1 Jelgavas Str., LV-1004, Riga, Latvia. E-mail: vija.klusa@lu.lv*

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Lunasin, a 43 amino acid residues-containing peptide (SKWQHQQD SCRKQKQGVNLTPCEKHIMEKIQGRGDDDDDDDDD) isolated from different plants (mostly soybeans and cereals), has been described as an anti-cancer, anti-inflammatory, cholesterol-lowering and anti-oxidant agent [1]. We have shown for the first time that synthetic lunasin, administered intracisternally in mice, possesses central activity demonstrating anti-dopamine effects in behaviour tests, thus indicating its potential anti-schizophrenic action [2]. Taking into account that schizophrenia is directly related to impairments also in other neurotransmitter systems, at least such as serotonin (5-HT)- and glutamatergic, the present study was focused on examination why and how lunasin may influence these systems by use of specific receptor agonists or antagonists. Moreover, we compared lunasin effects with those induced by its short fragment 33–35 (RGD), which is considered as a cell adhesion motif [1].

In this study we examined peptide effects on locomotor activity after their intranasal administration in ICR mice. Afterwards, the content of neurotransmitters and their metabolites were assessed by ultra-high performance liquid chromatography time-of-flight mass spectrometry (UHPLC-TOF-MS) method in the whole mice brain.

The obtained data demonstrated: (1) intranasal lunasin (1 nmol/mouse) and RGD (1 and 10 nmol/mouse) caused similar anti-dopamine effects (reduction of amphetamine hyperactivity); (2) RGD (10 nmol/mouse) and lunasin (1 nmol/mouse) likewise decreased hyperlocomotion caused by the $5\text{-HT}_{2A/C}$ agonist DOI, whereas they did not change glutamate receptor antagonist PCP effects; (3) neurochemical assessment showed that both peptides in a similar manner decreased elevation in contents of DA, NA, 5-HT, 5-HIAA, HVA caused by amphetamine. Peptides also reduced DOI-induced elevation of 5-HT and 5-HIAA content, and increased NA level after DOI injection.

In conclusion, lunasin and its fragment RGD influence not only the dopamine system, but also serotoninergic pathways. The present data indicate that RGD may play a putative role as the active centre of lunasin central effects. These findings open new vistas in future studies of these peptides as novel anti-schizophrenic agents.

Keywords: lunasin – RGD – 5-HT receptors – DOI

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A1.4

The Guide to PHARMACOLOGY: a collaboration between the British Pharmacological Society (BPS) and the Nomenclature Committee of the Union of Basic and Clinical Pharmacology (NC-IUPHAR)

Stephen P. H. ALEXANDER[1,*], Arthur CHRISTOPOULOS[2], Anthony P. DAVENPORT[3], Jamie A. DAVIES[4], Doriano FABBRO[5], Elena FACCENDA[4], Simon D. HARDING[4], Adam J. PAWSON[4], Joanna L. SHARMAN[4], Christopher SOUTHAN[4] and Michael SPEDDING[6]

[1]School of Life Sciences, University of Nottingham Medical School, Nottingham, UK; [2]Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, Victoria, Australia; [3]Clinical Pharmacology Unit, University of Cambridge, UK; [4]Centre for Integrative Physiology, University of Edinburgh, UK; [5]PIQUR Therapeutics, Basel, Switzerland; [6]Spedding Research Solutions SARL, Le Vesinet, France

**Corresponding author: Stephen P. H. Alexander, School of Life Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, UK. E-mail: steve.alexander@nottingham.ac.uk*

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The IUPHAR/BPS Guide to PHARMACOLOGY [1] is an expert-curated, freely-available online database of human drug targets and their ligands. It provides focussed overviews, key references and pharmacological characterisation of 2,800 targets organised into the major classes of molecular targets. The database includes > 9000 distinct ligand molecules, including approved drugs, investigational small molecules, endogenous and synthetic peptides, and antibodies.

The content of the database is largely generated by more than 800 researchers worldwide, arranged in 90+ expert subcommittees of the Nomenclature Committee of IUPHAR (the International Union of Basic and Clinical Pharmacology). Frequent interactions between these subcommittees and the expert curators, based at the University of Edinburgh, ensures currency of the information presented. Biennial publication of an extract from the online GtoPdb in the *British Journal of Pharmacology* allows a snapshot of the major pharmacological targets. The Concise Guide to PHARMACOLOGY 2015/16 [2] presented, in tabular form, nine sections of G protein-coupled receptors, ligand-gated ion channels, voltage-gated ion channels, other ion channels, nuclear hormone receptors, catalytic receptors, enzymes, transporters and other proteins. The Concise Guide to PHARMACOLOGY 2017/18 is due for publication in the autumn of 2017. These publications present tabular versions of the online database for ready comparison of selective pharmacological tools (agonists, antagonists, modulators, channel blockers, substrates, inhibitors and labelled ligands), together with brief overviews and suggested further reading.

Funding from the British Pharmacological Society and the Wellcome Trust allows free availability of this resource. The Wellcome Trust

have also funded a recent expansion of the database into immunopharmacology [3]. Not only have we assembled expert subcommittees to provide data on the targets and ligands of immunopharmacological relevance, but we have increased the scope of the online database to include immunological cell types, processes and diseases.

All of the data presented in Guide to PHARMACOLOGY are also available for download or for accessing in machine-readable format to allow consumers working with other databases or resources more ready access to our content.

Acknowledgements: We are grateful for support from the British Pharmacological Society, IUPHAR, the University of Edinburgh and the Wellcome Trust.

Keywords: databases – receptors – ion channels – enzymes – transporters

Links and reference

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3. **IUPHAR/BPS Guite to IMMUNOPHARMACOLOGY:** www.guidetoimmunopharmacology.org (last accessed 04/10/2017)

A1.5

The contributions of Professor Modris Melzobs to the pharmacology of Latvia: commemorating his 90th anniversary

Staņislavs JANKOVSKIS[1,*], Antons SKUTELIS[2], Jānis BALTKĀJS[3] and Ardijs RANKS[2]

[1]*Faculty of Medicine, Rīga Stradiņš University, Riga, Latvia;*
[2]*Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia;*
[3]*Latvian Society of Pharmacology, Riga, Latvia*
**Corresponding author: Staņislavs Jankovskis, Faculty of Medicine, Rīga Stradiņš University, 16 Dzirciema Str., LV-1007, Riga, Latvia. E-mail: stanislavs.jankovskis@rsu.lv*

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Professor Modris Melzobs was born in 1927 in the homestead "Ieviņi" in Alūksne parish. He studied at the Primary School in Paideri, at the Cesvaine Gymnasium, and graduated from Alūksne Secondary School with excellence in 1947. In 1953, he graduated from the Riga Medical Institute (RMI). From 1953 to 1957, he was a post-graduate student at the RMI, and started his career as lecturer at the Department of Pharmacology of the RMI. From 1966 to 1994 M. Melzobs was Head of the Department of Pharmacology, received a degree of Doctor of Medical Science, and a degree as Habilitated Doctor of Medicine, and was elected Professor. While leading the Department, he managed to maintain the Latvian language in pharmacology. He wrote four textbooks and ten teaching materials in pharmacology in Latvian, and took care of the young generation of scientists. Under his supervision seven doctoral theses have been defended, and he was a reviewer of 20 doctoral theses.

Professor Melzobs is the author of 230 scientific publications as well as of five inventions. His scientific areas of interest include: studies of cholinergic and adrenergic processes, calcium antagonists and antioxidant compounds. He presented scientific results at pharmacological conferences in Paris, Helsinki, Prague, Warsaw, San Francisco, as well as in the congresses of the Baltic Republics and the USSR. He published 75 popular articles devoted to science. In addition, Prof. Melzobs was a member of the Editorial Board of the journal *Pharmacology and Toxicology* (Moscow, Russia), and a Member of the Board of the USSR Pharmacology Society. He was the first President of the Latvian Society of Pharmacology (from 1972 to 1994). From 1998 Prof. Melzobs was State emeritus scientist. He was the first Chair of the Drug Register Committee (from 1997 to 1999) at State Agency of Medicines of the Republic of Latvia.

Professor Modris Melzobs has been awarded medals of outstanding scientists, such as Kravkov, Schmiedeberg, Grindel. Prof. Melzobs was a colleague with outstanding intelligence, versatile knowledge, and a high culture of behavior. He has devoted 45 years of his life to pharmacology, nearly 30 he taught pharmacology to thousands of young Latvian physicians, dentists and pharmacists.

Keywords: Melzobs, Modris – pedagogical activities – scientific activities – administrative activities

A1.6

Forty-five years of the Latvian Society of Pharmacology

Baiba JANSONE* and Vija Zaiga KLUSA

Department of Pharmacology, Faculty of Medicine, Riga, University of Latvia
**Corresponding author: Baiba Jansone, Department of Pharmacology, Faculty of Medicine, University of Latvia, 1 Jelgavas Str., LV-1004, Riga, Latvia. E-mail: baiba.jansone@lu.lv*

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In 2017, the Latvian Society of Pharmacology (LSP) celebrates the 45th anniversary. The Society was established in 1972 and its first President was Professor Modris Melzobs (1972–1994). From 1994 till 2012, the Society was led by Professor Vija Klusa and since 2012 by Professor Baiba Jansone. The pharmacological community in Latvia unite specialists from various research institutes (University of Latvia, Latvian Institute of Organic Synthesis, Rīga Stradiņš University, Latvian Biomedical Research and Study Centre, JSC "Grindeks" and others) involving research scientists, academics, industry specialists and students of life sciences about 50 active members. The LSP organizes biannual meetings and international conferences to discuss recent achievements in pharmacology, awards Honorary Members and commemorates outstanding Latvian pharmacologists. This year the society will commemorate the first president Prof. Modris Melzobs remembering his 90th anniversary.

The LSP, in honor of Oswald Schmiedeberg (1838–1921), the founder of experimental pharmacology, who was born in Latvia (near Talsi) and whose grandparents were Latvians, has established the Oswald Schmiedeberg's medal in 1998, which is awarded to Honorary Members of the Society. They received this medal for the important contributions in the field of pharmacology in Latvia, facilitating the development of the education and research in pharmacology.

Today, pharmacology in Latvia has evolved into among the leading scientific disciplines and is recognized internationally. The main scientific areas of pharmacology include molecular pharmacology, neuropharmacology, drug discovery, particularly search for novel compounds capable to halt neurodegenerative, cardiovascular and endocrine diseases, the role of mitochondria and animal models in various diseases. Latvian pharmacologists have a fruitful collaboration with specialists of Estonia, Lithuania, USA, Norway and other countries. More information about recent research achievements in pharmacology in Latvia is published in the special issue entitled 'Countries in focus: Pharmacology in the Baltic States' in *Pharmacological Research*, 2016 [1].

Since 1997, the Society has affiliated with the Latvian Academy of Sciences. In 1994, the LSP has joined the IUPHAR and from 2010 the Federation of European Pharmacological Societies (EPHAR).

Acknowledgements: To contribution of the members and Honorary members of the Latvian Society of Pharmacology in the development of Pharmacology in Latvia.

Keywords: Latvian Society of Pharmacology – Oswald Schmiedeberg's medal – 45th anniversary – neuropharmacology – drug discovery

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A1.7

Monitoring of ligand binding to receptors using fluorescence anisotropy-based assay

Ago RINKEN*

Institute of Chemistry, University of Tartu, Estonia
**Corresponding author: Ago Rinken, Institute of Chemistry, University of Tartu, Ravila 14a, EE-50411, Tartu, Estonia.*
E-mail: ago.rinken@ut.ee; website: http://gpcr.ut.ee/

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G protein-coupled receptors (GPCRs) transduce signals into cells via guanosine nucleotide binding regulatory proteins (G proteins). As GPCRs respond to different stimuli and modulate various signal transduction pathways inside the cells, they have become important targets for treatment of many diseases. The ligand binding to the receptor is the first, but often also most crucial step in the signal transduction pathway. During the last decade, in addition to conventional radioligand binding, several fluorescence-based methods have been implemented for characterization of ligand binding to GPCRs. We have implemented fluorescence anisotropy-based assay, which allows on-line monitoring of ligand binding and obtain valuable kinetic data. The ratiometric nature of the assay requires high concentrations of receptors, which we could achieve with implementation of budded baculovirus particles, which display GPCRs on their surfaces [1]. Interpretation of the kinetic results of the non-pseudo first-order reactions is also more demanding and requires special attention. Simple one-step binding schemes and competitive reactions can be solved with analytical algorithms with several simplifications [2], while in most cases integrative global analysis is required to achieve physically meaningful kinetic parameters. We have already achieved working fluorescence anisotropy/baculovirus kinetic assays for monitoring of ligand binding to melanocortin (MC_4R) [3], neuropeptide Y (NPY_1R), serotonin ($5\text{-}HT_{1A}R$) [4], dopamine (D_1DAR) and muscarinic (M_2mAChR) receptors.

Acknowledgements: The work was supported by Estonian Research Council grant IUT20-17.

Keywords: fluorescence anisotropy – budded baculoviruses – ligand binding – G protein-coupled receptors – kinetics

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A1.8

Developing new therapies for rare Batten disease

Maija DAMBROVA[1,2,*] and Valerjans KAUSS[3]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia; [3]Laboratory of CNS Active Compounds, Latvian Institute of Organic Synthesis, Riga, Latvia
**Corresponding author: Maija Dambrova, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.*
E-mail: maija.dambrova@farm.osi.lv

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The neuronal ceroid lipofuscinoses (NCLs), also referred to as Batten disease, denote fatal genetic lysosomal storage disorders that are the most common of the rare neurodegenerative childhood diseases, affecting approximately 14,000 world-wide. It is a devastating and severely debilitating group of genetic diseases. There are no curative treatments yet offered in the clinic for any type of NCL anywhere in the world. The EU-funded Horizon 2020 consortium BATCure aims to develop the first effective treatments for three genetically distinct NCL subtypes caused by mutations in intracellular transmembrane proteins. The goal of BATCure is to provide and test novel therapeutic leads, and to increase our understanding of the underlying biochemical and molecular basis of Batten disease, to use this knowledge to design new therapeutic options and develop new tools for diagnostics and the monitoring of treatments. BATCure partners are driving concerted activities to create new models, tools and technologies for developing and testing therapies, further delineate disease biology and gene function to identify new therapeutic target pathways, facilitate effective evaluation of preclinical therapies and improve diagnostics, extend a comprehensive natural history beyond the brain to include metabolic changes, identify new and repurpose existing small-molecule therapy, triage new compound treatments in zebrafish and mouse models. The Latvian Institute of Organic Synthesis will be involved in the medicinal chemistry and innovative drug discovery BATCure activities aiming to improve ADME/tox profile and brain bioavailability of active compounds, as well as participate in the preclinical drug development. In addition to novel compounds, also hits from FDA collection of 1,500 compounds, after validation in secondary assays and *in vivo*, could be immediately tested in patients to allow repositioning of known drugs as correctors of Batten disease. BATCure provides a mechanism to involve patients and their families to inform and fully contribute to therapy development and prepare for clinical trials. By the end of the project, BATCure expects to have a lead therapy ready for a clinical trial and developed faster diagnostics suitable for pre-symptomatic testing and monitoring efficacy of new treatments.

Acknowledgements: BATCure consortium is funded under the EU Horizon 2020 research innovation programme under grant agreement 666918.

Keywords: neuronal ceroid lipofuscinoses – rare disease – Batten disease – drug discovery and development

A1.9

Oncolytic cancer virotherapy with Rigvir®

Pēteris ALBERTS* and Andra TILGASE

Research and Development, International Virotherapy Center, Riga, Latvia

**Corresponding author: Pēteris Alberts, Research and Development, International Virotherapy Center, LV-1050, Riga, Latvia. E-mail: peteris@virotherapy.eu*

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An oncolytic, non-pathogenic ECHO-7 virus, adapted and selected for melanoma that has not been genetically modified (Rigvir®) was registered in 2004 in Latvia for melanoma therapy. Here, we describe two retrospective post-marketing studies. In the first study, Caucasian patients (n = 79) who had surgical excision of the primary melanoma tumour and diagnosis verified histologically were included; all were free of disease after surgery [1]. Survival was analysed by multivariate Cox regression. Current international guidelines advise no strict treatment for stage I–II melanoma patients; thus, treatment with Rigvir® was offered. 52 patients received Rigvir® and 27 were observed. The study was approved by the respective ethics committee. Rigvir® significantly prolongs survival in sub-stage IB–IIC melanoma patients following surgery compared to patients who are under observation. The hazard ratio for patients under observation *vs.* treated with Rigvir® is statistically significantly different: 6.27 for all, 4.39 for sub-stage IIA–IIB–IIC, and 6.57 for sub-stage IIB–IIC patients. Safety assessment of adverse events graded per NCI CTCAE did not show any value above grade 2 in Rigvir®-treated patients. These findings are supported and extended by the following 3 case reports: a patient diagnosed with melanoma stage IV M1c, a small cell lung cancer stage IIIA, and a histiocytic sarcoma stage IV [2]. All patients started Rigvir® treatment within a few months after diagnosis. The degree of regression of the disease was determined by CT. Safety assessment of adverse events graded per NCI CTCAE showed no value above grade 1 during Rigvir® treatment. Using current standard treatments, the survival of the 3 patients described is low. In contrast, the patients described here were diagnosed 4.5, 8.0 and 7.6 years ago, and their condition has improved and been stabile for over 2.5, 7.5, and 5 years, respectively. Taken together these results suggest that the mortality of sub-stage IB, IIA, IIB, and IIC melanoma patients treated with Rigvir® was 4.39–6.57 times less than for those under observation, and that Rigvir® can successfully be used in long-term treatment of patients with melanoma stage IV M1c, small cell lung cancer stage IIIA, and histiocytic sarcoma stage IV.

Keywords: Echovirus 7 – immunotherapy – melanoma – oncolytic virotherapy – Rigvir®

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A1.10

The impact of cancer extracellular vesicles on mesenchymal stem cell phenotype

Una RIEKSTINA[1,*], Aija LINĒ[2] and Ruta MUCENIECE[1]

[1]*Pharmacy Study Program, Faculty of Medicine, University of Latvia, Riga, Latvia;* [2]*Latvian Biomedical Research and Study Centre, Riga, Latvia*

**Corresponding author: Una Riekstina, Pharmacy study program, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: una.riekstina@lu.lv*

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Mesenchymal stem/stromal cells (MSCs) are versatile adult stem cells with multilineage differentiation potential that reside in adult organ stroma. MSCs are adherent cells with a spindle-like morphology, which express CD29, CD44, CD73, CD90, and CD105 cell surface markers and lack expression of hematopoietic markers CD45 and HLA-DR [1]. Importantly, MSCs are active producers of paracrine factors that may play an important role in the modulation of inflammatory conditions. Tumor microenvironment (TME) could be regarded as a site of chronic inflammation, and currently the MSC role in the TME is under debate. A considerable evidence demonstrates that MSCs may polarize into two subsets: a tumor-suppressing type of MSCs and tumor-supporting type, ***e.g.*** carcinoma-associated fibroblasts (CAF) [2].

Extracellular vesicles (EVs) secreted by malignant cells are communication tools between cells in TME. Cancer cell-derived EVs may contribute to the cancer metastasis by modulating the MSC functions in the TME [3].

In the present study, we have examined the uptake of primary colorectal cancer (CRC) SW480 and metastatic CRC SW620 cell line-derived EVs in the MSCs and the effect of tumor EV on MSC phenotype and paracrine functions.

CRC EVs were labelled with RNA-specific SytoRNA select dye and uptake was examined by confocal microscopy. Cell surface marker expression was analyzed by flow cytometry, CAF differentiation was assessed by quantitative PCR analysis, secretory factors were assessed by ELISA and Luminex assays.

Our results show that CRC EVs are taken up in MSCs and co-localized with endoplasmatic reticulum in the cell cytoplasm. EVs had no effect on MSC cell surface marker expression whereas the expression of TERT, FAP and α-SMA markers was increased indicating a shift towards CAF phenotype. Additionally, the expression of several inflammatory chemokines and cytokines (***e.g.*** IL-8, VEGF) was induced after MSC exposure to CRC EVs.

We conclude that CRC EVs may serve as a tool to modulate MSC phenotype and paracrine functions within TME.

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Keywords: mesenchymal stem cells – cancer – extracellular vesicles

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A1.11

Cannabinoids: complexity at every level on the path to therapeutic exploitation – plant cannabinoids, endocannabinoids, target receptors, transforming enzymes

Stephen P.H. ALEXANDER*

School of Life Sciences, University of Nottingham Medical School, Nottingham, UK

**Corresponding author: Stephen P.H. Alexander, School of Life Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, UK. E-mail: steve.alexander@nottingham.ac.uk*

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The *Cannabis* plant is amongst the oldest cultivated plants; exploited for its fibre and for the unique metabolites it contains. Despite the centuries of contact, understanding of the nature of the unique metabolites (which may number up to 100) present in the plant is very much more recent. The two best studied are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is a tricyclic component responsible for the psychoactivity associated with consuming preparations from the *Cannabis* plant, while CBD is a related bicyclic structure with promise as an anti-epileptic. The synthetic pathway in the *Cannabis* plant involves carboxylated versions of these agents, with evidence for extensive variation in content dependent on genetics, geography, cultivation method, anatomical region, harvesting and storage.

The molecular targets of THC in human are two G protein-coupled receptors, CB_1 and CB_2, which are activated by two families of fatty-acid derivatives, the most well-understood of which are anandamide (*N*-arachidonoylethanolamine) and 2-arachidonoylglycerol. Although these are both eicosanoid derivatives, they are synthesised and hydrolysed by independent routes, and have been reported to act at numerous molecular targets beyond CB_1 and CB_2 cannabinoid receptors. Both anandamide and 2-arachidonoylglycerol have been identified to be hydrolysed by multiple hydrolases, as well as being subject to oxidation by cyclooxygenase, lipoxygenase and cytochrome P450 routes. Furthermore, the endocannabinoids are capable of activating ligand-gated ion channels, such as the TRPV1 vanilloid receptor, and nuclear hormone receptors, such as the peroxisome proliferator-activated receptors. The enzymes which hydrolyse and transform these endocannabinoids also have a range of endogenous metabolites which act as substrates. These further substrates (and products) are biologically active in their own right, many with profiles extending beyond the receptors, channels and enzymes mentioned above.

The challenge for the next phase of cannabinoid research is to acknowledge and exploit this complexity to generate therapeutically useful agents from the many selective pharmacological tools currently available. An additional obstacle for cannabinoid research is the growing number of countries and states permitting medicinal and/or recreational *Cannabis*. Dealing with issues like quality control and delivery of the preparations of *Cannabis* to minimise harm and maximise 'benefit' will not be trivial.

Keywords: *Cannabis* – cannabinoids – G protein-coupled receptors – hydrolytic enzymes – ion channels

A1.12

How, why, and when to train the pharmacological targets for prevention of "second brain" injuries?

Shyam Sunder CHATTERJEE*

Retired pharmacologist; Pharmacology Research Laboratories, Dr. Willmar Schwabe GmbH & Co., Karlsruhe, Germany

**Current corresponding address: Shyam Sunder Chatterjee, Stettiner Straße 1, D-76139 Karlsruhe, Germany.*
E-mail: shyam.chatterjee@web.de

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Injuries and/or malfunctioning of the enteric nervous system, often referred to as "the second brain", leads to metabolic disorders and malnutrition- (both under- and over-nutrition)-associated physical and mental health problems. Although numerous pharmacological targets regulating the functions of the second brain and their functional modulators are now known, prevention and cure of malnutrition-triggered, or -associated, diseases and their syndromes still continue to be a major challenge for all systems of medicine, modern or alternative or complementary or not. Observations made during efforts to precondition a few well-known pharmacological targets involved in physiological functions of the second brain led us to identify several multi-targeted phytochemicals, fairly low daily oral doses of which afford protection against diverse spectrums of psychopathologies associated with, or caused by, malnutrition-triggered diseases and their symptoms. Food phytochemicals and most other pharmacological tools used during these efforts are the ones identified during efforts to decipher several paradoxical, or not yet easily explainable, observations made in Riga and elsewhere with several drugs leads from traditionally known medicinal plants identified during the second half of the past millennium in our research groups as therapeutic options for treatments of cognitive disorders [1]. In this presentation, potential uses of some cost- and time-saving and more predictive rodent bioassays now often used in our research groups for better understanding of quantitative systems pharmacology of bioactive substance necessary for judging their therapeutic potentials, or for drug discovery and development purposes, will be pointed out.

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Keywords: quantitative systems pharmacology – phytochemicals – preconditioning – allostatic load – bioassays *in vivo*

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A1.13

Traumatic brain injury-induced cognitive decline: mechanisms and treatment options

Liga ZVEJNIECE[1,*], Edijs VAVERS[1], Baiba SVALBE[1], Lasma LAUBERTE[1], Gundega VIKMANE[1] and Maija DAMBROVA[1,2]

[1]Laboraory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Liga Zvejniece, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.*
E-mail: liga@farm.osi.lv

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Traumatic brain injury (TBI) is the leading cause of disability and death in Europe among young adults and children and an increasing problem in the elderly. In the past decades, tremendous clinical and pre-clinical efforts were undertaken to understand the acute, life-threatening pathophysiological events occurring acutely after TBI. The first aim was to develop therapeutic procedures able to reduce the formation of brain edema, intracranial hypertension, cerebral

ischemia, and secondary loss of brain tissue in order to save the patient's live. In the past few years, it became more recognized that also weeks, months or even years after the initial event additional morbidity may occur, which significantly contributes to the overall burden of head injury. These long-lasting sequels of TBI range from sleep disorders, fatigue syndrome, depression, neuro-endocrine deficits, epilepsy, and psychiatric disorders to chronic neurodegeneration leading to parkinsonism, cognitive decline and dementia. Across all levels of TBI severity, attention, processing speed, episodic memory, and executive function are the most commonly affected. Data from the few available human studies suggest that chronic TBI is associated with brain atrophy and long-lasting neuroinflammation for up to 18 years after TBI. These findings were corroborated by longitudinal experimental studies in mice and rats showing progressive brain atrophy after TBI for up to one year after injury and long-lasting activation of microglia, and invaded monocyte invasion from 24 hours up to 1.5 years after the experimental TBI. The important role for neuroinflammation in TBI is further supported by a reduced lesion volume and improved behavioral outcome upon neutralization of IL-1β, a pro-inflammatory cytokine which may lead to changes on the synaptic level. The paradox of neuroprotection in TBI is that, despite a long list of potential neuroprotective agents active under experimental conditions, no compound has demonstrated protection in clinical trials. The ultimate aim of the CnsAflame project is to determine the underlying causes of chronic TBI to facilitate the development of an effective cure and run a multi centre preclinical trial of a drug candidate.

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Keywords: traumatic brain injury – drug discovery and development – pharmacotherapy

A1.14

Pharmacological modulation of serum bilirubin levels: can we achieve a neuroprotective action?

Mojca KRŽAN* and Lovro ŽIBERNA

Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana, Slovenia

**Corresponding author: Mojca Kržan, Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, University of Ljubljana, Slovenia. E-mail: mojca.limpel@mf.uni-lj.si*

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Bilirubin is an endogenous antioxidant with anti-inflammatory and anti-thrombotic activity, and is inversely correlated with risk of the outbreak of different diseases of the cardiovascular system, such as ischemic heart disease, hypertension, diabetes type II, metabolic syndrome, obesity. Bilirubin is present in various chemical forms in the blood, namely, conjugated with glucuronic acid (direct bilirubin), unconjugated bound to serum albumin (indirect bilirubin) and unconjugated-unbound (free bilirubin). Approximately 85% of the total bilirubin produced is derived from the heme moiety of hemoglobin, while the remaining 15% is produced from the red blood cell precursors. Bilirubin is normally rapidly taken up by hepatocytes where it is conjugated with glucuronic acid and thus becoming inactive but suitable for excretion. Non-conjugated serum bilirubin is 99% albumin bound. The only bioactive form is the free bilirubin, which is not measured routinely in the clinical setting, but has been recently identified to be around 10 nM in serum. Importantly, nanomolar concentrations of bilirubin can protect cells from the 10,000-fold molar excess of oxidants when both substances are added to cell cultures (*in vitro* conditions). This remarkable effect has been explained that bilirubin is acting as antioxidant, is itself oxidized to biliverdin, and then recycled by biliverdin reductase back to bilirubin. We hypothesize that modulation of serum bilirubin values is possible by pharmacological interventions acting on (i) increasing bilirubin synthesis (biliverdin reductase [BVR] induction, heme-oxygenase-1 [HO-1] induction); (ii) decreasing bilirubin metabolism (hepatic UDP-glucuronosyltransferase [UGT1A1] inhibition); (iii) decreasing bilirubin elimination (organic anion transporters [OATP] and bilitranslocase [BTL] inhibition); or (iv) by displacing it from albumin (drug interaction with bilirubin–albumin complex in blood serum). Since oxidative stress and inflammation are important pathophysiological factors in neurodegenerative diseases, the modulation of endogenous antioxidants can be a vital therapeutic approach in scavenging excess ROS, thereby preventing neuronal degeneration in a post-oxidative stress scenario. Paradoxically, high levels of serum bilirubin (>300 µmol/l) have neurotoxic effects. To exploit the novel idea of using bilirubin as endogenous neuroprotective mediator, our research group will investigate concentration-dependent effects of bilirubin on astrocytes, neurons, and microglia, as well as in whole animals in both healthy and increased oxidative stress conditions.

Keywords: bilirubin – antioxidant – neuroprotection

A1.15

Extracellular vesicles as a potential novel therapeutic tools against neurodegenerative diseases

Augustas PIVORŪNAS*

Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

**Corresponding author: Augustas Pivorūnas, Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, LT-08406, Vilnius, Lithuania.*

E-mail: augustas.pivoriunas@imcentras.lt

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We and others have demonstrated that extracellular vesicles (EVs) display neuroprotective and anti-inflammatory properties. Increasing evidence suggests that neuroinflammation has a causal role in the pathogenesis of chronic neurodegenerative diseases, therefore, new therapies directed against neuroinflammatory processes may be beneficial. In the present study we investigated the effects of EVs derived from human dental pulp stem cells (DPSCs) on migration and phagocytic activity of human microglial cells. To determine phagocytic activity of immortalized human microglial cells (purchased from ABM) we used apoptotic bodies (AB) derived from ReNcell VM human neural stem cells (Millipore). ABs were prepared and labeled according to the described protocol, with some modifications [1]. The microglial polarization into M1 and M2 states was induced according to the protocol described by Gaikwad and Heneka [2]. EVs were purified by differential ultracentrifugation from DPSCs grown in serum- and xeno-free medium. Control and EV-treated M0, M1 and M2 cells were stained with CellTrace calcein green AM (Thermo Fisher Scientific) and incubated with ABs at a ratio 3:1 for 2 hours. Digital images of randomly selected fields were captured by confocal microscope (Leica SP8) and phagocytic activity calculated according to the following formula: number of microglial cells containing engulfed ABs / total number of counted cells × 100. Internalization of phagocytosed material was verified by using Z stacks acquired through confocal microscopy. For wound-healing assays we used 2-well silicone inserts (Ibidi) and Leica SP8 live-cell imaging system. Time-lapse microscopy revealed that EVs significantly promoted migration of unpolarized M0 cells. We also detected increased phagocytic activity of M1 and M2 microglial cells (by 40% when compared with M0 cells). Importantly, EV treatment increased phagocytic activity of M0 and M2 cells by 46% and 17%, respectively. By contrast, EVs did not affect phagocytic activity in M1 cells.

Our findings demonstrate that EVs derived from human dental pulp stem cells can act as potent immunomodulators of human microglial cells.

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Keywords: extracellular vesicles – neuroinflammation – human microglial cells – human dental pulp stem cells

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A1.16

Neuroprotective action of gammapyrone, a GABA-containing peptide-mimicking compound

Vladimirs PILIPENKO[1,*], Karina NARBUTE[1], Ulrika BEITNERE[1], Jolanta PUPURE[1], Juris RUMAKS[1], Baiba JANSONE[1], Edijs VAVERS[2], Janis POIKANS[2], Egils BISENIEKS[2] and Vija KLUSA[1]

[1]Department of Pharmacology, Faculty of Medicine, University of Latvia, Riga, Latvia; [2]Latvian Institute of Organic Synthesis, Riga, Latvia

**Corresponding author: Vladimirs Pilipenko, Department of Pharmacology, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: vladimirs.pilipenko@lu.lv*

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A decrease in gamma-aminobutyric acid (GABA) system inhibitory activity is closely associated with the progression of Alzheimer's disease (AD) [1]. Deficiency in GABAergic signaling potentiates neuroinflammation, early hallmark of AD [2]. Gammapyrone (GMP) is a 1,4-dihydropyridine (DHP) compound containing one "free" and one "crypto" (incorporated into DHP cycle) GABA molecule joined via a peptide bond, thus creating a peptide-mimicking compound [3]. GMP, an atypical DHP, does not block neuronal calcium channels, but in a dose of 0.05 mg/kg demonstrated memory facilitation in naïve rats exposed to conditioned avoidance response test [4]. We suggested that in early AD-type model rats GMP may show spatial memory facilitation and normalize cell neurotransmitter balance.

We determined binding affinity of GMP for $GABA_{A1}$ (α1β2γ2) and $GABA_B$ receptors by using [^{3}H]muscimol and [^{3}H]CGP 54626, respectively. AD-type model rats (280 ± 20 g) were obtained by intracerebroventricular injection of streptozotocin (STZ, 750 µg/10 µl). All animals were treated intraperitoneally with saline (control) or GMP (0.01 and 0.05 mg/kg). Two weeks after STZ administration, locomotor activity and spatial learning/memory performance were assessed in the open field and water maze tests, respectively. Cortical and hippocampal expression was assayed immunohistochemically for astrocyte marker glial fibrillary acidic protein (GFAP), neuronal survival marker calbindin (CB), GABA-synthesizing glutamate decarboxylase-67 (GAD67) and acetylcholine-cleaving acetylcholinesterase (AChE). The expression of dopamine-synthesizing tyrosine hydroxylase (TH) was detected in the substantia nigra.

GMP demonstrated very low binding affinity for both $GABA_A$ and $GABA_B$ receptors. STZ administration produced deficits in spatial learning/memory in the water maze test, not influencing locomotion. STZ also induced remarkable neuroinflammation (astrogliosis), increase in AChE expression and slight decrease in GAD67 expression.

Both doses in STZ-treated rats significantly improved spatial learning/memory, protected against neuroinflammation, decreased acetylcholine cleavage. In the smallest dose GMP normalized cortical GAD67 expression while it did not bind to GABA receptors. CB and TH expression was not influenced by either STZ or GMP.

We conclude that the GABA-containing compound GMP provides neuroprotection in early AD-type model rats by targeting neuroinflammation, reducing acetylcholine cleavage, and cortical GABA, while not binding to GABA receptors. One may suggest that GMP action is provided via non-specific allosteric mechanisms. The neuroprotective activity of GMP indicates its putative usefulness in early stages of AD also in human beings.

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Keywords: GABA – gammapyrone (GMP) – streptozotocin – memory – neuroinflammation

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A1.17

Claudins and claudin mimetics: tight junction proteins in normal and ischemic blood–brain barrier

Ingolf E. BLASIG[1,*] Olga BREITKREUZ-KORFF[1], Lars WINKLER[1], Sophie DITHMER[1], Philipp BERNDT[1], Andre REX[2], Memet KAYA[3], Stephan LIEBNER[4], Hartwig WOLBURG[5] and Rosel BLASIG[1]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin-Buch, Germany; [2]Charité Berlin, Germany; [3]University of Istanbul, Turkey; [4]University of Frankfurt, Germany; [5]University of Tübingen, Germany

**Corresponding author: Ingolf Blasig, Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Str. 10, D-13125 Berlin, Germany. E-mail: iblasig@fmp-berlin.de*

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The blood–brain barrier (BBB) limits drug delivery to the brain. BBB's role in stroke and BBB modulation are not understood. The BBB-forming endothelium, paracellularly sealed by tight junction (TJ) proteins, ensures brain homeostasis and metabolite exchange. As far as known, claudin-5 (Cldn5) dominates the TJs and the paracellular tightening of the BBB. Contribution of other TJ proteins is unclear. We therefore elucidated the structure and function of TJs upon stroke and administration of claudin mimetics. Mice were analysed for BBB permeability (small/large molecules), expression (mRNA/protein) and morphology of the TJs *in vitro* after hypoxia (cell models, capillaries) and *in vivo* without and after transient middle cerebral artery occludion (MRI, electron microscopy, immunohistochemistry, BBB opening, infarct/oedema area). To manipulate cerebral TJs, claudin mimetics (peptides, small molecules) were designed/screened. We discovered that Cldn3 tightened the BBB for small molecules, limited endothelial endocytosis and transcytosis of

proteins, complemented TJ morphology, prevented inflammatory processes, and regulated TJ proteins (Cldn1, Cldn5, occludin). Acute hypoxia of isolated mouse brain capillaries did not affect the BBB-specific TJ marker Cldn5 in presence of Cldn3. In Cldn3 deficiency, Cldn5 declined at the TJs. In postischemic infarction, Cldn3 accounted for increased infarct volume due to increased swelling of the affected brain. The claudin modulators increased permeability through cell-culture models of cerebral barriers (bEnd, MDCK-Cldn5) and through the BBB of intact mice. Cldns 5, 3 and 1 contribute to the intactness of the BBB under physiological and pathological conditions, protect the BBB in stroke but prevent detumescence of the injured area, hence worsening infarct outcome. Thus, modulation of Cldns paracellularly tightening the BBB might help to improve stroke recovery as well as cerebral drug delivery.

Keywords: blood–brain barrier – cell contacts – tight junction proteins – drug delivery – stroke

A1.18

Aβ oligomer-eliminating D-enantiomeric peptides enhance cognition and impede neurodegeneration even by oral application

Dieter WILLBOLD[1,2,*]

[1]Institut für Physikalische Biologie, Heinrich-Heine-Universität, Düsseldorf, Germany; [2]Institute of Complex Systems, ICS-6: Structural Biochemistry, Research Centre Jülich, Germany

*Corresponding author: *Dieter Willbold, Institut für Physikalische Biologie, Heinrich-Heine-Universität, Düsseldorf, Germany; and Institute of Complex Systems, ICS-6: Structural Biochemistry, Research Centre Jülich, Germany. E-mail: dieter.willbold@hhu.de*

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Several lines of evidence suggest a central role of amyloid β peptide (Aβ) in the pathogenesis of Alzheimer's disease (AD). More than Aβ fibrils, small soluble and prion-like propagating Aβ oligomers are suspected to be the major toxic species responsible for disease development and progression. Therefore, eradication of these Aβ oligomers is our principal objective for therapy of AD. Previously, we have identified the fully D-enantiomeric peptide D3 by mirror-image phage display selection and showed that it was able to specifically eliminate Aβ oligomers and convert them into non-toxic species. D3 was able to reduce plaque load in transgenic AD mouse models and improved cognition even after oral application [1]. More recently, we developed derivatives of D3 with improved properties during a lead optimization strategy that focused primarily on the Aβ oligomer elimination efficiency.

We used our newly developed Aβ-QIAD (quantitative determination of interference with Aβ aggregate size distribution) to quantitatively measure Aβ oligomer elimination efficiency and thus target engagement [2]. Morris water maze and novel object recognition experiments in several transgenic mouse models were used to measure cognition enhancement of the compounds. Our most promising D3 derivative was able to enhance cognition and learning behavior even in 18-month-old transgenic AD mice with full-blown pathology even after oral application. SHIRPA and Rotarod assays were used to follow neurodegeneration in the TBA2.1 mouse model and its retardation by our compounds. Using HPLC and LC/MS, we investigated the stability of the compounds under various conditions. As expected from D-peptides, D3 and its derivatives showed superior pharmacokinetic properties, such as long half-lives and high oral bioavailability [3,4]. The presented compounds were able to eliminate Aβ oligomers as well as to enhance cognition and slow down neurodegeneration even after oral application.

D-enantiomeric peptides that specifically and efficiently eliminate Aβ oligomers are able to enhance cognition and impede neurodegeneration even when applied orally.

Keywords: amyloid β oligomers – D-enantiomeric peptides – oligomer elimination – therapy

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Posters

A2.1

Development of theranostic nanosystems on the base of amphiphilic 1,4-dihydropyridines with styrylpyridinium moieties

Klavs PAJUSTE[1,2,*], Martins RUCINS[1], Ludmila JACKEVICA[1], Iveta KANEPE-LAPSA[1], Mara PLOTNIECE[1,3], Dace TIRZITE[1], Ruta MUCENIECE[2], Arkadij SOBOLEV[1], Ilona DOMRACHEVA[1], Karlis PAJUSTE[1] and Aiva PLOTNIECE[1]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Medicine, University of Latvia, Riga, Latvia; [3]Department of Pharmaceutical Chemistry, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Klavs Pajuste, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.*
E-mail: klap@inbox.lv

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Background: The combination of diagnostic and therapeutic properties into the same agent for theranostic purposes now is focussing more and more on multifunctional nanomaterials giving "theranostic nanoparticles" [1]. Pyridinium amphiphiles on 1,4-dihydropyridine (1,4-DHP) scaffold having self-assembling properties, capable to form liposomes can be used for DNA delivery [2, 3] or in perspective for drug delivery.

Objectives: The aim of this work is the development of new nanosystems forming molecules which includes: (1) synthesis of new cationic moieties containing amphiphiles; (2) evaluation of biological activities: cytotoxicity, compound-generated reactive oxygen species (ROS) activity and ability to inhibit the calcium channels.

Methods: Cationic 1,4-DHPs were synthesized according to Pajuste *et al.* [3]. Cytotoxicity of 1,4-DHPs *in vitro* was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on two monolayer tumour cell lines—HT-1080 and MH-22A in

comparison with their action on normal mouse fibroblasts. The Neutral red uptake (NRU) assay was performed on 3T3 cells according to Stokes *et al.* [4]. Data from the *in vitro* tests was used for estimation of the starting dose for acute oral toxicity (LD_{50}) tests in rodents. Effects of total reactive oxygen species production after tested compound exposure in the HT-1080 cell line using CM-H_2DCFDA as a fluorescent probe. Ca^{2+} channel antagonist/agonist activities were assayed by changes of intracellular Ca^{2+} concentrations in A7R5 aorta smooth muscle cells.

Results: This type of compounds is interesting due to self-assembling properties and 1,4-dihydropyridine cycle because the 1,4-DHP moiety acts as an active linker. Establishing structure–activity relationships is a promising tool for design and development of putative theranostic agents. We have synthesized more than thirty pyridinium amphiphiles by targeted modification of 1,4-DHP substituents at 3,5-positions, phenyl substituent at the position 4 and 2,6-pyridinium moieties; compared their cytotoxicities, estimated LD_{50}, ability to inhibit the calcium channels, radical scavenging activity. For all synthesized amphiphiles, evaluation of fluourescence was performed. All fluorescence spectra were recorded with excitation at 410 nm and emission between 400 and 760 nm. All spectra were acquired under identical conditions. Only eight compounds possessed significant fluorescence.

Conclusions: The obtained data confirmed that properties of the synthesized compounds were dependent on the positions and nature of the substituents.

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Keywords: amphiphilic 1,4-dihydropyridines – styrylpyridinium moiety – cytotoxicity – generated ROS activity – Ca^{2+} channel antagonist/agonist

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A2.2

Solution structure of lunasin peptide

Rihards Aleksis[1,*], Kristaps Jaudzems[1], Ruta Muceniece[2] and Edvards Liepinsh[1]

[1]*Latvian Institute of Organic Synthesis, Riga, Latvia;* [2]*Faculty of Medicine, University of Latvia, Riga, Latvia*

**Corresponding author: Rihards Aleksis, Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga LV-1006, Latvia.*

E-mail: aleksis@osi.lv

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Background: Lunasin is a 43 amino-acid peptide with anti-cancer, antioxidant, anti-inflammatory and cholesterol-lowering properties. The amino acid sequence of the peptide contains nine aspartic acid residues (at the C-terminal end), an Arg-Gly-Asp cell adhesion motif and a predicted helix spanning residues Glu^{23}–Ile^{30} with homology to a conserved region in chromatin-binding proteins. It has been established that the poly-aspartyl region is the main active sequence responsible for colocalization with hypoacetylated chromatin and for inhibition of core histone acetylation. The anti-cancer activity is most likely mediated through inhibition of histone H3 and H4 acetylation [1–3]. Although the mechanism of action of lunasin has been characterized to some extent, its exact three-dimensional structure remains unknown.

Objectives: Develop a novel recombinant lunasin production method and determine the peptides structure in solution.

Methods: Bacterial expression and chromatographic methods were used to produce and purify lunasin. The peptide was characterized by NMR, CD and MALDI-TOF mass spectrometry.

Results: Our study reveals that lunasin can exist in a reduced or oxidized state with an intramolecular disulfide bond depending on solution conditions. Analysis of $^{13}C_{\alpha,\beta}$ secondary chemical shifts indicates that sequence regions Trp^3–Lys^{12} and Pro^{21}–Ile^{30} have high propensity for α-helical structure while the poly-aspartyl tail has a propensity to form an extended (β-sheet) conformation. The secondary chemical shift profiles are almost identical for both lunasin forms. The low number of interresidue NOEs observed in the spectra, the small $^{13}C_{\alpha,\beta}$ chemical shift differences and backbone dynamics indicate that the secondary structures are not stably folded [3].

Conclusions: Lunasin is an intrinsically disordered peptide. However, two sequence regions have a high propensity for α-helical structure and the C-terminal poly-Asp tail adopts an extended (β-sheet) conformation. Possibly, the identified transient secondary structure elements assume a stable fold upon interaction with histones H3 and H4. It is highly likely that the newly discovered cysteine redox properties are at least partially accountable for the antioxidant and anti-inflammatory effects of lunasin [3].

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Keywords: lunasin – solution structure – NMR spectroscopy – MALDI-TOF mass spectrometry – cysteine oxidation

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A2.3

Influence of structure and physico-chemical properties of synthetic lipid-like pyridinium amphiphiles on formation of magnetic liposomes

Oksana Petrichenko[1,*], Karlis Pajuste[2], Martins Rucins[2], Aiva Plotniece[2] and Andrejs Cebers[1]

[1]*Laboratory of Magnetic Soft Matter, Department of Physics, University of Latvia, Riga, Latvia;* [2]*Latvian Institute of Organic Synthesis, Riga, Latvia*

**Corresponding author: Oksana Petrichenko, Laboratory of Magnetic Soft Matter, Department of Physics, University of*

Latvia, 23 Zellu Str., LV-1002, Riga, Latvia.
E-mail: oksana.petricenko@lu.lv

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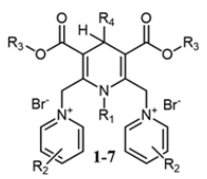
Background: The progress of theranostics combines diagnostic and therapeutic capabilities into single agent. In the Latvian Institute of Organic Synthesis (LIOS) were developed new self-assembling 1,4-dihydropyridine (1,4-DHP) amphiphiles with variations of the substituents at the cationic head-group of the molecule. The liposomes formed by these 1,4-DHPs represent a promising tool for the delivery of DNA into cells [1,2]. 1,4-DHP amphiphiles are also attractive because they contain the 1,4-DHP core as an active linker, which is an intrinsic structural part of many pharmacologically active compounds and drugs [3]. Magnetic liposomes (MLs) attract interest due to the numerous applications, such as magnetically directed drug carriers, in magnetic hyperthermia, gene transfection and theranostic agents, etc.

Objectives: The aim of this work is evaluation of influence of the 1,4-DHP structure and physico-chemical properties on the formation of the magnetic liposomes.

Methods: The reverse-phase evaporation (REV) method [4] was used for the MLs preparation. Compounds **1**–**7** were synthesized at LIOS and their structures are presented in Fig. 1. Compounds in solid state were tested by thermogravimetric (TG) and differential thermal analysis (DTA) (Shimadzu DTG-60). Produced magnetic liposomes were characterized by the dynamic light scattering (DLS) technique (Zetasizer Nano ZS) and optical microscopy (OM).



Comp.	R_1	R_2	R_3	R_4
1	H	H	$C_{12}H_{25}$	Ph
2	H	H	$C_{14}H_{29}$	Ph
3	H	H	$C_{16}H_{33}$	Ph
4	CH_3	H	$C_{12}H_{25}$	Ph
5	H	4-CN	$C_{12}H_{25}$	Ph
6	H	3-Ph	$C_{12}H_{25}$	Ph
7	H	H	$C_{12}H_{25}$	2-Napth

Figure 1: Structures of studied 1,4-DHP derivatives **1**–**7**.

Results: The REV method along with the use of 1,4-DHP amphiphiles has proved its applicability for the production of giant MLs size up to 10–20 μm (compounds **1** and **2**) and 4 μm for compound **3**. Compound **5** forms a MLs' uniform dispersion when the sizes of magnetic liposomes vary in the 1.5–2.0 μm range. All results are in good agreement with DLS data.

Conclusions: We can conclude that the non-polar alkyl chains elongation in the synthetic lipids molecule leads to a reduction in size of the MLs. The vacuum and accordingly evaporation process's duration affect the MLs size, morphology and the quantity of liposome. Linker part modification (1,4-DHP ring) isn't effective for the MLs production. All tested by TGA/DTA analysis compounds possess thermal stability at the temperature range below their melting points.

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Keywords: cationic amphiphiles – 1,4-dihydropiridine derivatives – magnetic liposomes – reverse-phase evaporation method

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A2.4

Cytotoxicity, self-assembling and physico-chemical properties of bifunctional lipid-like 4-(*N*-alkylpyridinium)-1,4-dihydropyridines as putative delivery systems

Pavels DIMITRIJEVS[1,2,*], Martins RUCINS[1], Oksana PETRICHENKO[3], Mara PLOTNIECE[1,2], Anita GULBE[1], Ludmila JACKEVICA[1], Marina GOSTEVA[1], Dace BANDERE[2], Ilona DOMRACHEVA[1], Karlis PAJUSTE[1], Arkadij SOBOLEV[1] and Aiva PLOTNIECE[1,2]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Department of Pharmacy, Rīga Stradiņš University, Riga, Latvia; [3]Laboratory of Magnetic Soft Matter, Department of Physics, University of Latvia, Riga, Latvia
**Corresponding author: Pavels Dimitrijevs, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.*
E-mail: pavels.dimitrijevs@gmail.com

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Background: Design of biologically active delivery materials is an ideal strategy for biomedical applications. Previously, cationic 1,4-dihydropyridine (1,4-DHP) amphiphiles capable of transfecting pDNA into cell lines *in vitro* were developed by our group [1].

Objectives: The aim of the study is characterization of physico-chemical, self-assembling and cytotoxic properties of 4-(*N*-alkylpyridinium)-1,4-dihydropyridines.

Methods: Cationic 1,4-DHPs were synthesized according to Rucins *et al.* [2,3]. Cytotoxicity of 1,4-DHPs *in vitro* was assessed using the MTT assay on two monolayer tumor cell lines: HT-1080 and MH-22A in comparison with their action on normal mouse fibroblasts. The NRU assay was performed on 3T3 cells according to Stokes [4]. Data from the *in vitro* tests were used for estimation of the starting dose for acute oral toxicity (LD_{50}) tests in rodents. Thermogravimetric (TGA) and differential thermal analysis were evaluated by Shimadzu DTG-60 analyzer. Samples for characterization of self-assembling properties by dynamic light scattering (DLS) measurements (Zetasizer Nano ZSP) were prepared by injection method.

Results: Nine cationic amphiphilic 1,4-DHPs containing 4-(*N*-alkylpyridinium) substituent and/or propargyl moiety/ies as pharmacophore groups were synthesized. Cytotoxicity tests showed that 4-(*N*-ethylpyridinium)-1,4-DHPs did not demonstrate any cytotoxic effect on tumor cell lines, their LD_{50} was defined as practically non-toxic. 4-(*N*-hexylpyridinium) and 4-(*N*-dodecylpyridinium)-1,4-DHPs possessed high cytotoxicity on tumor cell lines (IC_{50} 1–80 mM) and their LD_{50} was defined as slightly toxic or non-toxic. The average size of the nanoparticles varied from 52 to over 1000 nm for fresh samples, depending on the compound structure. TGA data demonstrated decomposition in one step and showed weight loss in the range of 179–280 °C.

Conclusions: Increasing of length of the alkyl chain at quaternized nitrogen in 4-(*N*-alkylpyridinium)-1,4-DHPs or the introduction of propargyl moieties in the 1,4-DHP molecule significantly influences the cytotoxicity against cancer cells. The 4-(*N*-alkylpyridinium)-1,4-DHPs form nanoparticles, but from all tested nanoparticles only the ones formed by 4-(*N*-dodecylpyridinium)-1,4-DHPs were stable after two weeks of storage. The presence of a cationic charge and *N*-dodecylpyridinium moiety at the 1,4-DHP cycle is essential for the formation of stable and homogenous nanoparticles. All tested compounds possess thermal stability at the temperature range below their melting points. These lipid-like compounds would be a promising tool for cancer therapy developments.

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Keywords: cytotoxicity – synthetic lipids – nanoparticles – dynamic light scattering

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A2.5

Search for HCA2, FFAR2 and FFAR3 receptor ligands in pyranopyrimidine and hexahydroquinoline ranges

Ilona MANDRIKA[1,*], Zenta KALME[2], Brigita VIGANTE[2], Ramona PETROVSKA[1], Egils BISENIEKS[2], Imanta BRUVERE[2], Janis POIKANS[2], Zaiga OGLE[2], Janis KLOVINS[1] and Gunars DUBURS[2]

[1]Latvian Biomedical Research and Study Centre, Riga, Latvia; [2]Latvian Institute of Organic Synthesis, Riga, Latvia

**Corresponding author: Ilona Mandrika, Latvian Biomedical Research and Study Centre, Ratsupites 1, Riga, LV-1067, Latvia. E-mail: ilona@biomed.lu.lv*

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Background: Hydroxycarboxylic acid receptor HCA2 (GPR109A, niacin receptor) and short-chain free fatty acid receptors FFAR2 (GPR43) and FFAR3 (GPR41) are G protein-coupled receptors expressed in human adipocytes, immune cells, such as macrophages, monocytes, neutrophils, and colon epithelial cells. The receptors are of interest as potential targets for treatment of various conditions and disorders related to dyslipidemia, diet-induced obesity and chronic inflammatory diseases such as arthritis, asthma, colitis and atherosclerosis [1, 2].

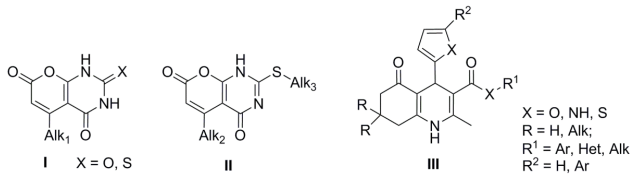
Objectives: Until now, pyranopyrimidine derivatives **I** have been studied only as niacin receptor ligands, but their *S*-alkyl derivatives **II** have been studied very little and occasionally. In turn, hexahydroquinoline derivatives have been studied only as ligands for FFAR2 and FFAR3 receptors—both agonists and antagonists.

Methods: Functional activity of synthesized compounds was assessed by modulation of forskolin-stimulated cAMP production in human Flp-In-293 cell lines that express recombinant HCA2, FFAR2 and FFAR3 receptors.

Results: In the present study we have synthesized novel representatives of compounds **I**–**III** (Fig. 1). Pyranopyrimidines of groups **I** and **II** were confirmed to be dual receptor (HCA2 and FFAR2, or HCA2 and FFAR3, respectively) ligands.

Hexahydroquinolines **III** showed specific affinity toward FFAR2 or FFAR3 receptors, but they were inactive toward the HCA2 receptor.

Data on some dual receptor ligands (Fig. 2) inhibiting forskolin-stimulated cAMP production are presented in Table 1.



I X = O, S **II** **III**

Figure 1: Derivatives of pyranopyrimidine (**I** and **II**) and hexahydroquinoline (**III**).

Compound A: **I** X = O $Alk_1 = CH_2CH_2CH_2CH_2Cl$
Compound B: **I** X = S $Alk_1 = CH_2CH_2CH_2CH_3$
Compound C: **II** $Alk_2 = CH_3$ $Alk_3 = CH_2CH{=}CHC_6H_5$
Compound D: **III** X = O R = H $R_1 = C_6H_4CH_3$-*o* $R_2 = H$

Figure 2: Dual receptor ligands.

Table 1: Inhibition of forskolin-stimulated cAMP production by some dual receptor ligands (EC_{50} or % units).

Receptors	A	B	C	D
HCA2	5.40×10^{-8} M	$3.5\cdot\times10^{-7}$ M	49%	NA
FFAR2	$3.8\ \times10^{-7}$ M	$7.4\ \times10^{-7}$ M	54%	NA
FFAR3	2.15×10^{-7} M	1.90×10^{-7} M	1.3×10^{-6} M	2.7×10^{-7} M

Conclusions: This study shows that several selective and potent FFAR2 and FFAR3 receptors agonists could be identified from a series of hexahydroquinoline derivatives.

Acknowledgements: The study was supported by the Latvian National Research Programme VPP-14-2-6.

Keywords: HCA2 receptors – FFAR2 receptors – FFAR3 receptors – ligands

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A2.6

Identification of targets in the energy metabolism pathways for possible treatment of sepsis

Marina MAKRECKA-KŪKA[1,*], Stanislava KORZH[1], Karlis VILKS[1,2] and Edgars LIEPINSH[1]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Biology, University of Latvia, Riga, Latvia

**Corresponding author: Marina Makrecka-Kūka, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic*

Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.
E-mail: makrecka@farm.osi.lv

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Background: Sepsis, a life-threatening organ dysfunction induced by infection, is a leading cause of death among critically ill patients. Current immunological pathways cannot fully explain the mechanisms of cellular dysfunction and organ failure in sepsis. Significant impairments in energy metabolism and mitochondrial dysfunction are associated with morbidity in sepsis. However, the role of energy metabolism and mitochondrial function in the progression of sepsis-induced multi-organ dysfunction is not fully understood.
Objectives: The aim of the study was to identify the energy metabolism pathways responsible for sepsis-induced multi-organ dysfunction in the experimental model of endotoxemia.
Methods: A single injection of LPS (10 mg/kg, i.p.) was used to induce endotoxemia in CD1 male mice. The control animals received a saline injection. During the experiment the animals were deprived of food. The plasma biochemical parameters, energy substrate uptake, mitochondrial function and gene expression in heart, brain and kidneys were determined 4 and 24 h after LPS injection.
Results: In LPS-induced mice model of sepsis the hypoglycemia and hyperinsulinemia were observed. In heart, brain and kidneys, the fatty acid uptake was decreased by 43%, 31% and 34%, respectively, while glucose uptake was decreased by 59%, 26% and 51%. 4 h after administration of LPS, the mitochondrial dysfunction was observed in kidney, but not in heart or brain. Moreover, increase in markers related to kidney function (blood urea and creatinine) also indicated on kidney damage. The LPS injection induced a decrease in mitochondrial fatty acid oxidation in heart, while electron transfer system oxidative phosphorylation capacity was similar to the control group. The gene expression measurements demonstrated that the genes related to inflammation (*IL1β, IL6, TNFα, iNOS*) are upregulated in all studied tissues, and particularly, in brain tissues. The genes related to energy metabolism (*CPT1A, CPT1B, PDHx*) and to mitochondrial function and biogenesis (*ATP5O, PGC1a*) were downregulated particularly in heart and kidneys.
Conclusions: The obtained results demonstrate that endotoxemia induces tissue-specific and multicomponent failure of energy metabolism pathways. The main strategy for energy metabolism correction for improvement of sepsis outcome could be simultaneous activation of fatty acid oxidation, reduction of hyperinsulinemia and protection of mitochondria.
Acknowledgements: This study was supported by ERDF project no. 1.1.1.2/VIAA/1/16/246.
Keywords: sepsis – energy metabolism – mitochondrial dysfunction

A2.7

Selective sigma-1 receptor antagonist NE-100 possesses pro-convulsive activity and induces seizures

Edijs Vavers[1,2,*], Liga Zvejniece[1], Baiba Svalbe[1], Lasma Lauberte[1], Grigory Veinberg[1] and Maija Dambrova[1,2]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia
**Corresponding author: Edijs Vavers, Latvian Institute of Organic Synthesis, Riga, Latvia. E-mail: edijs.vavers@farm.osi.lv*

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Background: The sigma-1 receptor (Sig1R) has been widely studied as a novel target to treat neurological disorders, including seizures. The anti-seizure activities of a number of selective Sig1R ligands have been studied mainly in glutamate- and opiate receptor-related chemoconvulsant-induced seizure models *in vivo*. Thus far, the anti-seizure activity of Sig1R ligands could not be fully concluded since agonist, antagonist and allosteric modulator have not been studied simultaneously using identical experimental settings.
Objectives: The aim was to test the seizure-modulating activity of the selective Sig1R agonist PRE-084, the selective Sig1R antagonist NE-100 and the positive allosteric Sig1R modulator E1R in GABA-ergic signalling-related chemoconvulsant-induced seizure models in mice.
Methods: The anti-seizure activities of selective Sig1R ligands were evaluated in pentylenetetrazol (PTZ) and (+)-bicuculline (BIC)-induced seizure models in SW mice. Animals received i.p. injection of saline or Sig1R ligand 60 min before PTZ or BIC i.v. infusion. Minimal doses of PTZ or BIC necessary to induce clonic and tonic seizures were considered as indices of seizure threshold.
Results: The Sig1R antagonist NE-100, at a dose of 25 mg/kg, demonstrated pro-convulsive activity on PTZ-induced seizures. The agonist PRE-084 did not change the thresholds of chemoconvulsant-induced seizures. The positive allosteric modulator E1R, at a dose of 50 mg/kg, showed anti-convulsive effects on PTZ- and BIC-induced clonic and tonic seizures. Surprisingly, NE-100 at a dose of 50 mg/kg induced convulsions *per se*. E1R significantly reduced the number of generalised seizures induced by NE-100.
Conclusions: The selective Sig1R antagonist NE-100 induced seizures that could be attenuated by E1R. Additionally, the obtained results suggest that Sig1R could be considered as a molecular target for new anti-convulsive drugs.
Acknowledgements: The current work was supported by the European Regional Development Fund, project no. 1.1.1.1/16/A/292.
Keywords: sigma-1 receptor antagonist – NE-100 – chemoconvulsant

A2.8

Influence of wolframin expression on dopamine receptor functions

Karl Rene Kõlvart*, Anni Allikalt and Ago Rinken

Institute of Chemistry, University of Tartu, Estonia
**Corresponding author: Karl Rene Kõlvart, Institute of Chemistry, University of Tartu, Ravila 14a, 50411, Tartu, Estonia.*
E-mail: karl.kolvart@gmail.com

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Backround: The *Wfs1* gene encodes wolframin, an ER-resident membrane protein, which is involved in regulation of several functions [1]. Loss of wolframin function, known as the rare disease Wolfram syndrome, is connected with several serious disorders, of which the causal mechanism is still not well understood [2].
Objectives: It has been shown that the *Wfs1* gene is expressed in almost all those brain regions where also D_1-like dopamine receptor genes are expressed [3]. Since it is also reported that the dopamine system is altered in *Wfs1*-deficient mice, we studied if it alters D_1-like receptor-specific ligand binding in the hippocampi of corresponding mice.
Methods: The binding of [^{3}H]SCH23390 to membranes of mice hippocampi were performed as it has been described for HEK 291 cell membranes in [4].
Results: Comparison of membranes of wild-type mice with membranes from *Wfs1* knockout mice revealed increased number of D_1-like receptors in latter case. This has been found in comparison of binding curves (pooled membrane samples) as well as in comparison of the number of binding sites determined at 4 nM [^{3}H]SCH23390.
Conclusions: Alterations in dopaminergic signalling connected with Wolfram syndrome may be caused at least in part by the upregulation of D_1-like receptors as it has been shown in *Wfs1* knockout mice.
Keywords: dopamine receptor – wolframin – radioligand binding assay

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A2.9

Immunosuppressant dosing accuracy: residual drug concentration versus estimation of the area under the curve

Aurelija NOREIKAITĖ[1,*], Franck SAINT-MARCOUX[2], Pierre MARQUET[2], Edgaras STANKEVIČIUS[1] and Edmundas KADUŠEVIČIUS[1]

[1]*Institute of Physiology and Pharmacology, Medical Academy, Lithuanian University of Health Sciences, Kaunas, Lithuania;* [2]*Laboratory of Clinical Pharmacology, Faculty of Medicine, University of Limoges, France*

**Corresponding author: Aurelija Radzevičienė, Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, 9 Mickevičiaus Str., LT–44307 Kaunas, Lithuania.*
E-mail: aurelija.noreikaite@lsmuni.lt

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Background: Therapeutic drug monitoring of immunosuppressants is essential, due to large intra- and interindividual variability of their pharmacokinetics and narrow therapeutic index. Determination of drug exposure by $AUC_{0–24}$ requires multiple samples and this method is not feasible in clinical practice. Various approaches as trough concentration (C_0), single concentrations within the dosing period (as C_2) and limited sampling strategies have been examined.
Objectives: To assess immunosuppressant dosing accuracy by performing drug blood monitoring in two different methods: determining C_0 and the area under the concentration–time curve ($AUC_{0–12}$).
Methods: Immunosuppressants (cyclosporine A, tacrolimus, everolimus and sirolimus) concentration–time profiles of whole blood, and mycophenolate mofetil (MMF) concentration–time profile of whole blood serum was measured 12 h after the first dose. Mycophenolic acid (MPA) in serum was determined by HPLC; other immunosuppressants concentrations in blood were determined by LC-MS method. $AUC_{0–12}$ were calculated using the Bayesian estimation and 3-point limited sampling strategy.
Results: C_0 and $AUC_{0–12}$ were determined for 614 kidney recipients with a graft life >1 year.
Dosing accuracy has been evaluated by determining C_0 and $AUC_{0–12}$ and these parameters compliance within the immunosuppressants' therapeutic ranges. It was found that $AUC_{0–12}$ exposures were within the therapeutic range more often than C_0 values in patients treated with cyclosporine. Similar results were obtained by analyzing other immunosuppressants' $AUC_{0–12}$ and C_0 values compliances within therapeutic ranges.
Different results were obtained, while analyzing MPA concentrations in serum, where C_0 values were more often within the therapeutic range than $AUC_{0–12}$ exposures.
Moreover, it was found that immunosuppressants are administered at low doses more often when assessing C_0 versus $AUC_{0–12}$ in kidney recipients treated with CsA, while in other treatment arms immunosuppressants are administered at low doses more often when assessing $AUC_{0–12}$ versus C_0, except tacrolimus group, where overdosing is observed more often.
Conclusions: The study results showed that determinations of C_0 and $AUC_{0–12}$ give different results. This explains why we should not consider only one parameter before making decision and should take into account both pharmacokinetic indicators. These results also showed that mostly kidney recipients experience undertreatment, except when tacrolimus is administrated. Severe tacrolimus overdose is monitored by determining C_0.

Keywords: immunosuppressants – kidney transplantation – drug monitoring

A2.10

Expression of proteasome genes in kidneys of diabetic rats in presence of 1,4-dihydropyridine derivatives

Kristīne OŠIŅA[1,*], Evita ROSTOKA[1,3] and Nikolajs SJAKSTE[1,2,3]

[1]*Institute of Biology of the University of Latvia, Salaspils, Latvia;* [2]*Latvian Institute of Organic Synthesis, Riga, Latvia;* [3]*Department of Medical Biochemistry, Faculty of Medicine, Riga, University of Latvia*

**Corresponding author: Kristīne Ošiņa, Institute of Biology, University of Latvia, 3 Miera Str., LV-2169, Salaspils, Latvia.*
E-mail: kristine.osina@lu.lv

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Background: The ubiquitin–proteasome system (UPS) that is responsible for protein degradation might be involved in the pathogenesis of diabetes mellitus (DM) and its complications [1]. A group of 1,4-dihydropyridine derivatives (1,4-DHPs) possess antidiabetic properties and might be prospective compounds for treatment of DM complications [2].
Objectives: In this study we tested effect of some 1,4-DHPs on several proteasome gene expression in kidneys of diabetic rats.
Methods: DM was induced in Wistar rats by streptozotocin (STZ) (50 mg/kg) injection. Nine days after DM approval rats were treated for three days with AV-153-Na (0.5 mg/kg), J-9-125, glutapyrone, metcarbatone and etcarbatone (0.05 mg/kg or 0.5 mg/kg). mRNA expression level of *Psma3*, *Psma6* and *Psmc6* genes in kidneys was measured with qPCR. Reference gene: *RNA polymerase II*.
Results: Induction of DM led to increased expression of *Psma3*, *Psma6* and *Psmc6* genes in kidneys of rats. Treatment with 1,4-DHPs increased the expression of the three genes in all control groups, except *Psmc6* expression in the group treated with AV-153-Na. In diabetic rats, the expression of the tested genes was increased by AV-153-Na, glutapyrone (0.5 mg/kg) and metcarbatone, while etcarbatone caused elevated expression only for *Psma3*, and J-9-125 (0.5 mg/kg) for *Psma6* and *Psmc6* genes.
Conclusions: 1,4-DHPs increased the expression of *Psma3*, *Psma6* and *Psmc6* genes in kidneys of control and diabetic rats.

Acknowledgements: The work was funded by the University of Latvia in the frame of the project "Topical clinical and basic investigations in biomedicine and pharmacy".

Keywords: 1,4-dihydropyridine derivatives – diabetes mellitus – gene expression – proteasome genes

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A2.11

Screening for mitochondrial DNA mutations associated with both antibiotic-induced and non-syndromic deafness in the ethnic Latvian population

Viktorija IGUMNOVA[1,2,*], Lauma VEIDEMANE[2], Egija ZOLE[2], Ilva POLE[2,3], Anda VĪKSNA[2,3], Dace BANDERE[1] and Renāte RANKA[1,2]

[1]Department of Pharmaceutical Chemistry, Rīga Stradiņš University, Riga, Latvia; [2]Laboratory of Molecular Biology, Latvian Biomedical Research and Study Centre, Riga, Latvia; [3]Centre of Tuberculosis and Lung Diseases, Riga East University Hospital, Latvia

**Corresponding author: Viktorija Igumnova, Department of Pharmaceutical Chemistry, Rīga Stradiņš University, 16 Dzirciema Street, LV-1007, Riga, Latvia; Laboratory of Molecular Biology, Latvian Biomedical Research and Study Centre, 1 Ratsupites, LV-1067, Riga, Latvia. E-mail: viktorija.igumnova@gmail.com*

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Background: Ototoxicity is an irreversible side effect of aminoglycosides and could manifest as either cochlea damage with permanent hearing loss or vestibular damage with dizziness, ataxia and/or nystagmus [1]. Several mutations in mitochondrial DNA in the 12S rRNA gene causes increased aminoglycoside attraction to 12S rRNA subunit causing disrupted protein synthesis and death of the cell, and are linked to increased susceptibility to ototoxicity. The homoplasmic A1555G and C1494T mutations and m.961_962delTinsC(n) pathogenic variant in the 12S rRNA gene have been associated with aminoglycoside-induced hearing loss and non-syndromic deafness in certain populations. Other possible deafness-related mutations are T1095C and A827G [2,3].

Objectives: To analyse the prevalence of the mitochondrial mutations associated with antibiotic-induced and non-syndromic hearing loss in the ethnic Latvian population.

Methods: DNA samples of 191 ethnic Latvians were used in this study. The samples and information were obtained from the Genome Database of the Latvian Population (VIGDB). The study protocol was approved by the Central Medical Committee of Ethics in Latvia. A 1752-bp DNA fragment was amplified by PCR for the detection of C1494T and A1555G mutations, and the 420-bp DNA fragment was amplified to target mtDNA T961G, 956-960insC, T1095C and A827G mutations. Obtained PCR products were sequenced by the Sanger method using both forward and reverse sequencing primers.

Results: PCR amplicons of desired length (1752 bp and 420 bp) were successfully obtained from all DNA samples, and sequence data for all mutations included in this study were available. Sequence data analysis revealed that C1494T and T1095C mutations were not present in any of 191 samples used. A1555G mutation was identified in one, T961G (also related to the H11a haplogroup) in four, 956-960insC in five and A827G (also related to the H6a1a3a haplogroup) in two samples were identified.

Conclusions: The presence of aminoglycoside ototoxicity-related mutations in the Latvian population indicates the necessity to include additional ototoxicity-related mutation analysis in future studies in order to determine the feasibility of DNA screening for patients before administration of aminoglycoside therapy.

Acknowledgements: VPP programme "BIOMEDICINE".

Keywords: ototoxicity – aminoglycosides – mitochondrial DNA – single-nucleotide polymorphisms – Latvia

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A2.12

Colorectal cancer cell line-derived extracellular vesicles induce changes in mesenchymal stem cell phenotype and secretory properties

Ineta POPENA[1,*], Arturs ABOLS[2], Elina ZANDBERGA[2], Liga SAULITE[1], Kaspars JEKABSONS[1], Karlis PLEIKO[1], Aija LINĒ[2] and Una RIEKSTINA[1]

[1]Pharmacy Study Program, Faculty of Medicine, Riga, University of Latvia; [2]Latvian Biomedical Research and Study Centre, Riga, Latvia

**Corresponding author: Ineta Popena, Pharmacy Study Program, Faculty of Medicine, University of Latvia, 1 Jelgavas Str., LV-1004, Riga, Latvia. E-mail: ineta.popena@lu.lv*

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Background: Solid tumours show accumulation of stromal fibroblasts that influence disease progression. The origin of cancer-associated fibroblasts (CAFs) is still unclear, with mesenchymal stem cells (MSCs) being considered as one of the possible contributors [1]. MSCs are multipotent cells positive for CD73, CD90 and CD105, and, besides their normal physiological roles, MSCs are also present in tumour microenvironment (TME) where they show tumour-promoting effects [2,3]. Extracellular vesicles (EVs) are membrane-bound vesicles containing different biomolecules including mRNA, miRNA, siRNA and other RNA fragments, and cancer cell-secreted vesicles are able to modulate stromal cell properties [4]. In colorectal cancer (CRC), the EV concentration in TME correlates to the invasive potential of cancer cells [5].

Objectives: The purpose of the study was to analyse CRC cell line-derived EV uptake and co-localisation in skin MSCs and to assess their effect on MSC properties.

Methods: EVs were isolated from SW480 and SW620 cell culture supernatants using size-exclusion chromatography and sepharose gel filtration. MSCs were incubated with SYTO RNA Select-labelled EVs alone or in the presence of uptake inhibitors covering different pathways. Next, MSCs were analysed for EV uptake and subcellular localisation using flow cytometry and fluorescence microscopy. MSCs were also analysed for alterations in cell phenotype, CAF gene expression and cytokine secretion using flow cytometry, qPCR and ELISA methods.

Results: CRC cell line-derived EVs enter sMSCs where they co-localise with cell nuclei and endoplasmic reticulum. The EV uptake is most effectively blocked with the dynamin-2 inhibitor dynasore. Exposure to cancer cell EVs results in down-regulated CD90

expression, increased VEGF and IL-8 secretion, altered FAP expression, upregulated chemokine gene expression as well as increased TERT expression in MSCs.

Conclusions: Alterations in MSC properties following exposure to EVs are associated with MSC shift towards cancer-associated fibroblast phenotype.

Acknowledgements: Supported by the Latvian Council of Sciences, Collaboration project no. 625/2014 "Cancer-derived exosomes – a source of novel biomarkers and therapeutic targets for gastrointestinal cancers".

Keywords: mesenchymal stem cells – extracellular vesicles – stromal cells – tumour microenvironment

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A2.13

Real-time monitoring of apoptosis in live cells with FRET biosensor

Maris-Johanna Tahk[1,*], Darja Lavogina[1,2], Olga Mazina[1,2] and Ago Rinken[1]

[1]*Institute of Chemistry, Tartu, University of Tartu, Estonia;* [2]*Competence Centre on Reproductive Medicine and Biology, Tartu, Estonia*

**Corresponding author: Maris-Johanna Tahk, Chair of Bioorganic Chemistry, Institute of Chemistry, University of Tartu, 14a Ravila, 50411, Tartu, Estonia. E-mail: maris-johanna.tahk@ut.ee*

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Background: Apoptosis or programmed cell death is a cellular defense mechanism, and its dysfunctions have been associated with several major diseases—for example, cancer. Apoptosis can occur via two different pathways, an internal pathway caused by cell stress or the external pathway triggered by ligand–receptor interaction. Both pathways activate a caspase cascade that leads to morphological changes and cell death. In the caspase family of proteases, there are seven caspases that are involved in apoptosis and can be classified as initiators (caspase-2, -8, -9, -10) or executioners (caspase-3, -6, -7) [1].

Methods: We have developed a method which uses FRET biosensor (Casper3-GR) and BacMam system for high-yield expression of sensor in both cancer and non-cancer cell lines [2]. When apoptosis is induced, time-dependent cleavage of the bond between two fluorescent proteins (TagGFP and TagRFP) is performed by caspase-3, leading to decrease in FRET. For measuring we use an automated microscope performing fluorescent imaging in two channels.

Results: Here, we show exhaustive validation of the method using HeLa cells and known apoptosis inducers like chemotherapy agents (bortezomib, MMAE) and compounds with a wide profile of potential targets (staurosporine). As a reference method, we used a viability assay utilizing resazurin dye which is reduced in live cells to fluorescent compound. By measuring data over 24 hours after adding the compound, we have found that different toxins have dissimilar FRET profiles in time, which reflects their efficiency and ability to modulate various cellular pathways.

Also, we could perform screening of novel as well as commercially available inhibitors of caspase-3—the compounds that can be viewed as protective agents against cell death. By inducing cell death with different toxins, we found that depending on the mechanism of binding of the caspase inhibitor (reversible *vs.* irreversible), the profile of FRET change is different.

Conclusions: In conclusion, we have developed a versatile method that combines advantages of FRET-based caspase-3 sensor and BacMam system. This method is easy to use, gives time-resolved information on the state of apoptosis in live cells, and can be combined with the 'classical' viability assays.

Keywords: apoptosis – FRET biosensor – caspase-3 – toxins – viability assay

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A2.14

Evaluation of small-molecule effectors of hepatitis B virus capsid assembly

Unda Nelda Dubova[1,*], Karīna Spunde[1], Irēna Timofejeva[1], Anda Sīpola[2], Brigita Vīgante[2], Gunārs Dubur[2] and Tatjana Kozlovska[1]

[1]*Latvian Biomedical Research and Study Centre , Riga, Latvia;* [2]*Latvian Institute of Organic Synthesis, Riga, Latvia*

**Corresponding author: Unda Nelda Dubova, Biomedical Research and Study Centre, 1 Ratsupites Str., Riga LV-1067, Latvia. E-mail: undadubova@inbox.lv*

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Background: Estimated 240 million persons worldwide are chronically infected with hepatitis B virus (HBV). Chronic hepatitis B (CHB) in up to 40% of cases progresses to liver cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC), a leading cause of cancer-related morbidity and mortality worldwide [1]. In spite of therapy with efficient inhibitors of HBV reverse transcriptase, a complete cure of CHB can rarely be achieved by this approach [2]. There is an urgent need for development of novel molecular targeted antiviral strategies to improve therapeutic outcomes for HCC. We are focused on antiviral strategy intended to suppress the self-assembly process of HBV core (HBc) protein as one of the promising ways to cure CHB without induction of drug resistance.

Objectives: The objective of the study is the discovery of new, small-molecule, antiviral drug candidates targeted on disruption of HBV capsid assembly.

Methods: The cytoplasmic expression of HBc gene driven by exogenously delivered recombinant self-replicating alphavirus RNA replicons were used for high level production of HBc and HBV capsids in eukaryotic cells. Drug candidates—heterylarylpyrimidines (HAPs) comprising a 1,4-dihydropyrimidine moiety—were obtained according to methods of synthesis of 1,4-dihydropyrimidine derivatives. The evidence of the HBc assembly was assessed by native agarose gel electrophoresis following by western blot analysis. Cytotoxicity of the compounds was estimated by phase-contrast microscopy and MTT viability assay.

Results: The method for direct assessment of HBV capsid assembly in cell culture was established and optimized. Dose-dependent effects on the level of cytoplasmic capsid release were assessed for 8 new HAPs—analogues of Bay-41-4109 [3]. Two compounds (V-4-84s, AS-M-I) showed promising effects by inducing a dose-dependent decrement of the relative quantity of assembled capsids in concentrations less than those needed to affect cell viability. Two other compounds (V4-93, AS-M-III) showed dose-dependent effects on HBc aggregation, possibly inducing disruption in capsid assembly.
Conclusions: Eight newly synthesized small-molecule effectors of HBV capsid assembly were evaluated for their ability to disrupt the capsid assembly in cell culture, promising candidates were selected for further evaluation of antiviral activity and discovery of mechanisms of action.
Acknowledgements: This study was supported by the National Research Programme „Biomedicine for Public Health" (BIOMEDICINE), project no. 3: „Development of novel anticancer drugs and immunotherapeutic approaches", provided by the Latvian Ministry of Education.
Keywords: heterylarylpyrimidines (HAPs) – hepatitis B virus – capsid assembly – Bay-41-4109
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A2.15

Results of screening of new salts of 7-thietanyl-3-propyl-xanthine derivatives on platelet aggregation under conditions *in vitro*

Almaz R. Khalimov[1,*], Felix Kh. Kamilov[1], Galiya A. Timirkhanova[1], Ferkat A. Khaliullin[1], Dina R. Kilsenbaeva[2], Aigul G. Safargalina[1], Diana E. Asanbaea[1], Bella R. Bayazitova[1] and Ilsiiar Z. Khalilova[1]

[1]Bashkir State Medical University, Ufa, Russia; [2]Republican Clinical Hospital named after G.G. Kuvatov, Ufa, Russia
**Corresponding author: Almaz R. Khalimov, Department of Biochemistry, Bashkir State Medical University, 3 Lenins Str., 450000, Ufa, Russia. E-mail: avsamorodov@gmail.com*
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Background: Preventing and control of bleeding is important in various areas of clinical medicine in the light of increasing numbers of patients with hemorrhagic manifestations, extensive use of anti-coagulants, increasing number of invasive diagnostic and treatment methods [1,2]. However, the drugs traditionally used in medical practice to control bleeding are often inefficient and unable to lead to effective reduction of blood loss.
Objectives: We have studied the influence of 25 firstly sythesized derivatives of 7-thietanyl-3-propylxanthine and medically applied therapeutic agents on the hemostasis system under conditions *in vitro* with donated human blood [3,4].
Methods: Experimental work was done *in vitro* with the blood of healthy male donors. The impact of firstly synthesized derivative xantine, aspirin and etamsylate on the functional activity of platelets under conditions *in vitro* was studied with the help of a laser analyzer of platelet aggregation Biola 230 LA (Russia). Adenosine diphosphate of 20 µg/ml exposure and collagen of 5 µg/ml exposure were used as an aggregation inducer, produced by Technology-Standard company in the city of Barnaul, Russia. Thrombelastography (TEG 5000, Haemoscope Corporation, USA) was performed in accordance with instructions of the manufacture and performed within 1 h of blood sampling. TEG parameters of reaction time, angle and maximal amplitude and conventional coagulation data of platelet count.
Results: We have defined different influence of the studied compounds on functional activity of platelets. The lithium salt of 2-[1,3-methyl-7-(dioxothietanyl-3)xanthinyl-8-thio]acetic acid shows hemostatic activity, which exceeds levelwise that of etamsylate.
Conclusions: The findings prove that it is neccessary and up-to-date to continue research of this number of derivatives influencing the hemostasis system as potential antiplatelets and hemostatic agents.
Keywords: xanthine derivatives – hemostasis – thrombelastography
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A2.16

A novel model for studying extracellular vesicle-mediated tumour and immune cell communication

Zane Kalniņa[1,*], Lilite Sadovska[1], Elīna Zandberga[1], Krizia Sagini[2], Una Riekstiņa[3], Alicia Llorente[2] and Aija Linē[1]

[1]Cancer Biomarker and Immunotherapy Group, Latvian Biomedical Research and Study Centre, Riga, Latvia; [2]Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital – The Norwegian Radium Hospital, Oslo, Norway; [3]Faculty of Medicine, University of Latvia, Riga, Latvia
**Corresponding author: Zane Kalniņa, Cancer Biomarker and Immunotherapy Group, Latvian Biomedical Research and Study Centre, Ratsupites 1 k-1, LV-1067, Riga, Latvia.*
E-mail: zane@biomed.lu.lv
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Background: Extracellular vesicles (EVs) have been described to have a significant role in intercellular communication and in the modulation of anti-cancer immune responses. There is an evidence that cancer-derived EVs can affect immune cell functions, but currently a method that can ensure immune cell supply with physiological amounts of cancer-derived EVs is lacking.
Objectives: The aim of the study was to develop a novel 3D co-culture model that would enable further studies of cancer-derived EV uptake in human immune cells in a nearly physiological setting.
Methods: The prostate cancer cell line PC3 was stably transfected with GFP-tagged CD63 and a high-expression clone was selected. CD63-GFP expression in EVs was verified by western blot. The CD63-GFP-expressing PC3 cells were cultured with human peripheral blood mononuclear cells (PBMCs) from healthy donors in 3D multicellular spheroid co-cultures. EV uptake by PBMCs was

analysed by flow cytometry and confocal microscopy with and without several endocytosis inhibitors.

Results: After co-culturing of PC3-CD63-GFP cells and PBMCs in a 3D multicellular spheroid setting, the highest uptake of CD63-labelled EVs was observed in CD19+ B cells, followed by CD8+ and CD3+ T cells. EV uptake in B cells was not affected by any of the tested endocytosis inhibitors, while the macropinocytosis inhibitor EIPA reduced EV uptake in CD3+ T cells.

Conclusions: This model allows to quantify the uptake of cancer-derived EV in human immune cells using continuous and probably nearly physiologically relevant amount of cancer-derived EVs that are produced in the co-culture microenvironment, thus mimicking the tumour–immune cell interactions in the tumour microenvironment.

Keywords: extracellular vesicles – cancer – immune cells – 3D spheroid co-culture – endocytosis

A2.17

Influence of anthocyanins on the adipogenic and chondrogenic differentiation of human adipose mesenchymal stem cells

Liga Saulite[1,*], Kaspars Jekabsons[1], Ineta Popena[1], Ruta Muceniece[1], Maris Klavins[2] and Una Riekstina[1]

[1]Faculty of Medicine, University of Latvia, Riga, Latvia; [2]Faculty of Geography and Earth Sciences, University of Latvia, Riga, Latvia

**Corresponding author: Liga Saulite, Faculty of Medicine, University of Latvia, 19 Raiņa Blvd., LV-1568, Riga, Latvia.*

E-mail: liga.saulite@lu.lv

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Background: Anthocyanins are flavonoids responsible for the pigmentation in plants [1]. Anthocyanins are known for their anti-oxidative, anti-inflammatory and anti-tumor properties [2]. The influence of anthocyanins on the reduction of obesity and diabetes has been a subject of discussion in recent years. It has been shown that consumption of anthocyanins lowers the risk of obesity and type 2 diabetes [3]. However, the effects of anthocyanins on the chondrogenic differentiation has not yet been studied in detail.

Objectives: Aim of this study was to evaluate the effect of anthocyanidins—malvidin, cyanidin, delphinidin—on the adipogenic and chondrogenic differentiation of human adipose mesenchymal stem cells (aMSCs).

Methods: The cytotoxicity of anthocyanidins was evaluated by the CCK-8 assay and the non-toxic concentration of 25 µM was used in the further experiments. aMSCs (purchased from ATCC) were differentiated into adipocytes and chondrocytes by Gibco® StemPro® differentiation kits for 21 and 14 days respectively. 25 µM malvidin, cyanidin and delphinidin (all from Sigma Aldrich) were added to the differentiation medium. Medium change was done each 2–4 days. The expression of adipogenesis genes *adiponectin*, *FABP4*, *LPL* and chondrogenesis genes *Sox9*, *Col2a1*, *aggrecan* and *TGF-β1* was analyzed by qPCR.

Results: Anthocyanidins induced a concentration-dependent cytotoxicity in aMSCs after 24–72 h incubation. 25 µM was assumed as optimal anthocyanidin concentration for the differentiation. All tested anthocyanidins decreased the expression of the adipogenesis marker *adiponectin*; however, only delphinidin decreased *FABP4* and *LPL* expression in aMSC after adipogenic differentiation. Malvidin increased the expression of chondrogenic markers *Sox9*, *Col2a1* and *TGF-β1*, delphinidin increased the expression of *Col2a1*, but cyanidin had no effect on chondrogenesis marker expression in aMSC after differentiation.

Conclusions: Anthocyanidins affect the differentiation efficiency of aMSC into adipogenic and chondrogenic lineages. Delphinidin has the highest activity in the reduction of adipogenesis and malvidin has a chondrogenesis-promoting capacity in aMSCs.

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Keywords: anthocyanidins – malvidin – cyanidin – delphinidin – mesenchymal stem cells

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A2.18

Cytotoxic, antiradical activity and limited stability of anthocyanidins in human cell cultures

Kaspars Jekabsons[1,*], Ilva Nakurte[2], Reinis Rembergs[1], Jana Namniece[1], Liga Saulite[1], Ineta Popena[1], Kristine Saleniece[1], Zane Dzirkale[3], Una Riekstina[1], Maris Klavins[4] and Ruta Muceniece[1]

[1]Department of Pharmacy, Faculty of Medicine, University of Latvia, Riga, Latvia; [2]Department of Physical Chemistry, Faculty of Chemistry, University of Latvia, Riga, Latvia; [3]Department of Pharmacology, Faculty of Medicine, University of Latvia, Riga, Latvia; [4]Department of Environmental Science, Faculty of Geography and Earth Sciences, University of Latvia, Riga, Latvia

**Corresponding author: Kaspars Jekabsons, Department of Pharmacy, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: kaspars.jekabsons@lu.lv*

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Background: Anthocyanins (ACs) are molecules where a sugar moiety is bound to another non-sugar functional group (aglycone: anthocyanidin; ACdn). Numerous studies have shown that both ACs and ACdns are biologically active. The ACdns are limited to a few structure variants, such as delphinidin, cyanidin, pelargonidin, peonidin and malvidin. Although effects of ACdns have been studied in antioxidant and cell proliferation assays, their metabolism in biological fluids and different cell line culture *in vitro* assays is still to be investigated [1, 2, 3].

Objectives: The aim of this study was to compare biological activity of malvidin (**M**), delphinidin (**D**) and cyanidin (**C**) in different human cell lines, and to study their metabolism in cell culture environment.

Methods: Influence on cell proliferation of ACdns (Sigma-Aldrich, USA) at concentrations of 25, 50 and 100 µM was investigated by using ViaCount and CCK-8 tests; antiradical activity was analysed using DPPH assay. Stability of ACdns in cell culture media after 24 h was evaluated by UHPLC-TOF-MS/MS method. The following human commercial cell lines have been used: monocytic leukemic cell line THP-1 (ECACC, UK), adipose mesenchymal stem cells (aMSCs), breast adenocarcinoma cell line MCF-7 and metastatic breast adeno-carcinoma cell line MDA-MB-231 (ATCC, USA).

Results: ACdns inhibited growth of THP-1 and aMSCs, whereas their effects on MCF-7 and MDA-MB-231 cell proliferation was negligible. **D** showed the most obvious anti-proliferative effect. **C** exerted stronger antiradical activity. ACdns were not detected in the cell supernatants after 24 h. Instead, we identified phenolic acids, the main metabolites of ACdns, namely, metabolite of **C**: protocatechuit

acid (PA), metabolite of **D**: gallic acid (GA), and metabolite of **M**: syringic acid (SA). Concentrations of PA and SA increased accordingly to the added concentration of ACdns with the exception of GA. GA was not identified in aMSC cell medium, but in THP-1 and MCF-7 cells the level of GA did not reflect the added amount of **D**.

Conclusions: ACdns possess cell line-selective cytotoxicity and limited life time in cell culture. The influence of solvents and oxidizing substances in cell media is under investigation.

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Keywords: anthocianidins – cell lines – cytotoxicity – antiradical activity – phenolic acids

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A2.19

Metabolic-targeted therapy with dichloroacetate and metformin: a novel treatment strategy for breast cancer

Laura MARTINKUTE* and Edgaras STANKEVICIUS

Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas, Lithuania

*Corresponding author: *Laura Martinkute, Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Zemales 29-3, LT-48269 Kaunas, Lithuania.*

E-mail: martinkutelaura@gmail.com

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Background: Most cancer cells produce energy by increasing aerobic glycolysis even in the presence of oxygen. This phenomenon is often referred as the Warburg effect [1]. Dichloroacetate (DCA) was observed to reverse the Warburg effect by inhibiting pyruvate dehydrogenase kinase and indirectly activating the gate-keeping enzyme pyruvate dehydrogenase. DCA shifts aerobic glycolysis towards mitochondrial glucose oxidation in cancer cells thus forcing them to attain apoptosis [2]. Metformin (Met), a widely used oral anti-diabetic agent, has been shown to have a strong anti-proliferative effect in many tumor cell lines.

Objectives: The aim of this study was to investigate the anticancer effect of DCA and Met in breast cancer *in vitro*. We hypothesized that these two agents could synergistically potentiate cytotoxic effects and induce cancer cell apoptosis.

Methods: MCF-7, MDA-MB-231 and MDA-MB-468 breast cancer cell lines were treated with different DCA and Met concentrations (1 mM, 5 mM, 10 mM and 20 mM) or their combinations. Cells were exposed to drugs for 24, 48 and 72 hours. Annexin V/PI-stained cells were analysed by flow cytometry. Flow-cytometry results were confirmed by viewing the cells under fluorescence microscope. The cell-growth-inhibitory effect of DCA, metformin and DCA–metformin was assessed by various cell viability assays.

Results: Dichloroacetate and metformin effectively sensitized breast cancer cell lines to apoptosis. The highest apoptotic cell rate was observed in MCF-7 breast cancer cell line after 24 hours of 20 mM of drug exposure.

Conclusions: This study demonstrates that targeting two key metabolic hallmarks of cancer is an effective anti-cancer strategy with therapeutic potential.

Acknowledgements: This research is part of a PhD project and is supported by funding of the Lithuanian University of Health Sciences.

Keywords: dichloroacetate – metformin – breast cancer – Warburg effect

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A2.20

Impact of long-chain acylcarnitines on muscle insulin sensitivity and interaction with Akt-related insulin signalling pathway

Karlis VILKS[1,2,*], Kristine VOLSKA[3], Elina MAKAROVA[1], Marina MAKRECKA-KUKA[1], Maija DAMBROVA[1,3] and Edgars LIEPINSH[1]

[1]Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Biology, University of Latvia, Riga, Latvia; [3]Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Karlis Vilks, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia. E-mail: karlis.vilks@farm.osi.lv*

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Background: Accumulation of acylcarnitines, the intermediates of fatty acid metabolism, has been linked to insulin resistance [1, 2], but molecular mechanisms of induced disturbances are still unclear.

Objectives: The aim of this study was to clarify the effects of elevated long-chain acylcarnitine (LCAC) concentration in skeletal muscle on protein kinase B (Akt)-related insulin signalling pathway.

Methods: In *in vivo* experiments, palmitoylcarnitine (PC) was administered intraperitoneally to male CD-1 mice at a dose of 50 or 100 mg/kg. Plasma glucose and insulin concentrations were measured 60 min after PC administration in the fasted and fed state. Additionally, radiolabelled deoxy-D-glucose ([^{3}H]DOG) uptake in muscles was determined. Muscle tissues were collected for further analysis. Differentiated C2C12 mouse myoblasts were incubated overnight with PC (at concentrations of 5 and 10 μM) and stimulated with insulin (100 nM for 15 min). To evaluate the LCAC-induced effect on Akt phosphorylation, cells and muscle tissues were analysed by western blot. Gene expression of *GLUT1*, *GLUT4*, *CPT1A*, *CPT1B* and *ACSL* in muscles was determined by quantitative RT-PCR.

Results: Administration of PC increased muscle LCAC content by 3-fold corresponding to short-term fasting. Blood glucose concentration was increased by 30% in fed and fasted states. Insulin concentration after PC injection was increased by 5-fold in fasted state and 2-fold in fed state. PC administration also significantly decreased insulin-dependent [^{3}H]DOG uptake in skeletal muscles. PC did not affect the expression of genes involved in muscle glucose transport and FA metabolism *in vivo*. PC treatment decreased Akt Ser^{473} phosphorylation in the C2C12 muscle cells and in animal muscle

tissue. However, insulin *in vitro* (at a concentration 100 nM) and *in vivo* (additional administration 0.3 U/kg) overcame the PC-induced effect on Akt phosphorylation.

Conclusions: Results demonstrate that increase in LCAC content induces muscle insulin resistance by impairing insulin signalling. These effects are not caused by changes in gene expression, but through the insulin signalling pathway by inhibiting Akt phosphorylation at Ser^{473}.

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Keywords: acylcarnitines – insulin resistance – protein kinase B

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A2.21

The comparative effects of carnitine and γ-butyrobetaine on elimination of meldonium: competition for OCTN2-mediated transport

Elina MAKAROVA[1,*], Eduards SEVOST JANOVS[2], Helena CIRULE[1], Olga ZARKOVA-MALKOVA[1], Dace HARTMANE[2] and Maija DAMBROVA[1,3]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Laboratory of Physical Organic Chemistry. Latvian Institute of Organic Synthesis, Riga, Latvia; [3]Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Elina Makarova, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia. E-mail: elina.makarova@farm.osi.lv*

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Background: The inhibition of organic cation transporter 2 (OCTN2) leads to a decrease in carnitine and acylcarnitine contents in tissues and energy metabolism optimization-related cardioprotective effects. The most potent clinically used inhibitor of OCTN2, the anti-ischemic drug meldonium, was recently included in the World Anti-Doping Agency List of Prohibited Substances and Methods due to possible enhancement of physical performance. In addition to the transport of endogenous carnitine, OCTN2 ensures the kidney reuptake of exogenous compounds, their transport to and accumulation in tissues and it could be responsible also for the observed unusually long elimination time of meldonium.

Objective: The aim of this study was to test the rate of meldonium washout after the end of the treatment and compare the effects on the washout duration of OCTN2 substrates, carnitine and γ-butyrobetaine (GBB), to evaluate the importance of competition for OCTN2 transport and pharmacokinetics of meldonium in mice.

Methods: Twenty-five male SW mice (6 weeks old, Tartu, Estonia) were divided into 5 groups. Twenty mice received meldonium (400 mg/kg) with drinking water for 2 weeks. One group was sacrificed after meldonium treatment; the other groups received water, GBB (200 mg/kg) and carnitine (200 mg/kg) with drinking water for one week to evaluate washout. Plasma, urine, and tissue samples were collected and stored at −20°C. The concentrations of meldonium, carnitine and GBB were measured using the UPLC/MS/MS method.

Results: Administration of carnitine and GBB effectively stimulated the washout of meldonium. GBB had a more pronounced effect on meldonium elimination than carnitine due to the higher affinity of GBB for OCTN2.

Conclusions: The competition of meldonium, carnitine and GBB for OCTN2-mediated transport determines the pharmacokinetic properties of meldonium. The unusually long washout period of meldonium after long-term treatment is determined by OCTN2-mediated transport that ensures a high muscle content of meldonium, while tissue clearance depends on relatively slow diffusion.

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Keywords: meldonium – carnitine – γ-butyrobetaine – pharmacokinetic

A2.22

Differences between open and closed head injury: evaluation of weight-drop model in experimental traumatic brain injury

Einars KUPATS[1,*], Edijs VAVERS[1,2], Janis KUKA[2], Baiba SVALBE[2], Baiba ZVEJNIECE[2], Liga ZVEJNIECE[2] and Maija DAMBROVA[1,2]

[1]Rīga Stradiņš University, Riga, Latvia; [2]Latvian Institute of Organic Synthesis, Riga, Latvia

**Corresponding author: Einars Kupats, Rīga Stradiņš University, Riga, Latvia. E-mail: einarskupats@gmail.com*

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Background: Traumatic brain injury (TBI) is one of the leading causes of mortality and morbidity in people under the age of 45 years and more than 75% of TBI cases are closed head injuries. To study closed head injury one of the most used experimental models is the weight-drop model. The incidence of skull fractures in the weight-drop model is more than 30%. We hypothesize that the heterogeneity of initial injury induces different pathophysiological mechanisms and neurological outcomes following TBI.

Objectives: The aim of the present study was to evaluate and compare potential risk factors of skull fractures and aspects of neuroinflammation between closed and open weight-drop induced TBI model.

Methods: The weight-drop TBI model was used to induce head injury in male SW mice. Interleukin (IL)-6, IL-1β and tumor necrosis factor alpha (TNFα) were measured by quantitative real-time PCR analysis in the hippocampus 12 h and 1 and 3 days after TBI with and without fracture. The neurobehavioral status of SW mice was assessed by the neurological severity score (NSS). To ensure exact anatomical reference and correlate skull thickness and respective force required to induce sufficient TBI, computed tomography scans were performed (30 keV, 0.95 mA, 250 ms at 720 projections). 2-mm and 5-mm cone tips were used in the weight-drop model to compare the impact difference on NSS and skull fracture incidence.

Results: Weight-drop impact with fracture induced a 3- to 10-fold difference in the expression levels of inflammatory genes IL-6, IL-1β and TNFα compared to animals without fracture. The average SW mice parietal bone thickness varied from 0.22 to 0.30 mm. Decreased parietal bone thickness was associated with an increased risk of fractures. Parietal bone fractures occurred in 10% using a 5-mm-diameter teflon-tipped cone, while a 2-mm-diameter cone induced fractures in 33% of cases. In addition, NSS was significantly higher in animals after TBI using a 5-mm cone.

Conclusions: To produce a homogenous type of injury and more reproducible NSS results, a 5-mm-diameter cone should be used in the weight-drop TBI model.

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Keywords: traumatic brain injury – weight-drop model – skull fracture – neuroinflammation

A2.23

Corticosterone induces DNA methyltransferases expression in rat cortical neurons

Mari URB[1], Kaili ANIER[1], Terje MATSALU[1], Anu AONURM-HELM[1], Indrek KOPPEL[2], Tõnis TIMMUSK[2] and Anti KALDA[1,*]

[1]*Department of Pharmacology, University of Tartu, Estonia;*
[2]*Department of Chemistry and Biotechnology, Tallinn University of Technology, Tallinn, Estonia*
**Corresponding author: Anti Kalda, Institute of Biomedicine and Translational Medicine, University of Tartu, Ravila 19, 50411, Tartu, Estonia. E-mail: anti.kalda@ut.ee*

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Background: Corticosterone is the main glucocorticoid hormone involved in stress responses in rodents. It is established that corticosterone exerts its effects via glucocorticoid receptor (NR3C1) and mineralocorticoid receptor that regulate downstream gene expression during development and adulthood [1]. In our previous study, we have shown that maternal separation on postnatal day 15 (PND15) increases DNA methyltransferase (DNMT) 1, 3A and 3B expression levels in rat nucleus accumbens lasting into adulthood [2]. However, the exact mechanism how maternal separation alters DNMT expression is unclear.

We hypothesize that stress-induced NR3C1 stimulation may increase the expression levels of DNMT and alter long-term DNA methylation/demethylation balance in infant rat brain.

Objectives: Our aim is to evaluate the effect of corticosterone and maternal separation on the expression levels of DNMT in rat cortex and cortical neurons.

Methods: Wistar rats were separated from mothers and littermates on PND 2–14 for 15 minutes (MS15) or 180 minutes (MS180) per day. Animal-facility-reared (AFR) group animals were not separated. Rats were decapitated on PND15, and *Dnmt1*, *Dnmt3a* and *Dnmt3b* mRNA levels in rat prefrontal cortex were measured with RT-qPCR. Plasma corticosterone levels were measured with ELISA. Increased relative DNMT3A protein levels were detected by western blotting in MS180 rats.

Results: Increased plasma corticosterone levels were detected in maternally separated rats. Higher mRNA and protein levels of DNMT3A, and higher mRNA levels of DNMT1 and DNMT3B in rat cortex at postnatal day 15 suggest that elevated corticosterone upregulates DNMTs expression. In rat primary cortical neurons, corticosterone treatment increased mRNA levels of DNMT3A and DNMT3B, which was attenuated by the glucocorticoid receptor antagonist mifepristone. NR3C1 was enriched in *Dnmt3a* promoter after 1 h corticosterone treatment.

Conclusions: The following results suggest that elevated corticosterone upregulates *Dnmt3a* and *Dnmt3b* expression: (1) mRNA levels of *Dnmt3a* and *Dnmt3b*, and protein levels of *Dnmt3a* were increased in the prefrontal cortex of MS180 rats on postnatal day 15; (2) glucocorticoid receptor mediates *Dnmt3a* and *Dnmt3b* mRNA upregulation after corticosterone treatment in primary cortical neurons; (3) NR3C1 binding was detected at *Dnmt3a* promoter in primary cortical cells after corticosterone treatment.

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Keywords: glucocorticoid receptor – DNA methylation – maternal separation – prefrontal cortex – rat

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A2.24

Effects of alendronate on carbohydrate metabolism and behavior in young healthy rats

Silvia GANCHEVA* and Maria ZHELYAZKOVA-SAVOVA

Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University, Varna, Bulgaria
**Corresponding author: Silvia Gancheva, Department of Pharmacology and Clinical Pharmacology and Therapy, Faculty of Medicine, Medical University, 55 Marin Drinov Str., Varna 9002, Bulgaria. E-mail: silvi_gancheva@abv.bg*

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Background: Osteocalcin is a vitamin K-dependent protein that is synthesized by osteoblasts and incorporated in the bone matrix in its carboxylated form (cOC). During the process of bone resorption, osteocalcin undergoes a decarboxylation reaction and is released in the circulation in its un(der)carboxylated form (ucOC). Experimental studies performed on osteocalcin-deficient mice revealed that ucOC functions as a hormone regulating energy homeostasis [1] and controlling behavior [2].

Alendronate is a bisphosphonate used for treatment and prophylaxis of osteoporosis. It increases bone mineral density by inhibiting bone resorption. As a result, alendronate impairs the release of ucOC in the circulation and decreases its plasma concentration [3].

Objective: The aim of the present study was to estimate the effect of alendronate on carbohydrate metabolism and behavior in young healthy rats.

Methods: 24 male Wistar rats were divided in two groups: an experimental group, receiving alendronate subcutaneously three times weekly in a dose of 50 µg/kg body weight, and a control group receiving saline. The rats had free access to food and water. The duration of the study was 15 weeks. At the end of the experiment biochemical and behavioral tests were performed. Carbohydrate metabolism was evaluated by measuring the fasting blood glucose level (FBG) and performing an insulin tolerance test (ITT). The area under the BG–time curve (AUC) was calculated. To evaluate the locomotor activity, we used the open field test (OFT); we assessed anxiety by the social interaction test (SIT) and depression-like behavior by the forced swimming test (FST). Student's *t*-test was used for analysis.

Results: The FBG was higher in alendronate treated animals, as was the AUC from the ITT. Alendronate had no effect on locomotion in the OFT and on the immobility time in the FST. However, the time of social interaction in the SIT was significantly reduced in the alendronate-treated group indicating an anxiety-like behavior.

Conclusion: Alendronate treatment impaired carbohydrate metabolism and caused an anxiety-like behavior in rats. We are tempted to speculate that alendronate produced the observed effects by reducing the plasma level of un(der)carboxylated osteocalcin thus impairing its physiological role as a regulator of energy homeostasis and behavior.

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Keywords: alendronate – fasting blood glucose – insulin tolerance test – anxiety

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A2.25

Hypoglycaemia-induced alterations in vascular reactivity

Reinis VILSKERSTS[1,2,*], Rudolfs MEZHAPUKE[1] and Maija DAMBROVA[1,2]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Department of Pharmaceutical Chemistry, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Reinis Vilskersts, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia. E-mail: vilskersts@farm.osi.lv*

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Background: Hypoglycaemia is a life-threatening condition characterized by low blood glucose levels and it is capable to induce failure of organ function and their damage. Vascular tone is regulated by several mediators and hypoglycaemia alters their effects on the vascular wall. Altered vascular reactivity could cause inadequate blood and oxygen supply and thus propagate organ injury.
Objectives: To study the effects of hypoglycaemia on vascular reactivity to physiological mediators in different blood vessels in *ex vivo* experimental models.
Methods: Aortic and femoral artery rings were prepared from CD-1 male mice. The effects of hypoglycaemia on the vascular reactivity were assessed in vessel rings 30 min after subsequent incubation in Krebs-Henseleit buffer solutions containing 5.5, 2.2 and 5.5 mM glucose. The response to vasoconstrictors (angiotensin II, endothelin-1, phenylephrine and serotonin) was tested at the end of incubation with each buffer solution. The response to vasodilatators (acetylcholine, adenosine, bradykinin and histamine) was also tested after incubation with each buffer solution in blood vessel rings precontracted with phenylephrine to a submaximal level.
Results: Incubation of aortic rings in buffer solution with 5.5 mM glucose after hypoglycaemia enhanced phenylephrine-induced vasoconstriction by 30%. Fluctuations in glucose concentration did not alter vascular reactivity of aortic rings to serotonin and angiotensin II. In addition, after hypoglycaemia the response to all tested vasoconstrictors was not changed in femoral artery rings. Hypoglycaemia decreased acetylcholine-induced vasorelaxation in aortic rings and femoral artery rings. Decreased response to acetylcholine was present also after incubation of vessel rings of both vessels for 30 min in buffer solution with 5.5 mM glucose. Moreover, exposure of femoral artery rings to hypoglycaemia slightly impaired the relaxation response to adenosine and bradykinin.
Conclusions: The obtained results demonstrate that hypoglycaemia and fluctuations in glucose concentrations impair acetylcholine-induced vasorelaxation and enhance phenylephrine-induced vascular constriction in conductance arteries. Hypoglycaemia-induced alterations in vascular reactivity can contribute to the development of hypoxic organ injury.
Acknowledgements: This study was supported by the Latvian State Research Program BIOMEDICINE.
Keywords: hypoglycaemia – vascular reactivity – aorta – femoral artery

A2.26

The evaluation of long-time neurological disabilities in mice after traumatic brain injury

Baiba SVALBE[1,*], Edijs VAVERS[1,2], Gundega VIKMANE[1], Lasma LAUBERTE[1], Maija DAMBROVA[1,2] and Liga ZVEJNIECE[1]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Baiba Svalbe, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Aizkraukles 21, Riga, LV-1006, Latvia. E-mail: baibas@farm.osi.lv*

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Background: Each year approximately 1.5 million people in Europe suffer from acquired complications after traumatic brain injury (TBI). It has become evident that surviving patients often develop progressive brain atrophy, depression and dementia of unknown origin resulting in significant morbidity. The hypothesis of the current study is that acute TBI triggers a chronic neuroinflammatory response which causes progressive post-traumatic neurodegeneration, cognitive decline and dementia. Currently, we lack experimental models and treatments that could tackle these chronic complications induced by TBI.
Objectives: The aim of the study is to characterize and understand long-lasting neurodegenerative changes occurring after lateral fluid percussion injury (latFPI).
Methods: Male Balb/c mice were subjected to latFPI and compared with sham-operated mice. The behaviour tests were done before and at 1, 7, 30, 90 and 180 days post-injury. The sensory-motor function was assessed by the neurological severity score (NSS) test. The motor coordination of the animal was evaluated by the accelerated rotarod test. The passive avoidance response (PAR), Y-maze and Morris water maze (MWM) tests were used to assess cognitive functionality after latFPI. Depression-like behaviour was assessed by the tail suspension test.
Results: In the NSS score test, sensory-motor function was significantly impaired during the observation period of 180 days after latFPI compared with sham-operated mice. In the rotarod test, the motor coordination was reduced on post-injury days 1 and 7. In the MWM test, the spatial memory was significantly impaired on post-injury days 30 and 90. In the Y-maze test, the latFPI group exhibited significant impairment of working memory on post-injury day 180. In the tail suspension test latFPI mice did not exhibit depression-like behaviour.
Conclusions: LatFPI elicits immediate and long-lasting impairments of sensory-motor functions, while the memory impairment is associated with progressive post-traumatic neurodegeneration. This knowledge suggests novel therapeutic strategies which may protect the brain from chronic post-traumatic atrophy and functional decline.
Acknowledgements: The study was supported by the framework of EU-ERA-NET NEURON CnsAflame and the European Regional Development Fund project no. 1.1.1.2/VIAA/1/16/244.
Keywords: traumatic brain injury – fluid percussion injury – memory – sensory-motor function

A2.27

Pharmacokinetics of levofloxacin after single intravenous and subcutaneous administration in domestic goats (*Capra hircus*): a pilot study

Andrejs SITOVS[1,*], Cristina VERCELLI[2], Beata LEBKOWSKA-WIERUSZEWSKA[3] and Mario GIORGI[4]

[1]Department of Pharmacology, Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia; [2]Department of Veterinary Sciences, University of Turin, Italy; [3]Department of Pharmacology, University of Life Sciences, Lublin, Poland; [4]Department of Veterinary Sciences, University of Pisa, Italy

**Corresponding author: Andrejs Sitovs, Department of Pharmacology, Faculty of Pharmacy, Rīga Stradiņš University, 13 Pilsoņu Str., LV-1002, Riga, Latvia. E-mail: andrejs.sitovs@rsu.lv*

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Background: Fluoroquinolones are widely used for the treatment of bacterial infections in humans and veterinary medicine. However, development of bacterial resistance to fluoroquinolones has been reported [1]. This may lead to the loss of effectiveness in the treatment of bacterial infections with the drugs of this group. Levofloxacin, a third-generation fluoroquinolone approved for human medicine only, shows excellent antibacterial activity against a variety of microorganisms. Several pharmacokinetic studies showed that it could be used in veterinary species such as bovine, poultry and small ruminants.

Objectives: To study the pharmacokinetic profiles of levofloxacin after single intravenous and subcutaneous administration in goats.

Methods: Seven female, clinically healthy domestic goats (body mass 52–64 kg) received 500 mg per animal of levofloxacin via intravenous (4 goats) and subcutaneous (3 goats) administration. Blood samples were collected at 5, 15, 30, 45 minutes and 1, 1.5, 2, 4, 6, 8, 10, 24, 34 and 48 hours after the administration. Serum levofloxacin concentrations were analyzed using the HPLC system, according to an existing chromatographic method [2]. Enrofloxacin was used as an internal standard. Pharmacokinetics was fit according to bicompartimental analysis by WinNonlin 5.3 software.

Results: No adverse effects were observed during the experiment. Levofloxacin was detectable up to 24 hours after both subcutaneous and intravenous administrations. Mean plasma clearance (i.v. group) was 440.5 ± 99.3 ml/h/kg, elimination half-life 1.65 ± 0.26 h, and volume of distribution at steady state 905.5 ± 91.2 ml/kg. After s.c. administration, levofloxacin showed elimination half-life of 16.2 ± 10.0 h, maximum plasma concentration of 3,247.7 ± 727.0 ng/ml and time at the maximum drug concentration of 2.19 ± 0.16 h. This suggests the presence of a flip-flop phenomenon. Subcutaneous bioavailability was 93.4 ± 6.0%.

Conclusions: This is the first study that compares the intravenous and subcutaneous routes of administration of levofloxacin in domestic goats. The pharmacokinetic trend of levofloxacin appeared to be somewhat similar to that reported for other small ruminants [3]. Second phase of this crossover study will contribute more data.

Keywords: levofloxacin – goat – pharmacokinetics – flip-flop kinetics

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A2.28

Protective effects of pharmacologically decreased long-chain acylcarnitine contents in the preclinical models of diabetes and its complications

Kristine VOLSKA[1,2,*], Marina MAKRECKA-KUKA[1], Elina MAKAROVA[1], Janis KUKA[1], Reinis VILSKERSTS[1,2], Edgars LIEPINSH[1] and Maija DAMBROVA[1,2]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Kristine Volska, Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Aizkraukles 21, LV-1006, Riga, Latvia. E-mail: kristine.volska@farm.osi.lv*

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Background: Incomplete fatty acid oxidation and subsequent accumulation of fatty acid intermediates long-chain acylcarnitines have been linked to development of insulin resistance and cardiovascular diseases. We hypothesised that decreasing the long-chain acylcarnitine content may represent an effective strategy for the treatment of diabetes and cardiovascular complications related to diabetes.

Objective: To investigate the protective effects of the acylcarnitine concentration-lowering drug methyl-GBB in experimental animal models of diabetes, cardiac ischemia-reperfusion injury and atherosclerosis.

Methods: Female apolipoprotein E knockout (apoE$^{-/-}$) mice, C57BL/6 male mice fed with high-fat diet, male CD-1 mice, diabetic db/db mice, non-diabetic db/lean male mice and male Wistar rats were used for the experiments. To lower long-chain acylcarnitine contents, chronic methyl-GBB treatment was used. In diabetic mice models, glucose and insulin tolerance tests were performed. In apoE$^{-/-}$ mice, the TNFα concentration in the plasma, the amount of atherosclerotic lesions and the number of immune cells in atherosclerotic lesions were analysed. The effects of methyl-GBB treatment on infarct size were investigated in an isolated rat heart infarction model.

Results: Methyl-GBB treatment induced a substantial decrease in tissue and plasma long-chain acylcarnitine concentrations in both fed and fasted states of animals in all experimental models. Both in db/db and high-fat-diet-fed C57BL/6 mice methyl-GBB administration (5 mg/kg) improved insulin sensitivity and significantly reduced blood glucose and insulin levels. In apoE$^{-/-}$ mice, treatment with methyl-GBB at a dose of 10 mg/kg reduced the TNFα concentration in the plasma 2.4-fold and decreased the infiltration of macrophages and monocytes into the aortic lesions of the aortic root. Furthermore, methyl-GBB treatment reduced the size of atherosclerotic plaques by 36%. The methyl-GBB (20 mg/kg) pretreatment-induced decrease in acylcarnitine content protected against acute ischaemia-reperfusion-induced damage in the isolated rat heart model by decreasing the infarct size by 44%.

Conclusions: Methyl-GBB treatment decreases the acylcarnitine contents and attenuates the development of insulin resistance, atherosclerosis and diminishes the damage induced by ischemia-reperfusion. The pharmacologically reduced long-chain acylcarnitine content represents an effective strategy to improve insulin sensitivity and to protect the heart against ischemia-reperfusion-induced damage and development of atherosclerosis.

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Keywords: long-chain acylcarnitine – methyl-GBB – diabetes mellitus – atherosclerosis – ischemia-reperfusion injury

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A2.29

Alterations in the polysialylated neural cell adhesion molecule (PSA-NCAM) and ganglion cell density in the retina after experimentally induced diabetes in mice

Natalia LOBANOVSKAYA*, Monika JÜRGENSON, Anu AONURM-HELM and Alexander ZHARKOVSKY

Department of Pharmacology, Center of Excellence for Translational Medicine, University of Tartu, Estonia
**Corresponding author: Natalia Lobanovskaya, Department of Pharmacology, Center of Excellence for Translational Medicine, University of Tartu, 19 Ravila, 50411, Tartu, Estonia.*
E-mail: natalia.lobanovskaya@gmail.com

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Background: Diabetic retinopathy affects retinal ganglion cells (RGCs) and glial cells. The mechanisms of development of diabetic retinopathy are still unclear. Some studies have suggested a role for polysialylated neural cell adhesion molecule (PSA-NCAM) in the survival of RGCs [1]. PSA-NCAM belongs to the immunoglobulin superfamily of adhesion molecules. It is abundantly expressed by astrocytes and Müller cells in the adult retina in close proximity to RGCs.

Objectives: The aim of this study was to investigate whether or not diabetic retinopathy is associated with alterations in PSA-NCAM expression in the adult mouse retina.

Methods: Diabetes was induced in 2.5-months-old Swiss Webster mice by intraperitoneal injection of streptozotocin. Examination of the proteins of interest in the retinas from diabetic mice at 2 months after induction was performed using immunohistochemistry and western blot analysis.

Results: In diabetic mice, a considerable reduction in RGC density was observed. The reduced density of RGC was associated with a redistribution of PSA-NCAM. PSA-NCAM immunoreactivity was diminished in the inner part of the retina where RGCs are located, and enhanced in the outer layers of the retina. Previous studies have shown that matrix metalloproteinase 9 (MMP-9) is responsible for the reduction in PSA-NCAM levels in neuronal cells [2]. We found that MMP-9 expression was much higher in the inner part of diabetic retinas compared with the controls and this might explain the observed decline of PSA-NCAM in this portion of the retina.

Conclusions: We propose that MMP-9 induces shedding of PSA-NCAM in diabetic retina. Thus, the decreased levels of PSA-NCAM located in the inner part of the retina might be, at least in part, responsible for the observed loss of RGCs in diabetic mice.

Acknowledgements: This study was supported by the Estonian Science Council grant (institutional research founding) IUT23, the Archimedes Foundation and the European Regional Development Fund.

Keywords: diabetic retinopathy – retinal ganglion cell – matrix metalloproteinase 9 – PSA-NCAM

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A2.30

Anti-inflammatory effects of Jathyadi Thailam plant extracts *in vitro*

Somit KUMAR[1,*], Ramona PETROVSKA[2], Valdis PIRAGS[1], Tatjana TRACEVSKA[1] and Ilona MANDRIKA[2]

[1]Faculty of Medicine, University of Latvia, Riga, Latvia;
[2]Latvian Biomedical Research and Study Centre, Riga, Latvia
**Corresponding author: Somit Kumar, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia.*
E-mail: drsomit.kumar@gmail.com

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Background: Diabetic foot ulcer is one of the most common complications of diabetes mellitus. In the diabetic foot, the process of wound healing is impaired and associated with inflammation, immune cell infiltration and increased production of proinflammatory cytokines and chemokines. Jathyadi Thailam is an Ayurvedic herbal oil with antimicrobial properties which has been used in traditional medicine for the treatment of nonhealing wounds, ulcers, and eczema [1].

Objective: The present study evaluated the effects of polar and nonpolar plant extracts of Jathyadi Thailam on anti-inflammatory activities in lipopolysaccharide (LPS)-stimulated diabetic foot patient-derived fibroblasts *in vitro*.

Methods: Fibroblasts were isolated from the wound site by punch biopsy of 4 mm from a non-granulating part of the chronic ulcers to make sure we were studying chronic nonhealing tissue and not newly arrived fibroblasts during debridement. All tissue samples were minced into fine pieces and incubated in 1X Accutase for 30 min at 37 °C. The suspension was filtered through an infusion chamber, the cells were centrifuged, washed in phosphate-buffered saline (PBS) and resuspended in culture medium (Dulbecco's modified Eagle's medium; DMEM) with 10% fetal calf serum (FCS), 100 IU/ml penicillin and 100 IU/ml streptomycin [2]. Cells were seeded into cell-culture plates at a density of 1×106 cells/well and stimulated with 1 µg/ml LPS in the presence or absence of Jathyadi Thailam nonpolar (50 µg/ml) and polar (10 µg/ml) plant extracts for 4 h. Inflammatory chemokines and cytokines MCP-1, IL-8, IL-1β, and transcription factor gene transcripts were evaluated by quantitative real-time PCR [3].

Results: LPS stimulation markedly upregulated the proinflammatory cytokines interleukin MCP-1, IL-1β and IL-8 gene expression in fibroblasts. The nonpolar extract of Jathyadi Thailam (at 50 µg/ml) was able to inhibit LPS-stimulated MCP-1 (by 88–71.2%), IL-8 (by 14–61.2%) and IL-1β (by 77.7–60.3%) expression. Furthermore, treatment with the polar extract (at 10 µg/ml) led to the suppression of MCP-1 (by 63.5–49.2%), IL-8 (by 14–16%) and IL-1β (by 22.6–66.6%) of LPS-induced expression of inflammation.

Conclusions: These findings indicate that a polar fraction of Jathyadi Thailam plant extracts exhibited potent anti-inflammatory properties by suppressing LPS-stimulated proinflammatory gene expression as compared to a nonpolar fraction.

Keywords: Jathyadi Thailam – fibroblasts – inflammatory cytokines – non-healing diabetic wound

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A2.31

Antimicrobial properties of Indian traditional medicinal polyherbal formulation

Baiba ZANDERSONE[1,*], Somit KUMAR[1,2], Iveta LIDUMA[1], Sintija SAUŠA[1] and Tatjana TRAČEVSKA[1]

[1]Faculty of Medicine, Academic Center for Natural Sciences, University of Latvia, Riga, Latvia; [2]The Arya Vaidya Chikitsalayam and Research Institute, Coimbatore, India

**Corresponding author: Baiba Zadersone, Faculty of Medicine, Academic Center for Natural Sciences, University of Latvia, 1 Jelgavas Str., LV-1004, Riga, Latvia.*

E-mail: baiba.zandersone@gmail.com

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Background: Herbal medicine has existed worldwide since ancient times. Pharmacologically active constituents of many single Ayurvedic herbs show antibacterial, anti-inflammatory and antiaging properties, but there is an obvious need to do a systematic research on existing formulations. Ayurvedic herbal formulations contain many phytochemical constituents, which work synergistically with each other in producing pharmacological action [1]. It is necessary to prove the antibacterial efficacy of complex formulations, which is especially important for drug resistant bacteria treatment.

Objectives: A polyherbal formulation, Jathyadi Thailam, based on 13 herbs infusion in sesame oil, is used in traditional Indian medicine for chronic wounds and burns healing. The aim of this study was to perform an antibacterial testing *in vitro*, with methods modification for Arya Vaidya Pharmacy (AVP). The patented formulation should be tested in order to find the most effective antibacterial component combination, extracted by different methods.

Methods: Polar and nonpolar fractions were extracted using the Sohlet extraction procedure. The antibacterial efficacy of the crude herbal extract was tested by the agar dilution method and by the microdilution method for most common isolates from diabetic wounds. Both reference strains and clinical isolates were tested. Clinical isolates were collected from diabetic foot ulcers from the Latvian Riga East University Hospital.

Results: Equal inhibitory effect was shown by crude herbal extract fractions for susceptible *Staphylococcus aureus* 2848, *S. epidermidis* (BF+) and *Enterococcus faecalis* reference strains for both agar dilution and broth dilution methods (Table 1).

Table 1: Minimal bactericidal concentratons (mg/ml) of AVP polar and nonpolar extract (broth microdilution method).

Reference strains:	MBC (AVP polar crude extract)	(AVP nonpolar crude extract)
S. aureus ATCC 2848	7.78	7.78
MRSA ATCC 38592	15.56	31.12
S. epidermidis (BF+)	1.95	1.95
E. faecalis 29212	15.56	15.56
E. coli ATCC 25 922	125.00	No inhibition
P. aeruginosa ATCC 2843	No inhibition	No inhibition
P. aeruginosa MDR	–	62.25
P. mirabilis ATCC 432 351	No inhibition	No inhibition
K. pneumonia ATCC 2558	No inhibition	125.00

Conclusions: AVP formulation is more effective for Gram-positive bacteria than for Gram-negative bacteria. Further research is necessary to isolate fractions with higher antimicrobial activity of the formulation for Gram-negative bacteria.

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Keywords: antimicrobial herbal formulation

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A2.32

Physico-chemical characterization of polyprenol-loaded liposomes

Ilona VANAGA[1,2,*], Jerzy GUBERNATOR[3], Uģis KLĒTNIEKS[2], Laila PLAKANE[4], Līga PLAKANE[5], Ruta MUCENIECE[1] and Baiba JANSONE[1]

[1]Faculty of Medicine, University of Latvia, Riga, Latvia; [2]SIA "Silv EXPO", Riga, Latvia; [3]Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland; [4]Residency in Sports Medicine, University of Latvia; [5]Faculty of Biology, University of Latvia, Riga, Latvia

**Corresponding author: Ilona Vanaga, Department of Pharmacology, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia.*

E-mail: ilona.vanaga@silvexpo.lv

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Background: Polyprenols (PPs) are natural long-chain isoprenoid alcohols of the general formula H-$(C_5H_8)_n$-OH where *n* is the number of isoprene units. Conifer-tree needles are one of the richest sources of polyprenols. PPs are interesting with their wide range of biological activity and low toxicity; therefore, PP pharmaceutical formulations are to be investigated. It is proposed that liposomes could be appropriate vehicles for administration of PPs, since they are universal drug carriers that can accommodate both hydrophilic and hydrophobic compounds and are especially suitable for lipophilic substances [1].

Objectives: The objective of the study was to characterize novel conifer PP liposomes by their physico-chemical properties.

Methods: *Abies sibirica* PPs with a purity of ~80% and the phospholipid mixture Phosal 40 IP (contains at least 25–75% of phosphatidylcholine) were used to prepare liposomes according to a modified method originally from Zompero *et al.* [2]. Solubility of PPs was studied using the HPLC method. The incorporation efficiency of PPs was determined using a modified Stewart assay for phospholipid content and the HPLC method for PP content. Liposome size was detected at 25°C by volume weighting and PDI index using Zetasizer Nano ZS. Liposome visualization was realized via light microscopy (Nikon Eclipse 90i with Nomarski contrast); microstructure and morphology imaging was acquired via TEM (Tesla BS 540 JEOL 100) using both positive and negative staining methods.

Results: A positive correlation was found between the phospholipid mixture concentration and the solubility of PPs with unlimited solubility of 80% PPs being reached at 25% of Lipoid P75 mixture in 96% ethanol at 24°C. A negative correlation was found between the incorporation efficiency of 80% PPs and their dissolved ratio in the Phosal mixtures with highest efficiency being reached at ratio 1:40 for both PP/Phosal 40 IP and PP/Phosal 75 SA mixtures. Liposome size was discovered to be polymodal with the main peak at about 1,360 nm (90% of the volume) and 2 smaller populations at size

307 nm (~5%) and 62 nm (~5%). The visual assessment by microscopy showed liposomes to be multilamellar (MLVs) with varying shapes and sizes, and confirmed the Zetasizer findings.

Conclusions: The physico-chemical characterization of PP MLV liposomes confirms the development of a new PP pharmaceutical formulation.

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Keywords: conifer polyprenols – liposomes – physico-chemical properties

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A2.33

Analysis of chemical composition and traditional medicinal use in Latvia of bird cherry flowers *Padus avium*

Inga SILE[1,*], Edita ROMANE[1], Kirill SHUBIN[2], Solveiga GRINBERGA[2] and Maija DAMBROVA[2,3]

[1]Department of Dosage Forms Technology, Faculty of Pharmacy, Rīga Stradiņš University, Latvia; [2]Latvian Institute of Organic Synthesis, Riga, Latvia; [3]Department of Medicinal Chemistry, Faculty of Pharmacy, Rīga Stradiņš University, Latvia

**Corresponding author: Inga Sile, Department of Dosage Forms Technology, Faculty of Pharmacy, Rīga Stradiņš University, 16 Dzirciema Str., LV-1007, Riga, Latvia. E-mail: inga.sile@rsu.lv*

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Background: Over 1,900 beliefs containing information about medicinal plant usage in Latvian-populated territory were found in folklore materials. In total, 216 genera belonging to 81 families were mentioned [1]. Nowadays, many medicinal properties of plants have been discovered from experience accumulated from a long history of their use. Modern technologies allow analyzing the identity and pharmacological effects of substances found in plants more precisely [2]. More studies should be done in order to validate certain plant usage in traditional medicine.

Objectives: The aim of this study was to collect data about medicinal plants in Latvian folk beliefs and analyze chemical composition of fresh and dried flowers of bird cherry *Padus avium* Mill. [*P. racemosa* (Lam.) Gilib.].

Methods: Among the top ten plants mentioned in folk beliefs, bird cherry was selected for detailed analysis. The flowers of *P. avium* in a fully flowering stage were harvested from two collection sites, Small Jugla and Alūksne (Latvia). A part of the collected material was used fresh and the other one was first dried at room temperature. Diethyl ether was used as an extractant. Chemical composition of the flower extracts was investigated by gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) methods.

Results: The most frequently used *P. avium* parts according to folk beliefs were bark, fruits, flowers and leaves. In Latvian traditional medicine they have been used as teas, tinctures (both externally and internally) and as fresh material for topical application to treat headache, toothache, pain in ears, neck and stomach, diarrhoea, cough, erysipelas, bruises, swelling. About 100 compounds were found in *P. avium* flowers by GC-MS analysis. Twenty two compounds are present in more than 1% relative concentration in flower extracts of *P. avium*. More than 10 phenolic components (flavonoids) and their glycosides were found by LC-MS analysis.

Conclusions: There was no significant difference of chemical composition between fresh and dried flowers. A large number of compounds were identified in bird cherry flowers, and some of them are known to possess anti-inflammatory, antidiarrheal and skin whitening effects.

Keywords: *Padus avium* – bird cherry – extraction – GC-MS – LC-MS

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A2.34

Evaluation of the effects of *Padus grayanae* Maxim.'s dry extract on the indices of peripheral blood and biochemical parameters of experimental animals as a stage of preclinical research

Ainagul ANUVARBEKOVA*, Ashirali ZURDINOV, Tamara SABIROVA and Isabek ISMAILOV

Department of Basic and Clinical Pharmacology, Kyrgyz State Medical Academy, Bishkek, Kyrgyzstan

**Presenting author: Ainagul Anuvarbekova, Department of Basic and Clinical Pharmacology, Kyrgyz State Medical Academy, ul. Akhunbaeva 92, Bishkek, Kyrgyzstan. E-mail: ainashka77@mail.ru*

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Background: *Padus grayanae* Maxim. (Rosaceae Juss.' family), which naturally grows in Japan, was planted in the laboratory of trees and shrubs, the botanical garden, the National Academy of Sciences of the Kyrgyz Republic. As it was established earlier, the aqueous and alcohol extracts obtained from the aerial parts of the plant possess immunomodulatory effects [1, 2].

Objectives: The main purpose of the study is to investigate the effect of *Padus grayanae* Maxim.'s dry extract on biochemical parameters and indices of peripheral blood as a chronic toxicity research.

Methods: The main object of the research, dry extract of the *Padus grayanae* Maxim., was obtained by freeze-drying [3] according to the requirements of the monograph of the 6th edition of the European Pharmacopoeia [4].

The research of the phytoextract toxicity as a chronic experiment was conducted in accordance with generally accepted good laboratory practices (GLP) using 80 white rats. The research substance was dissolved in water before the administration and then was introduced orally using a tube 1 time a day in doses of 300 mg/kg, 600 mg/kg and 900 mg/kg within 1 and 3 months.

Results: Conducted laboratory experiments did not reveal any toxic effects of the drug. It was determined that the introduction of the phytoextract for 1 and 3 months significantly increased the level of hemoglobin, erythrocytes and leucocytes in peripheral blood.

A significant decrease of glucose and cholesterol levels in peripheral blood of experimental animals was defined. Changes in other biochemical parameters detected during the introduction of the substance to rats, did not go beyond the physiological norms and recovered within one month after cancellation of its using to the level of control values.

Conclusions: As a result of chronic research, it was found that during intragastrical administration to rats in the dose range investigated, the dry extract of *Padus grayanae* Maxim. has no pathological effects on the main homeostatic constants. It shows to be safe.

Keywords: dry extract – *Padus grayanae* Maxim. – toxicity

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A2.35

Effects of *Aronia melanocarpa* fruit juice and chlorogenic acid on anxiety-like behavior in chronically treated ovariectomized rats

Milena TODOROVA[1,*], Miroslav EFTIMOV[1], Antoaneta GEORGIEVA[1], Krasimir KUZMANOV[2] and Stefka VALCHEVA-KUZMANOVA[1]

[1]Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria; [2]Vivarium, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

**Corresponding author: Milena Todorova, Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov", 55 Marin Drinov Str., 9000 Varna, Bulgaria.*

E-mail: milena.todorova@mu-varna.bg

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Background: *Aronia melanocarpa* fruits are one of the richest sources of polyphenols, amongst them proanthocyanidins, flavonoids and phenolic acids, mainly chlorogenic acid (CGA) and neochlorogenic acid. Polyphenols can cross the blood–brain barrier and thus act centrally. They have been demonstrated to possess anti-anxiety and antidepressant effects in young/healthy rats [1, 2, 3].

Objectives: This study aimed to determine the effects of *Aronia melanocarpa* fruit juice (AMFJ) and CGA on anxiety-like behavior in chronically treated ovariectomized (OV) female rats using the social interaction (SI) test.

Methods: All experimental animals (female Wistar rats; $n = 70$) were divided into 5 groups, each of 14 animals: control, OV, OV+AMFJ5, OV+AMFJ10 and OV+CGA. Rats from groups OV, OV+AMFJ5, OV+AMFJ10 and OV+CGA were ovariectomized. Controls were sham-operated. After 14 days recovery period, the animals were treated daily orally for 60 days as follows: control and OV groups with distilled water (10 ml/kg); OV+AMFJ5 and OV+AMFJ10 groups with AMFJ at doses of 5 ml/kg (diluted with distilled water to 10 ml/kg) and 10 ml/kg, respectively, and OV+CGA group with CGA 20 mg/kg dissolved in distilled water (10 ml/kg). At the end of the experimental period, the changes in rat SI behavior were registered. The prolongation of SI time served as a measure of an anti-anxiety effect.

Results: In ovariectomized rats, SI time was significantly shorter ($p < 0.001$) compared with that of the sham-operated controls. SI time of rats belonging to group OV+AMFJ5 did not differ from that of the control group and was significantly longer ($p < 0.05$) than that of OV group. SI time of OV+AMFJ10 group did not differ from that of OV group and was significantly shorter ($p < 0.001$) than that of the controls. SI time of rats belonging to group OV+CGA was longer than that of OV group and did not differ significantly from that of the control group. The lack of effect of AMFJ at the higher dose in OV+AMFJ10 group might be the result from the decrease of locomotor activity probably due to sedation (unpublished data).

Conclusion: CGA and the low AMFJ dose administered chronically showed an anxiolytic-like effect in ovariectomized rats.

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Keywords: ovariectomized rats – anxiety – *Aronia melanocarpa* fruit juice – chlorogenic acid

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A2.36

Assessment of *Chaenomeles maulei* fruit juice effects in the forced swim test in rats

Vesela BORISOVA*, Miroslav EFTIMOV and Stefka VALCHEVA-KUZMANOVA

Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov", Varna, Bulgaria

**Corresponding author: Vesela Borisova, Department of Pharmacology and Clinical Pharmacology and Therapeutics, Faculty of Medicine, Medical University "Prof. Dr. Paraskev Stoyanov", 55 Marin Drinov Str., 9000 Varna, Bulgaria;*

E-mail: vessela90@abv.bg

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Background: Depression is a chronic psychiatric disorder manifesting with lowered pleasure, mood and interest. Although there are many highly effective antidepressants available, there is an increasing interest in natural antidepressants with fewer side effects. The main bioactive compounds in *Chaenomeles maulei* fruits are polyphenols known for their antidepressant-like activity.

Objectives: The objective of the present study was to assess the forced swim test behavior of rats treated with *Chaenomeles maulei* fruit juice (CMFJ).

Methods: The animals used were 64 male healthy Wistar rats treated orally with CMFJ for either 14 or 30 days. They were divided in eight groups with 8 animals, four groups for each treatment period. CMFJ was given at 2.5, 5 and 10 ml/kg doses and the control groups were treated with distilled water. We assessed the immobility time as a measure of behavioral despair.

Results: After 14 days of administration all doses of CMFJ significantly decreased the immobility time of the rats ($p < 0.05$) in comparison to the control group. After 30 days treatment, the doses of 2.5 and 5 mg/kg significantly shortened the immobility time ($p < 0.05$ *vs.* control) while the effect of the 10 ml/kg dose was not statistically significant.

Conclusion: The forced swim test is based on the assumption that immobility reflects a measure of behavioral despair [1]. Some antidepressants produce antidepressant-like effects by reducing immobility in addition to motor stimulation [2]. CMFJ decreased the immobility time in the FST after 14 and 30 days treatment which might be

due either to antidepressant-like activity or increased locomotor activity.

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Keywords: forced swim test – antidepressant – *Chaenomeles maulei* – polyphenols

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A2.37

From forest to pharmacy: studies of pharmaceutically valuable compounds in wild black crowberry (*Empetrum nigrum*)

Zane Vincevica-Gaile[1,*], Sabine Strauta[1], Jorens Kviesis[1], Ruta Muceniece[2] and Maris Klavins[1]

[1]Department of Environmental Science, University of Latvia, Riga, Latvia; [2]Department of Pharmacy, Faculty of Medicine, University of Latvia, Riga, Latvia

**Corresponding author: Zane Vincevica-Gaile, Department of Environmental Science, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: zane.gaile@lu.lv*

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Background: Black crowberries (*Empetrum nigrum*) are higher plants distributed mostly in a circumboreal area of the Northern Hemisphere, including forests of Latvia. Although crowberries are described to be useful in ethnomedicine due to antioxidative, antimicrobial, anti-inflammatory and diuretic activity, their chemical composition has not been studied widely [1].

Objectives: The aim of this study was to identify pharmaceutically active compounds in berries and foliage of *Empetrum nigrum* collected in forests of Latvia to promote their use in new pharmaceutically valuable products.

Methods: Berries and foliage of black crowberries were hand-picked in the end of summer, 2015, in nature reserve Moorland of Cena (Latvia). Extracts from berries were obtained using different methods, but 5% solution of formic acid in 96% ethanol, enhancing extraction by ultrasound was assessed as the most promising; extract of foliage was obtained using chloroform. UPLC, GC-MS analysis of extracts were performed to detect anthocyanins, lipids and flavonoids. Antiradical activity was detected using DPPH, total polyphenols with Folin–Ciocalteu reagent, and carbohydrates by the phenol–sulphuric acid method [2].

Results: Total content of polyphenols in berries was 2.80–8.32 g/100 g (as gallic acid equivalent, GAE); total flavonoids: 0.77–11.57 g/100 g (as quercetin dihydrate equivalent, QDE); sum of carbohydrates: 4.20–8.82 g/100 g (as glycose equivalent); antiradical activity varied 12–15% depending on the extraction method. Concentrated extract of black crowberries contained polyphenols 36.20 g/100 g (as GAE) and flavonoids 36.25 g/100 g (as QDE), thus resulting in 69.5% antiradical activity. Concentrated extract of polyphenols contained 48.7 mg/g of anthocyanins and it was possible to identify 13 anthocyanins, predominantly of those delphinidin-3-*O*-galactoside, cyanidin-3-*O*-galactoside and peonidin-3-*O*-galactoside which are more stable than arabinosides [3]. In total, 17 lipid compounds were detected, predominantly linoleic acid, linolenic acid and oleic acid. Nine compounds were identified in extract of foliage (mostly dihydrochalcones and flavanones) indicating potential antibacterial and antifungal activity [4].

Conclusions: High levels of polyphenols, especially anthocyanins (13 anthocyanins were identified by UPLC) with elevated antiradical activity were detected in extract of *Empetrum nigrum* berries. Berries and foliage of black crowberries can be a good source of phytosterols and unsaturated fatty acids; thus, their use for production of phytopharmaceutical products is promising.

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Keywords: anthocyanins – antiradical activity– lipids – polyphenols – forest berries – *Empetrum nigrum*

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A2.38

Berries of *Vaccinium* spp.: a valuable source of polyphenols with potential for application in diverse pharmaceuticals

Linards Klavins[1,*], Jorens Kviesis[1], Vizma Nikolaeva[2], Martins Boroduskis[2] and Maris Klavins[1]

[1]Department of Environmental Science, University of Latvia, Riga, Latvia; [2]Faculty of Biology, University of Latvia, Riga, Latvia

**Corresponding author: Linards Klavins, Department of Environmental Science, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: linards.klavins@gmail.com*

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Background: Plants are an important source for the development of new pharmaceuticals. As prospective source, plants traditionally used in food and ethnomedicine, can be considered, for example, berries of *Vaccinum* spp. (cranberries, lingonberries, blueberries and others). It has been demonstrated that these berries contain various polyphenols in high concentrations. Extracts of *Vaccinium* spp. berries have a role in developing novel pharmaceutically active ingredients [1].

Objectives: The aim of this study was to obtain polyphenolic extracts from 5 different *Vaccinium* spp. berries, evaluate antiproliferative activity against dermal fibroblasts and antimicrobial activity of these extracts.

Methods: Bog cranberries, lingonberries, American cranberries, bilberries and blueberries were harvested in the summer/autumn of 2016 in the bogs and forests of Latvia. Berry press residues were

used for the extractions. Various extraction methods were tested (Soxhlet, microwave, ultrasound-assisted extraction etc.), composition of extraction solvent was optimized using response surface approach (RSM) [2]. Obtained optimal extracts were purified using XAD7-HP sorbent. The polyphenolic composition was described using HPLC-PDA and ORBITRAP-MS. DPPH, ABTS and FRAP were used to determine the antioxidative potential. Antimicrobial activity was determined using agar diffusion method. Dermal fibroblast cell cultures were employed to determine the effects of polyphenol extracts (cell proliferation and flow cytometry).

Results: Performed investigation of possibilities for the extraction of berry phenolics revealed ultrasound-assisted extraction to be the method of choice. Response surface methodology was used to identify the optimal solvent concentrations used for the extraction of total polyphenols and anthocyanins, ethanol and methanol acidified with trifluoroacetic acid gave the highest extraction outcome. In total, 37 individual polyphenols were identified and quantified, allowing to perform chemotaxonomic analysis. The prepared polyphenol concentrates (polyphenolic contents up to 56%) were described to have a high radical-scavenging activity, which was measured *in vitro* by cell cytometry; also, the extracts' ability to interrupt proliferation of dermal fibroblasts was observed. Moreover, the extracts show antimicrobial activity against various pathogenic microorganisms (*Bacillus*, *Micrococcus*, *Staphylococcus*).

Conclusions: A strategy to produce berry extracts with high polyphenol concentrations was created and optimized. Obtained polyphenol extracts showed outstanding antiradical and antimicrobial properties, in addition, antiproliferative properties were observed, demonstrating plausible anticancer features.

Acknowledgements: This study was supported by the European Regional Development Fund within project no. 1.1.1.1/16/A/047 "Genus Vaccinium berry processing using 'green' technologies and innovative, pharmacologically characterized biopharmaceutical products".

Keywords: polyphenols – *Vaccinium* – antiradical activity – berries – lipids

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A2.39

Ultrasound and antibiotic induced vascular relaxation

Aurelija RADZEVIČIENĖ[1], Silvijus ABRAMAVICIUS[1,*], Vaida ZEMAITYTE[2], Vytautas OSTAŠEVIČIUS[3], Vytautas JŪRĖNAS[3] and Edgaras STANKEVICIUS[1]

[1]Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Kaunas, Lithuania; [2]Lithuanian University of Health Sciences, Kaunas, Lithuania; [3]Institute of Mechatronics Kaunas University of Technology, Kaunas, Lithuania

**Corresponding author: Silvijus Abramavicius, Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, 9 Mickevičiaus Str., LT–44307 Kaunas, Lithuania.*

E-mail: silvijus2@yahoo.com

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Background: Ultrasound is being used as a therapeutic modality (*e.g.* sonothrombolysis) [1]. By establishing the vasomodulatory effects of ultrasound, we provide the physiological basis for the development of therapeutic and rehabilitation devices. Some antibiotics (*e.g.* gentamycin) exhibit vasomodulatory effects [2]. Local potentiation of antibtiotic-induced vascular relaxation could potentially be used to modulate blood flow to vital organs (kidney) and thus likely be used for pharmacologic renal protection and reduce the incidence of acute kidney injury [3].

Objectives: (1) To investigate the vasomodulatory effects of ultrasound. (2) To investigate the vasomodulatory effects of antimicrobial drugs.

Methods: Segments of rat (Wistar) superior and small mesenteric arteries were dissected, isolated, and transferred into cold physiological saline solution and mounted in myographs (Dual Wire Myograph System – 420A) for functional studies. The arterial segments were stretched to their optimal lumen diameters for active tension development. Ultrasound (US) waves and antibiotics—gentamycin (GEN), ciprofloxacin (CIP), piperacillin and tazobactam (PT)—were applied on the vessels, precontracted with 10 µM of noradrenalin (NA). Acetylcholine-induced (10 µM ACh) relaxation was used as control and as proof of intact endothelial function in physiological saline solution (PSS) or PSS with high potassium concentration (KPSS). The changes in isometric tension (IT) were recorded. Vascular relaxation is presented as isometric tension and percentage change in mN from plateau of vascular contraction with 10 µM NA.

Results: The vessel segments were obtained from 4 Wistar male rats. The IT (mN) decreased in vessels precontracted with 10 µM NA and exposed to GEN (0.16 mg/ml) by 59.93%, exposed to CIP (0.03 mg/ml) by 20.50%, and exposed to US (50 amp) by 35.03%. In KPSS GEN (0.12 mg/ml) reduced the IT by 32.00%, GEN 0.04 mg/ml did not induce vascular relaxation. US (30 amp) reduced IT by 5.43% in KPSS, while GEN (0.04 mg/ml) and US (30 amp) reduced IT by 22.46%. PT did not exhibit vasomodulatory effects.

Conclusions: Ultrasound waves, gentamycin and ciprofloxacin induce vascular relaxation in rat mesenteric arteries and may have therapeutic applications with regards to vasomodulatory functions.

Keywords: vascular relaxation – ultrasound – gentamycin

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A2.40

Use of remote photoplethysmography in assessment of topical corticosteroid-induced skin blanching

Anastasija CAICA[1,*], Uldis RUBINS[2], Zbignevs MARCINKEVICS[1] and Andris GRABOVSKIS[2]

[1]Department of Human and Animal Physiology, University of Latvia, Riga, Latvia; 2Institute of Atomic Physics and Spectroscopy, University of Latvia, Riga, Latvia

**Corresponding author: Anastasija Caica, Department of Human and Animal Physiology, Faculty of Biology, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia.*

E-mail: anastasija.caica @lu.lv

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Background: Topically applied corticosteroids are frequently prescribed as local therapeutic treatment. The human skin-blanching

assay proposed by McKenzie [1], for the assessment of bioavailability of topical corticosteroids has been in use for decades. Hence, in spite of technological advancements, the intensity of the drug-induced blanching is still assessed subjectively by eye, as existing techniques (reflectance spectroscopy) are complex and expensive. The simple and cost-effective alternative to existing methods is remote photoplethysmography, with its ability to non-intrusively acquire signals by means of a light source and a video camera.

Objectives: To evaluate the reliability of remote photoplethysmography in assessment of topical corticosteroid-induced skin-blanching.

Methods: Five healthy volunteers gave informed consent and participated in the present pilot study. To produce skin blanching, an adhesive plaster (15×15 mm area) containing 0.02 ml of clobetasol-17-propionate ointment (Dermovate® 0.5 mg/g, GlaxoSmithKline), was applied on the volar aspect of the right forearm for 12 hours. Thirty minutes after plaster removal, remote photoplethysmography (rPPG) signal was acquired (camera Ximea xiQ, at 100 Hz per channel) from blanched and surrounding skin regions at 530 nm and 810 nm light source illumination. To compare signals, PPG AC and DC components were compared for both wavelengths and sites (blanched and intact skin).

Results: The corticosteroid ointment produced substantial blanching of non-glabrous skin in all subjects. However, there was no statistically significant difference between rPPG AC signal amplitude acquired from blanched and intact skin regions, whereas the blanched skin region displayed a statistically significant increase (8–12%) of DC component amplitude only at 530 nm illumination, indicating on a non-pulsatile nature of the observed vascular response, possibly originated from reduced functional capillary density due to long-lasting vasoconstriction of arterioles supplying superficial cutaneous vascular plexus. Using DC component mapping it was possible to automatically select and evaluate the blanched skin region.

Conclusions: Remote photoplethysmography can provide objective assessment of blanching intensity and in future could be utilised in determination of steroid topical bioavailability.

Acknowledgements: The support of University of Latvia project no. AAP2016/B047 is highly appreciated.

Keywords: vasoconstrictor assay – topical corticosteroids – remote photoplethysmography

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A2.41

Anticoagulative therapy for high-risk patients with atrial fibrillation: risk stratification and monitoring

Katrina Pukite[1,*], Ketija Apsite[2], Inga Urtane[1] and Oskars Kalejs[2,3]

[1]*Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia;*
[2]*Faculty of Medicine, Rīga Stradiņš University, Riga, Latvia;*
[3]*Center of Cardiology, Pauls Stradiņš Clinical University Hospital, Riga, Latvia*
* *Corresponding author: Katrina Pukite, Faculty of Pharmacy, Rīga Stradiņš University, 16 Dzirciema Str., LV-1007, Riga, Latvia.*
E-mail: katrina.pukite89@inbox.lv

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Background: Atrial fibrillation (AF) is the most common arrhythmia that increases by age, doubles for every decade after age of 50 years and reaches about 10% of patients ≥80 years [1]. Despite direct oral anticoagulants' (DOACs) predictable pharmacokinetics and pharmacodynamics, laboratory tests are necessary for effective and safe medical treatment, also for prediction and detection of thrombotic and bleeding events, as well as in situations when temporary discontinuation could be desirable [2].

Objectives: Identify and analyze the need of coagulation tests for AF patients with high cardiovascular risk in clinical practice.

Methods: Quantitative, analytic, cross-sectional clinical trial, during the period from October 2016 till June 2017, was performed at Center of Cardiology, Pauls Stradiņš Clinical University Hospital, Latvia. Data were collected from patients with non-valvular AF, under anticoagulative therapy ≥3 months, defined as a high-risk group by CHA_2DS_2-VASc score (more or equal to 2 or 3), men and women respectively. Data were analyzed using SPSS.

Results: Data were collected from 143 patients of whom 46.2% (n = 66) were male; the mean age was 69.7 (SD ± 9.9) years. About 2/3 (73.1%) of all patients the AF were longer than 1 year. The mean CHA_2DS_2-VASc score was 4.2 (SD ± 1.5). The most common co-morbidities were arterial hypertension (65.0%; 93), chronic heart failure (48.3%; 69), coronary artery disease (32.9%; 47), diabetes mellitus (24.5%; 35), and dyslipidemia (25.9%; 37). Almost half of patients (46.2%; 66) used DOACs, 31.5% rivaroxaban and 14.7% dabigatran, respectively; furthermore, 1.4% patients used DOACs with anti-aggregants. 49.7% (71) patients had increased risk of possible drug–drug interactions, most frequently with proton-pump inhibitors (16.8%; 24), amiodarone (24.5%; 35), anti-inflammatory drugs (49.0%; 70). The use of DOACs and possible drug–drug interactions increases by risk score, reaching the maximum score 3 (16.1%; 23) and the mean frequent score 4.4 of 86 (60.1%) AF patients, respectively.

Conclusions: DOACs usage correlates with CHA_2DS_2-VASc score with mean frequent score 4.4 of 86 (60.1%) AF patients, respectively. From all high-risk AF patients, 47.7% had potentially moderate or major risk of drug–drug interactions. For 60.1% of AF patients the monitoring of anticoagulative therapy should be considered.

Keywords: atrial fibrillation – CHA_2DS_2-VASc risk score – DOACs – drug monitoring – laboratory tests

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A2.42

Action of proanthocyanidin microdoses on pyruvate in blood

Jelena Krasilnikova[1,*], Galina Telysheva[2], Sarmite Janceva[2], Amelia Figueiro Vaz[1], Oda Tommeras[1] and Caroline Forsbo[1]

[1]*Rīga Stradiņš University, Riga, Latvia;* [2]*Latvian State Institute of Wood Chemistry, Riga, Latvia*
**Corresponding author: Jelena Krasilnikova, Department of Human Physiology and Biochemistry, Rīga Stradiņš University, 16 Dzirciema Str., Rīga, LV-1007, Latvia.*
E-mail: jelena.krasilnikova@rsu.lv

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Background: One of the most common chronic diseases is diabetes mellitus, which is characterised by elevated levels of glucose in blood plasma (hyperglycaemia). In this case, the glucose physiological process is characterised by the formation of high-energy ATP-form compounds adenosine triphosphate and nicotinamide adenine dinucleotide hydride (NADH), which accumulate in muscles and

internal organs. In diabetes mellitus patients, the pyruvate concentration in blood plasma exceeds 2.5 mg/dl, followed by disorders of the glucose enzymatic process and hampering of the accumulation of the energy compounds ATP and NADH in muscles and internal organs. The permanent disruption of the energy flow in diabetes mellitus patients causes the emergence of toxicity, oxidative stress, muscular dystrophy, endotheliopathy and neuropathy. Taking into account the glucose exchange disorders, with increasing pyruvate levels in blood plasma, the development of an agent that would lower it becomes a topical issue.

Objectives: The aim of the present study was to evaluate the effects of plant proanthocyanidin (PAC) on the reduction of the pyruvate concentrations in blood plasma.

Methods: Pyruvate levels in blood plasma were determined by the biochemical colorimetry method. The method is based on the fact that, for pyruvic acid in alkaline environment, the coloured 2,4-dinitrophenylhydrazine is formed. The investigation on the reduction of the pyruvate levels in blood was carried out in patients with diagnosed type 2 diabetes using series of 64 experimental studies. PAC were obtained from alder bark using single-stage extraction with 40% ethanol/water solution, with further purification using Sephadex LH-20.

Results: The microdose of PAC (50 µl), in *in vitro* conditions, reliably reduces the pyruvate level at its increase, which is promising for the reduction of the metabolic acidosis in patients with diabetes mellitus and can improve cell bioenergetics.

Conclusions: The presented data show that the proanthocyanidins from black and grey alder bark can be promising for the reduction of pyruvate levels in blood plasma.

Acknowledgements: The study was financed by the Bioeconomy grant "LigProBK" and COST Action FA1403 POSITIVe.

Keywords: proanthocyanidins – diabetes mellitus – pyruvate levels – hyperglycaemia

A2.43

Intake of polyprenol rich product SuperCell® HEPA improves efficiency of oxygen use in well-trained amateur floorball players

Laila PLAKANE[1,*], Ilona VANAGA[2], Līga PLAKANE3, Ruta MUCENIECE[4] and Uģis KLĒTNIEKS[2]

[1]Residency in Sports Medicine, University of Latvia; [2]SIA "Silv EXPO"; [3]Faculty of Biology, University of Latvia; [4]Faculty of Medicine, University of Latvia, Riga, Latvia

**Corresponding author: Laila Plakane, Residency in Sports Medicine, University of Latvia. E-mail: laila.plakane@gmail.com*

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Background: Polyprenols are linear polymers consisting of isoprene units and are found in almost all living bodies. It is known that polyprenols protect cell membranes from peroxidation and reactive oxygen species. Hepatoprotective abilities are described as well [1]. Polyprenols protect muscles from statin-induced muscle damage in rats by improving muscle strength and power [2]. However, there is lack of information about possible effect on athlete performance. In sports, food supplement use is very common, also in floorball. Although floorball exists for more than 30 years, there is no information about physiological characteristics of floorball players in comparison with other team sportsmen.

Objectives: The objective of the study was to evaluate adaptation to acute aerobic test loads and to determine polyprenol effects on physiological characteristics of well-trained amateur floorball players.

Methods: 30 male floorball players participated in this study, 16 of them were taking SuperCell® HEPA and 14 did not. All participants did VO_2 max exhaustive incremental cycling test before and after use of polyprenols with each step 2 minutes and increment by 25 W, where VO_2 peak and a number of additional cardiorespiratory variables (heart rate, cardiac output, stroke volume, arterial blood pressure, as well as ventilation, gas exchange and oxygen consumption) were measured to investigate cardiorespiratory adaptation.

Results: Polyprenol intake increased $VO_{2\,peak}$ (peak oxygen consumption) by 2.8 ml/min/kg while in the control group there were no changes, O_2/HR (oxygen pulse) increased by 0.9 ml/beat in the polyprenol-user group while it decreased by 0.4 ml/beat in the control group ($p = 0.019$). In the polyprenol-user group, P_{dia} (diastolic pressure) in the last cycling-test minute decreased by 4 mmHg while it increased in the control group by 10 mmHg ($p = 0.02$); SVc (cardiac output) in the polyprenol-user group increased by 2.9 ml while it decreased by 7.4 ml in the control group ($p = 0.04$). Average cycling test time increased by 1 min in the polyprenol-user group while it did not change in the control group.

Conclusions: Use of polyprenols increased the ability to tolerate high-intensity exercise in the cycling test and improved oxygen consumption efficiency by increasing $VO_{2\,peak}$, O_2/HR. Differences between polyprenol and control groups were statistically significant.

Acknowledgements: Research founded by SIA "Silv EXPO".

Keywords: polyprenols – floorball – aerobic capacity

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A2.44

Are the peptide-based compounds the treatment candidates for Alzheimer's disease?

Jolanta UPITE* and Baiba JANSONE

Department of Pharmacology, Faculty of Medicine, University of Latvia, Riga, Latvia

**Corresponding author: Jolanta Upite, Department of Pharmacology, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia. E-mail: jolanta.upite@gmail.com*

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Background: Alzheimer's disease (AD) is a neurodegenerative disease that affects more than 45 million people widespread [1]. Aggregation of intracerebrally generated amyloid β (Aβ) and tau proteins is the main neuropathological hallmark of AD [2]. The discovery of mutated Aβ to be protective in heterozygote family members in Italy and Island opens new roads for the treatment of amyloid protein depositions in the brain [3]. Recently, intensive research is focused on the role of short sequence peptides in dissolving Aβ aggregates in the central nervous system and providing their elimination from the brain through blood circulation.

Objectives: To advance our understanding about peptide-based Aβ aggregate inhibitors, based on natural Aβ sequence and containing modified amino acids.

Methods: A systematic search (1996–2017) of PubMed, Science Direct, EBSCO, Scopus, and Cochrane Library was performed by using key terms: AD, Aβ peptide, Aβ aggregation, and peptide-based AD inhibitors.

Results: The search yielded up to 658 records from 1996 to 2017. After screening of the titles and abstracts, 107 records met the criteria: AD, Aβ peptide, Aβ aggregation, peptide-based AD inhibitors, drug treatment and clinical trials. Widening of the knowledge of

mechanism relying on the basis of Aβ fibril formation and protein misfolding has expanded the research interest of usefulness of peptide-based Aβ aggregate inhibitors in AD. In the last 25 years researchers' interest was directed on molecular structures of peptides suitable for potential treatment of AD, such as D-amino acid- and modified amino acid-containing peptides, retro-inverso peptide inhibitors, endogenous dipeptides, and peptides based on C-terminus, peptides derived from functional site sequence, cyclic peptides. Most of the synthesized peptides tested in AD studies have showed positive results in transgenic mouse models, however, have failed at different phases of clinical studies. The reasons identified were difficulties to cross the blood–brain barrier and low bioactivity. Peptide-based therapeutic agents might be one of the potential option for AD treatment that need to be further developed; however, there are still needs also for effective biomarkers to diagnose the disease at an early stage, thus gaining the most of the benefit of the used treatment before the neurodegenerative processes in the brain becomes irreversible.

Conclusions: The results of extensive research studies made in the last 25 years have shown the potential role of short peptide-based Aβ aggregate inhibitors in the treatment of AD.

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Keywords: Alzheimer's disease – amyloid aggregation – amyloid β peptide – drug treatment

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A2.45

Increase of antibiotic inappropriate use among children: How can we help in pharmacy?

Sheroza IRISMETOVA* and Inga URTĀNE

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Rīga Stradiņš University, Riga, Latvia

**Corresponding author: Sheroza Irismetova, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Rīga Stradiņš University, Dzirciema iela 16, LV-1007, Riga, Latvia.*

E-mail: sheroza.irismetova@gmail.com

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Background: Antibiotic resistance is a growing problem especially among children due to lack of parents' knowledge about antibiotic use [1]. It can be reduced by finding out the primary difficulties regarding utilization in such cases as disease conditions, preparation, storage and choice of drink [2].

Objective: To analyze more common mistakes during antibiotic treatment in children made by their parents.

Methods: Prospective quantitative study was conducted from December 2016 to January 2017 in Kazakhstan and Latvia. Data such as age, gender, location, illness conditions, experience with suspension usage were obtained by anonymous questionnaire.

Results: Data were collected from 100 parents (50 from Kazakhstan and 50 from Latvia), mean age was 31.4 ± 4.8 years. Using independent samples *t*-test it was found that the average number of children differed statistically significant between countries: for Kazakhstan 2.6 ± 1.1 and for Latvia 1.4 ± 0.5 ($t = 7{,}03$; $p < 0.01$). More than half of the parents experienced antibiotic usage (66%). It was seen that Kazakhstan parents were likely to give antibiotics to children in comparison with negative attitude to it, 39% *vs.* 11% respectively ($p = 0.021$). Taking the Latvian population, there was no significant difference between usage, 27% *vs.* 23% accordingly. Most inappropriate usage of antibiotics was observed in cases such as flu (59.1%), cold (31.8%) and cough (28.8%) in both countries. The data showed cold and cough were treated twice frequently in Kazakhstan than in Latvia (21.2% *vs.* 10.6% and 19.7% *vs.* 9.1%). More than half of all study parents stored ready suspension at room temperature rather than in fridge (59.1% *vs.* 40.9%; $p = 0.012$). There was a representative difference among beverages such as tea and carbonated drinks compared to still water and juice (25.7% *vs.* 74.2%; $p = 0.007$).

Conclusions: One of the reasons of inappropriate use of antimicrobial agents was lack of parent's knowledge about adequate antibiotic treatment for their children. Most of the population in both countries had been giving antibiotics to their children in conditions such as flu when it was not necessary. Mistakes in suspension preparation, storage conditions and use of inappropriate beverages could affect the pharmacokinetics and action of antibiotic active substance.

Keywords: antibiotics – children – inappropriate use

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A2.46

Establishment of the Baltic Biomaterials Centre of Excellence (BBCE)

Maija DAMBROVA[1,*], Elina MAKAROVA[1], Arita DUBNIKA[2], Ilze SALMA[3], Mauro ALINI[4], Aldo BOCCACCINI[5] and Janis LOCS[2]

[1]Laboratory of Pharmaceutical Pharmacology, Latvian Institute of Organic Synthesis, Latvia; [2]Institute of General Chemical Engineering, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga Technical University, Latvia; [3]Department of Oral and Maxillofacial Surgery, Rīga Stradiņš University, Riga, Latvia; [4]AO Research Institute Davos, Switzerland; [5]Institute of Biomaterials, Friedrich-Alexander University of Erlangen-Nuremberg, Germany

**Corresponding author: Maija Dambrova, Latvian Institute of Organic Synthesis, 21 Aizkraukles Str., LV-1006, Riga, Latvia.*

E-mail: maija.dambrova@farm.osi.lv

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Background: The ageing population puts a high burden on the society in terms of costs of healthcare and loss of quality of life. The need for bone and joint replacement implants is increasing with older age, and musculoskeletal conditions are the second-greatest cause of disability globally.
Objectives: The main objective of the BBCE project is to establish the joint Baltic Biomaterials Centre of Excellence focused on the advanced biomaterial development for medical applications. Within the frame of critical-mass formation in research and development, also the cooperation between industry and research organisations will be encouraged, leading to technology transfer and delivery of new products in to the market.
Methods: BBCE will be based on the long-term strategic cooperation and collaboration between AO Research Institute Davos, Switzerland (ARI) and Friedrich-Alexander University of Erlangen-Nuremberg, Germany (FAU) on the one hand and three institutions from Latvia — The Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University (RTU RBIDC), the Latvian Institute of Organic Synthesis (LIOS) and Rīga Stradiņš University (RSU) — on the other hand. LIOS will be responsible for the preclinical studies of biomaterials developed by the RTU RBIDC. To consider potential clinical applications, the safety, biocompatibility and biological activity of biomaterials will be assessed in *in vitro* toxicity tests and *in vivo* model systems of tissue regeneration.
Conclusions: BBCE will ensure sustainable quality of the research and technology transfer potential in the field of biomaterials for medical applications, mainly focusing on areas possessing the best available scientific and research capacities, in particular, on research of biomaterials, advanced materials, nanotechnology, biomedicine and biopharmacy.
Acknowledgements: This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement no. 763721: BBCE, Baltic Biomaterials Centre of Excellence.
Keywords: biomaterials – medical devices – preclinical studies – excellence centre

A2.47

Consumption of antibacterial agents in outpatient and inpatient practice in Latvia

Valerija KUTUZOVA* and Janis KURLOVICS

Faculty of Medicine, University of Latvia, Riga, Latvia
**Corresponding author: Valerija Kutuzova, Faculty of Medicine, University of Latvia, 19 Raina Blvd., LV-1586, Riga, Latvia.*
E-mail: vk14053@lu.lv

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Background: Antimicrobial resistance is one of the greatest problems in nowadays medical care. Excessive and inappropriate usage of antibacterial agents is one of the reasons that have led to bacterial resistance.
Objectives: The aim of this study was to find out prescribing and usage principles of antibacterial agents in ambulatory and stationary sectors and analyse the compliance with guidelines, observe the tendency of consumption of antibacterial agents in Latvia compared with other European countries.
Methods: The 793 prescriptions of three open-type pharmacies (01.10.2016–31.12.2016) and consumption data on the antibacterial agents issued in two closed-type hospitals' pharmacies over a two-year period (01.01.2015–31.12.2016) were analysed.
Results: The beta-lactams (ATC code J01C) were the widely used of all antibacterial groups (47.50%) in Latvia, tetracyclines (J01A) were 15.65% from prescribed antibiotics and macrolides, lincosamides and streptogramins (J01F) took the third place 15.52%. In closed system outpatient pharmacies for enteral use the most often distributed antibacterial group was quinolones (J01M; 27.53%), but for parenteral use (other beta-lactam antibacterials, J01D; 16.65%). Analysis of prescription in community pharmacies showed that code of diagnosis was indicated only in 23.7% of prescriptions. 13.6% of prescriptions were redeemed longer than 4 days from prescribing. 54% of prescriptions with written code of diagnosis were prescribed by general practitioners, 10.1% by otorhinolaryngologists, and 8.5% by internists. The study found that 18.87% of prescribed antibacterial agents were not consistent with the guidelines.
Conclusions: The beta-lactams are the most widely used antibacterial agent group in ambulatory care in Latvia; in stationary care it was quinolones and other beta-lactams. It was found that code of diagnosis had been indicated only in a quarter of analysed prescriptions. General practitioners are the main prescribers of antibacterial agents and they most often had indicated a code of diagnosis comparing to other practitioners. It was observed that more than 10% of pharmacy clients did not start antibacterial therapy immediately; such an approach could rise the risk for medical outcome; however further research should be done to evaluate the background and reasoning. Almost 20% of prescriptions did not meet the guidelines, more strict control could be needed.
Keywords: antibacterial agents – resistance – tendency

A2.48

Structural studies of bacteria *Borrelia burgdorferi* protein BBP28

Jēkabs FRIDMANIS[1,*], Kalvis BRANGULIS[2], Mārtiņš OTIKOVS[1], Kristaps JAUDZEMS[1] and Edvards LIEPIŅŠ[1]

[1]Department of Physical Chemistry, Latvian Institute of Organic Synthesis, Riga, Latvia; [2]Department of Structural Biology, Latvian Biomedical Research and Study Centre, Riga, Latvia
**Corresponding author: Jēkabs Fridmanis, Department of Physical Chemistry, Latvian Institute of Organic Synthesis, 19 Aizkraukles Str., LV-1006, Riga, Latvia. E-mail: fridmanis.jekabs@osi.lv*

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Background: Lyme disease is the most widespread vector-transmitted disease in the United States of America [1]. However, in spite of its prevalence, a vaccine for this disease is not yet available. In recent years, considerable attention has been devoted to several *Borrelia burgdorferi* (causal agent for Lyme disease) outer surface proteins, like BBP28, that are upregulated during transmission from infected *Ixodes* genus ticks to mammals. These proteins are believed to play a significant role in the establishment and maintenance of infection and thus could serve as drug targets to prevent the transmission of the pathogenic bacteria [2].
Objectives: The objective of this research was to produce Lyme disease outer surface protein BBP28 in an *E. Coli* expression system and determine its molecular structure using nuclear magnetic resonance spectroscopy.
Methods: Site-directed mutagenesis, nuclear magnetic resonance spectroscopy, MALDI-TOF mass spectrometry, crystallography.
Results: During this study using NMR data two structures of BBP28 were determined: one for the full-length protein, and the other for a truncated version of BBP28 that lacked 27 amino acids of the unstructured N-terminal segment.
Conclusions: The structured part of BBP28 is formed by 5 α-helices and a 14-amino-acid-long C-terminal strand, covalently attached to a loop connecting helices 4 and 5.
Acknowledgements: The authors would like to thank Dr. Ina Baļķe and Dr. Dāvids Fridmanis for assistance in employment of MALDI-TOF mass spectrometer and sequencing of created expression plasmids.

Keywords: BBP28 – *Borrelia burgdorferi* – nuclear magnetic resonance – MALDI-TOF mass spectrometry

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A2.49

Aparecium: an easy-to-use data pipeline software for experimental data transformations, standardization and exporting

Tõnis Laasfeld*, Sergei Kopanchuk and Ago Rinken

Chair of Bioorganic Chemistry, Institute of Chemistry, University of Tartu, Estonia

**Corresponding author: Tõnis Laasfeld, Chair of Bioorganic Chemistry, Institute of Chemistry, University of Tartu, 14a Ravila, 50411, Tartu, Estonia. E-mail: tonis.laasfeld@gmail.com*

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Background: With the arrival of microplate-based assays, the amount of data that can be obtained from a single experiment has grown significantly. Furthermore, a single imaging experiment can create tens to even hundreds of Gigabyte of data. In many cases, the data from several different measurement systems need to be analyzed together in the case of global analysis of complex models. So far, the integration between the plate readers and the analytics software such as GraphPad Prism or SBToolbox2 has relied on MS Excel and unstandardized tabular formats, which are not suitable for large datasets.

Objectives and methods: In order to solve this problem we propose a specialized software, Aparecium, which can standardize experimental data and integrate it with experiment description, export the data to different commonly used analytics software depending on user needs. It also provides easy-to-use graphical user interfaces. In order to develop such a software, MATLAB was used for handling multidimensional datasets. Java, another popular programming language, was chosen for providing the user with more sophisticated and dynamic graphical user interfaces. Aparecium uses modular architecture and is developed in an object-oriented manner.

Results: Aparecium has several data import modules for reading output formats of several plate-reader manufacturers (BMG Labtech, PerkinElmer Inc., Biotek Instruments Inc.) and OME-TIFF images. The users can combine experimental data with experiment descriptions and save the data as MIDAS (Minimum Information for Data Analysis in Systems Biology) files. Aparecium has so far been used as a platform for developing a new automated image-based cell-size estimation (ICSE) assay for baculovirus quantification [1] as well as for GPCR studies.

Conclusion: Aparecium can address the stated problems by providing easy-to-use graphical user interfaces, allowing data import from different plate-reader manufacturers and export to several data analysis software. Aparecium is under active development and will provide even more useful functionality in the future. It is distributed under GNU GPL and can be downloaded from [2].

Acknowledgements: We would like to acknowledge Anni Allikalt, Maris-Johanna Tahk, Olga Mazina, Reet Link, Kadri Ligi, Darja Lavõgina and Santa Veikšina for suggesting new features, testing the software and pointing out flaws.

Keywords: data management – systems biology – computer vision – MATLAB – GPCR

Reference and link

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A2.50

The oncolytic effect of the ECHO-7 virus Rigvir® on cell viability *in vitro*

Andra Tilgase[1,*], Liene Patetko[2], Ilze Blāķe[2], Mārtiņš Borodušķis[2], Anna Ramata-Stunda[2] and Pēteris Alberts[1]

[1]Research and Development, International Virotherapy Center, Riga, Latvia; [2]Faculty of Biology, University of Latvia, Riga, Latvia

**Corresponding author: Andra Tilgase, Research and Development, International Virotherapy Center, LV-1050, Riga, Latvia.*

E-mail: andra@virotherapy.eu

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Background: The genetically non-modified, non-pathogenic ECHO-7 virus strain Rigvir®, selected and adapted for melanoma or subjected to targeted evolution, is the first approved oncolytic virus. It is a positive-sense single-stranded RNA, non-enveloped, icosahedric virus approximately 25–30 nm in diameter. The host for ECHO viruses is human. A recent retrospective study suggests that substage IB, IIA, IIB, and IIC melanoma patients treated with Rigvir® had a 4.39–6.57-fold lower mortality than those under observation only [1]; there was no untoward side effect or discontinuation of treatment. Safety assessment of adverse events graded according to NCI CTCAE did not show any value above grade 2.

Objectives: The present study was performed to test the effect of Rigvir® on the viability of several human cancer cell lines.

Methods: The effect of Rigvir® (1% or 10%, volume/volume) on the viability of FM-9, RD, AGS, A549, HDFa, HPAF-II, MSC, MCF7, HaCaT, and Sk-Mel-28 cell lines was measured using live cell imaging *in vitro*. PBMC viability was measured using flow cytometry. Cells were observed for 96 h after addition of Rigvir®. The presence of the virus was visualized by specific ECHO-7 antibody staining. Statistical difference between treatment groups was calculated using two-way ANOVA.

Results: Rigvir® (10%) reduced cell viability in FM-9, RD, AGS, A549, HDFa, HPAF-II and MSC cell by 67–100%. HaCaT viability was partly affected while Rigvir® had no effect on MCF7, Sk-Mel-28 and PBMC viability. Detection of ECHO-7 antibody in FM-9, RD, AGS, A549, HDFa, HPAF-II and Sk-Mel-28 cells suggests that the presence of ECHO-7 in the cells preceded or coincided with the time of reduction of cell viability. Rigvir® (10%) had no effect on PBMC cell count. The results suggest that Rigvir® *in vitro* reduces the viability of cells of human melanoma, rhabdomyosarcoma, gastric adenocarcinoma, lung carcinoma, and pancreas adenocarcinoma but not of PBMC. The presence of ECHO-7 in sensitive cells was confirmed using ECHO-7 antibodies.

Conclusions: The results suggest that the basis for the clinical benefit of Rigvir® is due to its oncolytic properties and that the effect of Rigvir® could be clinically tested in other cancers besides melanoma.

Keywords: Echovirus 7 – immunotherapy – melanoma – oncolytic virotherapy – Rigvir®

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