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P01
ENDOKRINI FGF: OD TEMELJNIH MEHANIZAMA DO TERAPIJSKIH MOGUĆNOSTI

ENDOCRINE FGFS: FROM BASIC MECHANISMS TO THERAPEUTIC OPPORTUNITIES (IUPHAR LECTURE)

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Tyrosine phosphorylation of cellular proteins plays an important role in the control of cell proliferation, differentiation, cell metabolism as well as other important cellular processes. Ligand binding to the extracellular ligand binding domain of receptor tyrosine kinases induces receptor dimerization, a step crucial for activation of the catalytic domain and for tyrosine autophosphorylation; both processes are mediated by an intermolecular process. A large family of growth factors such as platelet derived growth factor (PDGF), stem cell factor (SCF), colony stimulating factor (CSF), and nerve growth factor (NGF), among many others are dimeric proteins. These growth factors induce receptor dimerization by virtue of their dimeric nature. Canonical fibroblast growth factors bind to their receptor monovalently, and when added alone are unable to induce dimerization and activation of FGF-receptors. Canonical FGF-induced dimerization of FGF-receptors is mediated by heparin sulfate proteoglycans. Endocrine FGFs, FGF19, FGF21 and FGF23 are circulating hormones that regulate metabolic processes in a variety of tissues. They signal through FGFRs in a manner that requires a Klotho protein. It was proposed that Klotho proteins, which are cell surface proteins with tandem glycoside hydrolase domains, act as co-receptors for FGFR activation by endocrine FGFs, playing roles analogous to heparan sulfate proteoglycans in canonical FGF signaling. By determining crystal structures of free and ligand-bound β-Klotho extracellular regions, we show that β-Klotho in fact serves as a primary high-affinity cell-surface receptor for FGF21, with FGFR1c functioning as a catalytic subunit that mediates intracellular signaling.

P02

PLACEBO I LIJEKOVI: DIJELJENJE ZAJEDNIČKOG MEHANIZMA DJELOVANJA

PLACEBOS AND DRUGS: SHARING COMMON MECHANISMS OF ACTION (EPHAR LECTURE)

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Although placebos have long been considered a nuisance in clinical research, today they are an active and productive field of research and, because of the involvement of many mechanisms, the study of the placebo effect can actually be viewed as a melting pot of concepts and ideas for neuroscience. Indeed, there exists not a single but many placebo effects, with different mechanisms and in different systems, medical conditions, and therapeutic interventions. For example, brain mechanisms of expectation, anxiety, and reward are all involved, as well as a variety of learning phenomena, such as Pavlovian conditioning, cognitive and social learning. There is also some experimental evidence of different genetic variants in placebo responsiveness. The most productive models to better understand the neurobiology of the placebo effect are pain and Parkinson's disease. In these medical conditions, the neural networks that are involved have been identified: that is, opioid, cannabinoid, cholecystokinin, cyclooxygenase, dopamine modulatory networks in pain and part of the basal ganglia circuitry in Parkinson's disease. Overall, the main concept that is emerging today is that placebos and drugs share common mechanisms of action, which suggests a cognitive/affective interference with drug action.

P03

INKRETINSKI HORMONI ZA NEURODEGENERATIVNE BOLESTI

INCRETIN HORMONES FOR NEURODEGENERATIVE DISEASES

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Are diabetes drugs a treatment for Alzheimer's or Parkinson's? Despite considerable efforts, for most acute and chronic neurodegenerative diseases efficacious treatments affecting the underlying pathologies are unavailable. Preclinical studies suggest insulin and gut-derived hormones, in particular glucagon-like peptide 1 (GLP-1), as modifiers of both acute and chronic neurological diseases. A number of mechanisms such as anti-inflammatory properties of the hormones, gluco-regulatory mechanisms in the brain and repair of endothelial dysfunction have been proposed. Here, we will review both preclinical and clinical data that raise some optimism for future treatments. In particular, we will propose mechanisms of action and treatment targets for GLP-1 in the brain. In experimental models we have shown that certain anti-inflammatory pathways are activated by GLP-1 resulting in improved clinical outcomes. In humans we have demonstrated glucose homeostatic effects of GLP-1 and in Alzheimers' GLP-1 maintained glucose consumption significantly compared to placebo. Further, insulin, when administered intranasally, holds promise in neurodegenerative states. Here, we will review the evidence and propose new paths for exploration.

SIMPOZIJI / SYMPOSIA

S01 *MARIJAN KLARICA*: SIMPOZIJ „MARIN BULAT“: ČIMBENICI KOJI ODREĐUJU DINAMIKU MOLEKULA UNUTAR KRANIOSPINALNOG SUSTAVA / FACTORS THAT DETERMINE THE DYNAMICS OF MOLECULES WITHIN THE CRANIOSPINAL SYSTEM

S01/1
NEURORADIOLOŠKA DIJAGNOSTIKA POREMEĆAJA VOLUMENA I GIBANJA LIKVORA

NEURORADIOLOGY OF CSF VOLUME AND MOVEMENT IMPAIRMENTS

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4 Ruder Boskovic Institute, Department of Molecular Biology, Zagreb, Croatia

Development of advanced radiological techniques make possible to depict central nervous system in details, with excellent contrast between CSF and surrounding structures (bones and parenchyma). Therefore, it is feasible to perform precise segmentation of all CSF spaces and to quantify their volumes both in intracranial and spinal part. Aside from volumetric analysis advanced MR techniques (T2 space and phase-contrast sequences) are capable to depict and even quantify movement of CSF, especially in regions where these movements are pronounced as foramen of Monro, mesencephalic aqueduct and cranio-cervical junction. Radiological diagnostic procedures often show findings which are not in concordance with classical CSF hypothesis based on active secretion manly by choroidal plexuses, unidirectional CSF circulation and reabsorption in dural sinuses. Our clinical cases showed that choroidal plexuses are not necessary for CSF production and that blockage of mesencephalic aqueduct will not always induce hypertensive hydrocephalus as proposed by classical concept. Furthermore, application of contrast in spinal CSF space will almost always show distribution opposite to one proposed by classical concept. These radiological findings point out that classical hypothesis of CSF physiology is not able to explain numerous clinical cases which emphasize necessity for new approaches to this issues.

S01/2

RASPODJELA RAZLIČITIH MOLEKULA IZMEĐU INTERSTICIJSKE I CEREBROSPINALNE TEKUĆINE

DISTRIBUTION OF DIFFERENT MOLECULES BETWEEN INTERSTITIAL AND CEREBROSPINAL FLUID

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The cerebrospinal fluid (CSF) movement and its influence on substance distribution and elimination from the CSF system have been thoroughly analyzed and discussed in the light of the new hypothesis of CSF physiology. As a result, CSF movement is not presented as a circulation, but a permanent rhythmic systolic-diastolic pulsation in all directions. Such movement also represents the main force of substance distribution inside the CSF system. This distribution occurs in all directions, i.e., in the direction of the imagined circulation, as well as in the opposite direction, and depends on the application site and the resident time of tested substance, where longer resident time means longer distribution distance. Transport mechanisms situated on the microvessels inside the central nervous system (CNS) parenchyma play the key role in substance elimination from the CSF and interstitial fluid (ISF) compartments, which freely communicate. If a certain transport mechanism is not available at one site, the substance will be distributed by CSF movement along the CSF system and into the CNS region where that transport mechanism is available. Pharmacological manipulation suggests that the residence time and the substance travel distance along the CSF system depend on the capacity of transport mechanisms situated on CNS blood capillaries. Physiological absorption of the CSF into the venous sinuses and/or lymphatics, due to their small surface area, should be of minor importance in comparison with the huge absorptive surface area of the microvessel network.

S01/3

REVIZIJA KLASIČNOG KONCEPTA SEKRECIJE LIKVORA IZ KOROIDNOG SPLETA

REVISION OF THE CLASSIC CONCEPT OF CEREBROSPINAL FLUID SECRETION BY CHOROID PLEXUS

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The classic hypothesis presents the cerebrospinal fluid (CSF) as the "third circulation," which flows from the brain ventricles through the entire CSF system to the cortical subarachnoid space to eventually be passively absorbed into the superior sagittal sinus through arachnoid granulations. The choroid plexus represents a key organ in the classic CSF physiology and a powerful biological pump, which exclusively secretes CSF. Thereby, the CP is considered to be responsible for CSF pressure regulation and hydrocephalus development. The role of the CP in the CSF dynamics has been thoroughly analyzed by presenting arguments in favor of the thesis that the CPs are neither biological pumps nor the main site of CSF secretion; that they do not participate in regulation of ICP/CSF pressure; are not the reason for the existence of hydrostatic pressure gradient in the CSF system and that this gradient is not permanent (disappeared in the horizontal position); and that they do not generate imagined unidirectional CSF circulation, hydrocephalus development and increased ICP/CSF pressure. The classic hypothesis cannot provide an explanation for these controversies but the recently formulated Bulat-Klarica-Oreskovic hypothesis can. According to this hypothesis, CSF production and absorption (CSF exchange) are constant and present everywhere in the CSF system, and although the CSF is partially produced by the CP, it is mainly formed as a consequence of water filtration between the capillaries and interstitial fluid.

S01/4

MEHANIZMI RAZVOJA HIDROCEFALUSA NORMALNOG TLAKA

MECHANISMS OF THE NORMAL PRESSURE HYDROCEPHALUS DEVELOPMENT

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Clinical features of idiopathic normal pressure hydrocephalus (iNPH) are often very similar to those of other neurodegenerative diseases such as Alzheimer's disease, dementia with Lewy bodies, or extrapyramidal spectrum disorders including Parkinson's disease, progressive supranuclear palsy and corticobasal syndrome. This is not so unexpected considering that iNPH can also be regarded as a neurodegenerative disorder, and that a precise diagnosis is virtually impossible without the brain pathological analysis. Even then, a coexistence of multiple types of pathological processes can often be found in the brain. If not treated timely, iNPH can become severely debilitating. Differentiating between the classical iNPH and the rest of the mentioned disorders provides better long-term results of the cerebrospinal fluid (CSF) shunt treatment. For this reason, the identification of specific biomarkers to establish accurate diagnosis would be very useful. MicroRNAs (miRNAs) are small non-coding RNA molecules of 19–25 nucleotides that act epigenetically via posttranscriptional gene expression control, mainly regulating the stability and translation of the messenger RNA. In the last decade, there has been increased interest in their roles as biomarkers for various neurodegenerative diseases. The aim of our study was to find the potential CSF miRNA biomarkers for NPH mimics that can reliably distinguish them from iNPH patients, thus providing a better screening of the patients that would benefit more from the invasive procedure such as CSF shunt placement.

S01/5

JEDNOSMJERNA CIRKULACIJA LIKVORA NE POSTOJI: OPAŽANJA POMOĆU MRI TIME-SLIP TEHNIKE

THERE IS NO CEREBROSPINAL BULK FLOW: MRI TIME-SLIP OBSERVATION

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Cerebrospinal fluid dynamics was observed by magnetic resonance imaging (MRI) time spatial spin labeling inversion pulse (Time-Slip) technique to visualized CSF dynamics. There were numerous disagreements regarding classical CSF bulk flow theory before radioisotope (RI) cisternography became available. However, RI cisternography and CT cisternography lead one to believe there is unidirectional CSF bulk flow from the production site (choroid plexus) to arachnoid villi. External tracers that were used for RI cisternography or RI cisternography were biological inert macromolecules and believed to flow with CSF movement. Di Chiro who is one of a pioneer of RI cisternography observation, repeatedly mentioned these observations were limited to macromolecule tracer, not for small molecules. Needless to state, CSF was mainly composed of water, which is not macromolecule. Macromolecular tracer study can show the communication between where it is injected and where it is observed. However, it cannot trace CSF motion or bulk flow. Ideal tracer of the CSF motion is the CSF itself and Time-SLIP technique achieves exactly that. Observations of CSF motion by using Time-SLIP technique were very different from what we saw in external tracer studies. Cerebrospinal fluid dynamics have been extensively studied using cardiac gated phase contrast (PC) MRI for many decades. Conventional PC MRI technique, however, requires averaging CSF motion over multiple heart beats data acquisition to allow the depiction of "to-and-fro" CSF motion only within one cardiac cycle. CSF motion driven by respiration had been considered negligible until the significant effect of respiration on CSF dynamics was shown recently by real time Time-SLIP. Conventional PC-MRI use cardiac gate data acquisition so this method did not count respiratory driven CSF motion during the acquisition. Combination of cardiac pulse and respiration on CSF motion results physiological variability of CSF motion. There is no requirement to introduce exogenous tracer to trace CSF dynamics in Time-SLIP so that one can observe CSF dynamics non-invasively without disturbing CSF physiological condition. The CSF dynamics observation obtained by Time-SLIP showed different aspect of CSF dynamics than the classical CSF circulation theory that was believed for a century. An explanation of the principle of Time-SLIP is left to other publications.14-16 Briefly, as CSF itself is marked with RF pulses in MRI, the dynamics of the CSF can be observed as long as that marking persists. In Time-SLIP

technique, non selective inversion recovery pulse are applied to the whole field of view followed by selective inversion recovery pulse, where the observation and images are acquired after delay times. The images can be acquired one by one and then arranged in sequence on the time axis or can be acquired fully sequentially (real-time Time-SLIP). Images acquired in the former type have higher quality in terms of resolution etc. However, owing to the principles of this technique, the continuity of those images gets lost when they are displayed as a movie in the situation that driving force for CSF motion is inconstant. Time-SLIP technique permits observation of CSF dynamics over a 1-6 sec. period. Repeated studies are readily doable and can assess CSF dynamics in this method. This pulse labeling can non-invasively repeatedly tag a variable volume of CSF in the region of interest in any orientation and in any place in the central nervous system (CNS). Cerebrospinal fluid dynamics in pathophysiological condition in each individual case can be investigated using this method. Exchanging of CSF between the lateral ventricle and third ventricle was observed in normal physiological brain. Mixing motion in the third and fourth ventricle was observed. Pulsatile motions of CSF were observed entire subarachnoid space except for the cerebral convexity. Using images acquired with Time-SLIP MRI technique. CSF pulsations were traced using semi-automated tracking software called Dynatracer, which allows movement of the tagged CSF to be traced over the entire observation time. Tracing the CSF movement showed flow of the pulsatile nature synchronized to cardiac pulse and respiration but not bulk flow. These results indicated that there is no net unidirectional CSF flow as we see in the blood stream. In other words, CSF does not flow unidirectionally from a production site to an absorption site. This observation, that there is no CSF circulation, is in agreement with the recent CSF theory proposed by Bulat, Klarica and Oreskovic, which describes cerebral blood vessels as being responsible for CSF (water) transport thus there should be no CSF bulk flow from the choroid plexus in the lateral ventricle to the arachnoid villi at the cerebral convexity.

S02 *IVANA ŠUTEJ, KRISTINA PEROŠ*: SALIVARNI BIOMARKERI I DIJAGNOSTIČKI POTENCIJAL SLINE / SALIVARY BIOMARKERS AND SALIVA DIAGNOSTIC POTENTIAL

S02/1
CIRKULIRAJUĆI BIOMARKERI I EGZOSOMI U SALIVARNOJ DIJAGNOSTICI

CIRCULATING BIOMARKERS AND EXOSOMES FOR SALIVA DIAGNOSTICS

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The aim of our research activities at AIT, the Austrian Institute of Technology, is to define reliable biomarkers suitable for early and non-invasive disease diagnosis and prognosis. To this end we have been establishing and optimizing a whole range of multiplex capability technologies (e.g. microarrays, quantitative PCR, Luminex bead technology) to meet the special demands and challenges of diagnostic biomarker discovery - and validation in body fluids. Using this specific technology expertise we e.g. successfully discovered autoantibody- as well as DNA methylation -based diagnostic marker panels for the big 4 cancer entities (breast, colon, prostate, lung) in serum or plasma. Based on these success stories and the evident advantages of saliva as a diagnostic matrix our recent special interest is to go for saliva diagnostics and to evaluate saliva for its suitability for circulating biomarker-based diagnostics. Along these lines we will report on the evaluation of different commercially available strategies for isolation of exosomes from human serum and saliva. We will further present data from genome-wide microRNA – as well as DNA-methylation profiling in salivary - and serum-derived exosomes which will also include our experiences when comparing the performance of high-density microarrays and next generation sequencing for miRNA profiling. Last but not least we will report on first results of a research project where we are looking for salivary and plasma exosome-derived epigenetic biomarkers for early type 2 diabetes diagnosis.

S02/2

ULOGA SLINE U DIJAGNOZI POREMEĆAJA TEMPOROMANDIBULARNOG ZGLOBA

THE ROLE OF SALIVA IN THE DIAGNOSIS OF TEMPOROMANDIBULAR DISORDERS

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Introduction: Temporomandibular disorders (TMD) are most common orofacial pain disorders of non-dental origin with the prevalence of 6.1–10.2% in general population. Despite signs and symptoms being well described, the underlying pathophysiological mechanisms is still unknown. The etiopathophysiology is multifactorial, involving a combination of factors such as parafunctions, micro- and macro traumas, genetic influences, physiological and psychological stressors. Several studies suggest that oxidative stress (OS) plays a role in the pathophysiological processes of many diseases including TMD.

Materials and methods: In our current project "The Role of Oxidative Stress and Opiorphin in TMD" supported by the Croatian Science Foundation, saliva was used to assess the (dis)balance in oxidative status for its potential role in the onset and/or the progression of TMD. Identification of OS biomarkers would objectively indicate implication of OS in TMD pain onset mechanisms, and provide basis for early detection, and potential target for therapeutic agents to prevent progression to more severe dysfunction.

Results: We found significant differences in expression of salivary OS biomarkers and antioxidants between TMD patients and healthy individuals. We have addressed the differences in oxidative status by considering pain intensity and specific TMD diagnostic subgroups. An association between higher levels of OS markers with higher pain intensity has been reported, as well as the decrease of OS markers during therapy period.

Conclusions: In this lecture the novel approach to understanding pathophysiological mechanisms in TMD and their links to OS, as well use of saliva as non-invasively available diagnostic biofluid will be discussed.

S02/3

SALIVARNI KALCIJ, MAGNEZIJ I FOSFAT U ODNOSU NA ORALNO I SISTEMSKO ZDRAVLJE

SALIVARY LEVELS OF CALCIUM, MAGNESIUM AND PHOSPHATE IN CORRELATION WITH ORAL AND SYSTEMIC HEALTH

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Concentrations of calcium, magnesium and phosphate in the saliva reflect many orodental functions of saliva: remineralisation processes, buffering ability, oral microbiota and periodontal bone metabolism. Referent values are established but affected by factors including saliva roles in food and bacteria clearance, mastication, lubrication, digestion, antimicrobial defence and buffering effect. Increased salivary calcium and phosphate concentrations may reflect systemic conditions or physiology processes, as increase with age and in saliva of menopausal women; or some disease processes, as in saliva of cystic fibrosis patients. Increased inorganic salivary calcium, phosphate, pH and flow rate are correlated with orodental conditions as well, like periodontitis and lower caries suspectibility. Salivary minerals measurement is still finding its way in dental experimental and clinical research. There are several different methods of saliva sampling and laboratory assessement of salivary minerals that may result in inconsistent findings. Measurements of salivary magnesium and phosphate by standard spectrophotometry or microwave assisted inductively coupled plasma mass spectrometry are consistent. Although, these two methods are not consistent for measurement of salivary calcium. It is important to be aware of how different laboratory methods may affect results for salivary calcium. In salivary mineral analysis, sampling and laboratory methods are improving but it is still complex to clearly correlate findings with clinical outcomes.

S02/4
SLINA I CIJELJENJE SLUZNICE USTA

SALIVA AND HEALING OF ORAL MUCOSA

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Physiological roles of saliva are inseparable from the main physiological roles of oral cavity – eating and speaking – which saliva supports by moisturizing and lubricating oropharyngeal tissues. Saliva is also indispensable for maintaining health of both hard (mineralized) dental tissues as well as oral mucosa. However, when lesions affecting oral mucosa do occur, can saliva promote healing? It has been suggested that several active substances present in saliva could be beneficially involved in the healing process. These include growth factors like epidermal growth factor and vascular endothelial growth factor, and, particularly, histatins, a family of cationic histidine-rich salivary peptides. Proposed mechanisms of action include influence on the infiltration of inflammatory cells, accelerating migration and proliferation of oral epithelial cells and fibroblasts, inciting remodeling of the extracellular matrix, and promoting proliferation of endothelial cells and forming of new blood vessels. Revealing a role of a particular salivary substance in the healing of mucosal lesions allows for the possibility of designing and conducting studies exploring their therapeutic potential. Effective and safe novel therapeutics promoting regeneration of oral mucosa and relieving pain would be particularly beneficial in conditions which substantialy impair patients' quality of life and are often difficult to prevent or to treat such as mucosal changes (mucositis and ulcerations) related to hyposalivation, and oral mucositis occuring as a side effect of chemotherapy and head and neck radiotherapy.

S02/5

SALIVARNA DIJAGNOSTIKA DANAS I SUTRA - IMPLEMENTACIJA I POMOĆ U KLINIČKOJ PRAKSI

SALIVARY DIAGNOSTICS TODAY AND TOMORROW - IMPLEMENTATION AND HELP IN CLINICAL PRACTICE

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The methodology for saliva assay has been improving with time due to the ease in its collection and the emergence of advanced technology. Biomarkers of exposure can be used to predict risks while biomarkers of disease can be used for screening, diagnosis, prognosis, outcome determination, or combinations. Since saliva is produced in mouth, use of saliva in diagnostics was first investigated and applied for oral diseases, and therefore it progressed the most until now. Afterwards it started to infiltrate in biomarkers research for various systemic diseases. Within the last two-and-a-half decades scientists and technology discovered biomarkers, some of them ready to be included in diagnostics of specific disease and some need more further investigations. The most significant progress in research and implementation in conventional diagnosis and treatment of diseases with saliva diagnostics as complement has been made in periodontal disease, Sjögren's syndrome. oral cancer, cardiovascular disease. renal insufficiency, neuro-degenerative and neuropsychiatric disorders, microbe infections. orofacial pain and cancer. Future: Saliva diagnostic tests have the potential to be used within a broad spectrum of applications that include population-based screening programs, confirmatory diagnosis, risk stratification, prognosis determination and therapy response monitoring. Further development in point of care diagnostic test for ambulatory or even home use with saliva as specimen would improve global health in world population. Although there are few available salivary tests on market, until saliva becomes a certified diagnostic test that can replace the conventional ones, all the research values must be compared with the existing accepted methods.

S03 *SUZANA MATANOVIĆ MIMICA*: AKTUALNE TEME IZ PODRUČJA KLINIČKE FARMAKOLOGIJE / CURRENT TOPICS IN THE FIELD OF CLINICAL PHARMACOLOGY

S03/1
LIJEKOVI KOJI UTJEČU NA ŽIVOTNI STIL - IZMEĐU DIJAGNOZA I ETIČKIH DILEMA

LIFESTYLE DRUGS - BETWEEN DIAGNOSES AND ETHICAL DILEMMAS

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The term 'lifestyle drugs' (LD) was mentioned for the first time in the second half of the last century. More than twenty different definitions are quoted, but it is common for all that LD are natural or synthetic substances that individuals use for non-medical reasons, as their own choice, in order to improve their quality of life. For many, the former means a better mood, for others higher psychic and / or physical endurance, and for the third, more beautiful (mostly younger) look. In a wider sense, LD include drugs of abuse, but also "drugs for fraud", as it is the case with substances used for doping in sports. Many of these substances are officially approved medicines for certain medical indications. Modern age imposes new life values and styles, but also new ways to achieve them. "Diagnosis" can be set up without a doctor, from the Internet and social networks you can acquire knowledge about the medicine free of charge, online shopping saves precious time, preserves discretion and personal comfort, bypassing relatively strict regulatory norms. In addition to the questionable aspect of the production, procurement and use of these substances, numerous ethical dilemmas arise. Are "true" patients discriminated against in an unfair way? Is it easy to draw a line between medical and lifestyle indications? What will happen when a LD stops working? How will the world look when all people become younger, more muscular and tight? How to motivate young athletes to fight honestly and without deception? How to treat the negative consequences of the use of LD? Finally, lifestyle - medical diagnosis of XXI century or not?

S03/2

KAKO INTERPRETIRATI REZULTATE KLINIČKIH ISPITIVANJA LIJEKOVA?

HOW DO WE INTERPRET RESULTS OF CLINICAL TRIALS OF MEDICATIONS?

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Introduction: Critically reviewing the design, endpoints, and results of clinical trials can be challenging to health care professionals. However, this process is important for applying research results to clinical practice.

Materials and methods: The purpose of this presentation is to provide an overview of key points to consider in the interpretation of clinical trials results.

Results: Fundamental aspects of clinical trials include study designs, chosen endpoints, analyses, and methods of handling missing data along with potential sources of bias and uncertainty. The important factors to consider are the validity of the study methods, clinical importance of the results and the relevance of the results for the clinical practice. This presentation will discuss some common issues that can arise during the interpretation process. Identifying sources of error and understanding study limitations helps clinicians evaluate the validity of clinical data, ultimately influencing clinical decision making and patient care.

Conclusions: Effective interpretation of clinical trial data allows for the integration of high-quality clinical research into clinical practice. Evidence from clinical trials directly impacts clinical decision making, which ultimately guides patient management.

S03/3

ONKOLOŠKI LIJEKOVI S NOVIM MEHANIZMOM DJELOVANJA - KLINIČKI ZNAČAJ

ANTICANCER DRUGS WITH NOVEL MECHANISM OF ACTION- CLINICAL SIGNIFICANCE

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Over the last two decades, there has been a shift in the focus of cancer therapy from conventional cytotoxic drugs to more specific targeted therapies directed to cancer cells. Three main approaches include immunotherapy, targeted therapies and precision (personalised) medicine approach based on comprehensive genomic profilling. All of them are dedicated to one main goal: to limit collateral destruction of normal tissues, while enhancing cancer cells destruction. First generation of immune checkpoint inhibitors directed against cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1) has undoubtly revolutionalised treatment of several cancer types. They unlock host immune response againts tumour cells with durable responses in those patients who respond. There are new generation of immune modulators (NGIMs) in different phases of clinical development. Among them are indoleamine-2,3-dioxygenase 1, lymphocyte activation gene-3 and IL15 as the most prominent targets. New inhibitory pathways are under investigation with drugs blocking LAG-3, TIM-3, or B7/H3 pathway, but agonists of stimulatory checkpoint pathways such as OX40, ICOS, and CD40 also. Molecules targeting components of tumour microenvironment like IDO and TLR are also among novel approaches. Drugs targeting cancer epigenetics are also among novel approaches. Epigenomics include a wide range of heritable changes in gene expression, which do not come from any alteration in DNA sequences. Aberrant DNA methylation, histone modifications, and expression of long non-coding RNAs (lncRNAs) are key epigenetic mechanisms associated with cancer initiation and progression.

S03/4

BIOSLIČNI LIJEKOVI I EKSTRAPOLACIJA INDIKACIJA

BIOSIMILAR DRUGS AND EXTRAPOLATION OF INDICATIONS

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A biosimilar is defined as a biological medicine highly similar to already approved biological medicine. As every brand biological is modified many times during its life cycle, comparability exercises are undertaken to demonstrate that efficacy and safety are not adversely affected. The same scientific principles is applied to demonstrate biosimilarity of a new molecule; the process of biosimilar approval by regulatory bodies includes the same standards of pharmaceutical quality, efficacy and safety, as for brand biologics. The similarity has to be shown in terms of structure, biological activity and efficacy, safety and immunogenicity. During the clinical development, it is mandatory to demonstrate similarity of clinical efficacy and safety as with the reference product, not clinical efficacy per se. The most sensitive and homogeneous patient population should be chosen for those trials. Extrapolation of indications means approval of a biologic in an indication not specifically studied during clinical development. This is only possible if scientifically justified and based on overall comprehensive comparability data, such as analytical, non-clinical, pharmacokinetics, pharmacodynamics, clinical (efficacy, immunogenicity). For the concept of extrapolation the most essential part is similarity of physicochemical and functional properties of a molecule. The aim of the extrapolation is to avoid unnecessary clinical studies in a target population. The examples of extrapolations of indications: biosimilar infliximab has been studied only in patients with rheumatoid arthritis and ankylosing spondylitis but has also been approved for Crohn's disease and ulcerative colitis; biosimilar rituximab has been studied only in patients with advanced follicular non-Hodgkin lymphoma but has also been approved for patients with diffuse large B cell non-Hodgkin's lymphoma and chronic lymphocytic leukaemia; biosimilar trastuzumab has been studied only in early breast cancer (which is a sensitive and homogeneous population) and has been approved also for metastatic breast cancer; biosimilar filgrastim has been studies in breast cancer patients with chemotherapy-induced neutropenia but has been approved for the treatment of neutropenia of various etiologies and for the mobilization of peripheral blood progenitor cells in patients and healthy donors.

S03/5

POSTOJE LI JOŠ NEKE DILEME OKO ANTIKOAGULANTNE TERAPIJE?

ARE THERE STILL SOME DILEMMAS ABOUT THE ANTICOAGULANT THERAPY ?

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Thromboembolic diseases are of major clinical concern due to their high prevalence and consequences, which are often fatal. Venous thromboembolism (VTE) is estimated to be the third most common cardiovascular disorder after coronary heart disease and stroke internationally. Anticoagulant and thrombolytic therapy options are available for the treatment of thromboembolic disease During the last 60 years, vitamin K antagonists (VKAs), which include coumarin derivatives (eg, warfarin), has been the only oral anticoagulant used; however, new substances with anticoagulants effects, referred to as new oral anticoagulants, have recently been discovered. Compared with VKAs, this new generation of oral anticoagulants (non-vitamin K antagonist oral anticoagulants, NOACs) has more predictable anticoagulant responses, and NOACs have been shown to be effective in the prevention and treatment of VTE and in the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation (AF). The advantages of NOACs over VKAs are their high efficacy in preventing stroke in AF, lower incidence of major bleeding, convenience of use, minor drug and food interactions, predictable PK and PD, rapid onset and offset of action, short half-life, and lack of the need for laboratory monitoring. Still, several years after their introduction, some disadvantages of NOACs should be mentioned, such as their higher cost, and even limited experience with these drugs. Also NOACs should not be used in patients with severe renal and hepatic disease and in patients with mechanical heart valves. No clear instructions for use in renal failure, extremly expensive antidotes, and monitoring the therapeutic effect are some of the open questions about their use.

S04 *ZDRAVKO LACKOVIĆ, IVICA MATAK*: NEUROGENA UPALA I GLAVOBOLJE / NEUROGENIC INFLAMMATION AND HEADACHE

S04/1
UPALNA SIGNALIZACIJA PARENHIMA KAO POKRETAČ NEUROGENE UPALE DURE

PARENCHYMAL INFLAMMATORY SIGNALING AS A DRIVER OF DURAL NEUROGENIC INFLAMMATION

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Cortical spreading depression (CSD) is the neurophysiological correlate of migraine aura. It is generally accepted that the CSD-induced migraine headache emerges as a result of parenchymal and subsequent meningeal sterile inflammatory signalling. CSD triggers a parenchymal inflammatory cascade first by opening pannexin-1/P2X7 large-pore channels and formation of the inflammasome complex in neurons. The inflammasome processes pro-caspase-1 to caspase-1, which cleaves pro-IL-1β and release IL-1β. Activation of pannexin-1 also leads to release of high-mobility group box 1 (HMGB1) from neurons. Both HMGB1 and IL-1β cause translocation of NF-kappa B to nucleus in astrocytes, which induces synthesis of pro-inflammatory enzymes such as COX-2 and iNOS. Astrocytes then start releasing algesic mediators such as prostaglandins and NO into the CSF and activate the nociceptive nerves around pial and dural vessels, leading to headache. Stimulation of nociceptors also triggers the dural neurogenic inflammation, which can sustain hours to days lasting headache. In the absence of CSD (i.e. migraine without aura), the same cascade of events may be triggered during intense excitatory activity when glycogen-derived glucose and lactate from astrocyte processes cannot match the increased energy demand for restoration of the synaptic transmembrane gradients of glutamate, K+, and Ca2+, which can create the conditions for activation of neuronal Pannexin-1. Interestingly, migraine triggers such as sleep deprivation, stress may induce transcriptional changes in astrocytes that reduce glycogen availability, therefore, increase susceptibility to migraine without aura as well as decrease the CSD threshold, hence, predisposes to migraine with aura.

S04/2

AKUTNI FARMAKOLOŠKI TRETMAN EPIZODNE MIGRENE: SISTEMATSKI PREGLED

THE ACUTE PHARMACOLOGICAL TREATMENT OF EPISODIC MIGRAINE: A SYSTEMATIC REVIEW

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Introduction: It has been indicated that there are few reliable trials about pharmacological interventions to relieve the pain of migraine in children and that using paracetamol or NSAIDs as first-line agents is "widely accepted good clinical practice during acute attacks unless contraindicated". The World Health Organization Essential Medicines List (WHO EML) contains limited number of analgesics. Two analgesics for treatment of acute migraine attacks in WHO EML for children are ibuprofen and paracetamol.

Materials and methods: We searched Embase, CDSR, CENTRAL, DARE and MEDLINE databases up to 18 April 2017. We analyzed randomized controlled trials (RCTs) and systematic reviews (SRs) about efficacy and safety of ibuprofen or paracetamol for treatment of acute migraine attacks in children. We conducted meta-analysis and assessments of evidence with GRADE, Cochrane risk of bias tool, and AMSTAR.

Results: Three RCTs (201 children) and 10 SRs on ibuprofen and/or paracetamol for acute migraine attacks in children were included. Meta-analysis indicated that ibuprofen was superior to placebo for pain-free at 2 h or pain relief at 2 h, without difference in adverse events. There were no differences between paracetamol and placebo, or ibuprofen and paracetamol. Ten SRs that analyzed various therapies for migraine in children were published between 2004 and 2016, with discordant conclusions.

Conclusions: Limited data from poor quality RCTs indicate that ibuprofen and paracetamol might be effective analgesics for treating migraine attacks in children. Inclusion of ibuprofen

and paracetamol as antimigraine medicines for children in the WHO EML is supported by indirect evidence from studies in adults.

S04/3

NEUROGENA UPALA DURE UZROKOVANA PUTEM BOLI: KARAKTERIZACIJA I ANALGETSKI PROFIL

PAIN-EVOKED NEUROGENIC INFLAMMATION OF THE CRANIAL DURA IN RODENTS: CHARACTERIZATION AND ANALGESIC PROFILE

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Introduction: Migraine headache pathophysiology has been associated with trigeminovascular system activation and dural neurogenic inflammation (DNI). Clinical evidence suggest the link between pain in the orofacial area with migraine. Thus, we characterized the possible relation of DNI and different types of orofacial pain in rats.

Materials and methods: Occurrence of DNI was characterized in various neuropathic pain models, as well as in orofacial inflammatory pain, by detection of extravasated plasma proteins and histology. Response to different antimigraine and analgesic drugs was examined by spontaneous pain measurement or mechanical allodynia assessment, and subsequent DNI analysis.

Results: Different neuropathic pain (infraorbital or greater occipital nerve constriction), acute and chronic inflammatory stimuli in the orofacial area (formalin snout injection, complete Freund's adjuvant into temporomandibular joint) lead to DNI, but not the peripheral mononeuropathies. Pain-evoked DNI is accompanied by extravasation of plasma proteins and tissue infiltration with different types of inflammatory cells. Its occurrence and intensity depend on the intensity and duration of painful stimuli, and can be blocked by different analgesic drugs (triptans, opioids, local anesthetics etc). Interestingly, the anti-inflammatory effect of botulinum toxin A and morphine on DNI was dependent on central opioidergic system.

Conclusion: DNI, as a non-specific reaction to painful stimuli in the trigeminal area, may be associated with different types of headaches. Analgesic efficacy of different analgesics and anti-migraine drugs is associated with the reduction of DNI. Modulation of the effects of

morphine and BoNT/A by opioid antagonists suggest important role of endogenous opioids on pain-evoked DNI.

S04/4

RINOSINUSITISOM IZAZVANA NEUROGENA UPALA I SPONTANA BOL U ŠTAKORA: MOGUĆA VEZA SA SINUSNOM GLAVOBOLJOM

RHINOSINUSITIS-INDUCED NEUROGENIC INFLAMMATION AND SPONTANEOUS PAIN IN RATS: IS THERE A LINK WITH „SINUS“ HEADACHES?

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Introduction: Overlapping symptoms and comorbidity of different pathologies related to nasal and paranasal structures with migraine suggest a pathophysiological link between these disorders. However, the mechanisms involved in this association are unclear. Herein, we investigated if deep intranasal inflammatory stimulation might evoke migraine-associated trigeminovascular activation and central pain transmission.

Materials and Methods: The rat nasal mucosa at the border of nasal cavity and maxillary sinus was stimulated with low doses of pro-inflammatory substances capsaicin and formalin. Mucosal and dural tissue neurogenic inflammation was quantified by extravasation of Evans blue-plasma protein complexes. Behavioral and neuronal response to formalin was quantified by rat grimace scale and pain-associated c-Fos neuronal activation in the trigeminal nucleus caudalis. Involvement of trigeminal activation was assessed by botulinum toxin type A injections into the trigeminal ganglion (2 U/kg).

Results: We found that nasal inflammatory stimulation provoked the neurogenic inflammation within cranial meninges, with correlating intensities of peripheral nasal and intracranial meningeal inflammation. Formalin-evoked painful behavioral score correlated with the number of c-Fos-expressing activated neurons in the bilateral trigeminal nuclei related to nociception. BoNT/A injected into the trigeminal ganglion reduced the inflammation in the nasal mucosa, suggesting a link between trigeminal nerve activation and nasal neurogenic inflammation.

Conclusion: Nasal inflammation induces behavioral responses related to central pain transmission and headache-related trigeminovascular changes, which might account for overlapping symptoms and comorbidity of nasal/paranasal inflammatory disorders with migraine.

S04/5

SAŽETAK SIMPOZIJA: MJESTO NEUROGENE UPALE U PATOFIZIOLOGIJI GLAVOBOLJA

SYMPOSIUM SUMMARY: THE PLACE OF NEUROGENIC INFLAMMATION IN THE PATHOPHYSIOLOGY OF HEADACHES

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The etiology of migraine, trigeminal neuralgia, temporomandibular joint disorders and other form of pain in trigeminal region is not known and could be very different. In this symposium we investigated possible association of those types of pain with neurogenic dural inflammation and responsiveness to botulinum toxin.

S05 SPONZORIRANI SIMPOZIJ: NOVARTIS: SRČANO ZATAJENJE / SPONSORED SYMPOSIUM NOVARTIS: HEART FAILURE

S05/1

SAKUBITRIL VALSARTAN: ZNANSTVENA PODLOGA

SACUBITRIL VALSARTAN: THE SCIENCE BEHIND

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The discovery and development of sacubitril/valsartan is an amazing journey and an excellent example of academia-industrial collaborations. A decline in left ventricular systolic function leads to activation of three major compensatory neurohormonal systems in an attempt to increase cardiac output (CO): sympathetic nervous system (SNS), renin-angiotensin-aldosterone system (RAAS) and natriuretic peptide system. Long-term over-activation of the SNS and RAAS in HF are thought to be harmful and blockade of these pathways has been the focus of current HF therapies. In contrast to the RAAS and SNS, the natriuretic peptide system is a potentially beneficial counter-regulatory system in HF. Neprilysin inhibition may enhance the effects of natriuretic and other vasoactive peptides, which include vasodilation, diuresis and natriuresis, reduced sympathetic tone, reduced aldosterone, and antifibrotic and hypertrophic effects. Sacubitril/valsartan is a first-in-class angiotensin receptor neprilysin inhibitor (ARNI). ARNIs overcome the challenges associated with previous approaches to neprilysin inhibition. It demonstrated a greater benefit in reducing the risk of cardiovascular death and hospitalization compared with current RAS-based standard of care in patients with heart failure with reduced ejection fraction, and is a breakthrough therapy for heart failure. This presentation will review the key moments of sacubitril/valsartan development, share with the audience the inspiring story, including the scientific background and clinical development strategies.

S05/2

SRČANO ZATAJENJE: ZNAČENJE PODATAKA IZ PRAKSE

HF: WHY REAL WORLD DATA MATTER

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Angiotensin receptor neprilysin inhibitor (ARNI) sacubitril/valsartan simultaneously blocks the angiotensin II type I receptor and inhibits the enzyme neprilysin. Neprilysin inhibition augments endogenous biologically active natriuretic peptides (NP) and other vasoactive compounds, with increased generation of cGMP, reported to be reduced in myocardial cells in HFpEF. Moreover, NP augmentation induces diuresis, vasodilation, natriuresis,and can reduce myocardial fibrosis and improve myocardial relaxation. In a phase II trial of HFpEF, PARAMOUNT-HF (Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction), sacubitril/valsartan compared with valsartan, reduced NT-proBNP (N-terminal pro-B-type natriuretic peptide) at 12 weeks and reduced both left atrial volume and New York Heart Association (NYHA) class at 36 weeks compared with valsartan. These data provided the rationale for the design of PARAGON-HF. The PARAGON-HF trial is a randomized, double-blind, parallel group, active-controlled, event-driven trial comparing the long-term efficacy and safety of sacubitril/valsartan and valsartan in patients with chronic HFpEF (left ventricular ejection fraction ≥45%), New York Heart Association functional class II to IV symptoms, elevated natriuretic peptides, and structural heart disease evidenced by echocardiography. The primary outcome is the composite of CV death and total (first and recurrent) HF hospitalizations. The secondary endpoints include change in the KCCQ score (clinical summary score for HF symptoms and physical limitation) and change in NYHA functional class from baseline to 8 months, time to first occurrence of composite renal outcome (≥50% decrease in eGFR relative to baseline, end-stage renal disease or renal death) and time to all-cause mortality.

S05/3

NEDOSTACI TERAPIJE KOD ZATAJENJA SRCA S OČUVANOM EJEKCIJSKOM FRAKCIJOM

UNMET NEEDS IN HFPEF

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The European Society of Cardiology guidelines separate patients with HF to either reduced EF (<40%, HFrEF), mid-range EF (40% to 49%, HFmrEF), and preserved EF (≥50%, HFpEF). The evidence suggests that HFpEF is a separate syndrome from HFrEF. The classical risk factors for developing HFpEF include age, female gender, hypertension, metabolic syndrome, diabetes, obesity, renal dysfunction, waist-to-hip ratio, and physical inactivity. Recent studies have revealed that multiple diastolic and nondiastolic abnormalities in cardiovascular function contribute to the pathophysiology of HFpEF. In HFpEF the driver of myocardial dysfunction comes from systemic comorbidities and endothelial inflammation. LV diastolic dysfunction, or increased LV filling pressure from LV stiffening and abnormal relaxation, is a fundamental mechanism of HFpEF. Overall CV mortality in HFpEF is lower than in HFrEF, however the majority of deaths in HFpEF are due to CV causes. There is a high rate of HF hospitalizations in HFpEF and HFrEF, however a higher rate of non-HF readmissions is observed among patients with HFpEF.

S06 *DINKO VITEZIĆ*: KLINIČKA FARMAKOLOGIJA I FARMAKOEKONOMIKA / CLINICAL PHARMACOLOGY AND PHARMACOECONOMICS

S06/1

KINIČKA FARMAKOLOGIJA I FARMAKOEKONOMIKA - VAŽNOST U PROCJENI LIJEKOVA

CLINICAL PHARMACOLOGY AND PHARMACOECONOMICS: THE IMPORTANCE IN DRUG ASSESSMENT

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Continual rise is in health care costs is a fact, in Croatia and worldwide, and it is not always connected with additional health benefits. Health care spending on medicines is in the focus because it is one of the largest areas within health care, precisely measured and relatively easily manageable in comparison to other health care areas. Pharmacoeconomics is defined as a scientific discipline which analyses the values of one medicine or medical intervention to another by using different types of evaluation. Clinical pharmacology as a basis for rational pharmacotherapy includes also economical aspects of therapy. From the last decade of the 20th century clinical pharmacology evolved and incorporate the principles of pharmacoeconomics and health technology assessment. Clinical pharmacologists today, according to their education and professional practice, have a specific knowledge not only to analyze, but also to gather evidence and be active in preparation, conduct and interpretation of pharmacoeconomic evaluations. Further, clinical pharmacology is involved in the assessment of the value of the new medicinal product in comparison to the others in the same field so the decision about the acceptance, delay or rejection of the new therapy could be firmly grounded.

S06/2

FARMAKOEKONOMSKE ANALIZE U KLINIČKIM ISPITIVANJIMA LIJEKOVA

PHARMACOECONOMIC ANALYSES IN CLINICAL TRIALS

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Introduction: Pharmacoeconomics identifies, measures, and compares the costs and consequences of drug therapy to healthcare systems and society. Combining costs and outcomes indicates whether a particular healthcare intervention represents good value for money. Pharmacoeconomic analysis is now often a part of clinical trials comparing different forms of treatment intervention, considering that the efficiency of a healthcare intervention in practice may differ from its apparent efficacy in the clinical trial.

Materials and methods: The purpose of this presentation is to provide an overview of the emerging need for clinical trials assessing the economic value of interventions given that many countries now consider evidence of economic value along with clinical effectiveness for regulatory and reimbursement decisions.

Results: The growing prevalence and cost of chronic progressive diseases has increased the number of economic evaluations alongside clinical trials. The number of stakeholders interested in both clinical and economic trials is growing, and now includes governments, payers (insurers), health care professionals, and patients. As decision makers increasingly demand evidence of economic value for health care interventions, conducting high-quality economic analyses alongside clinical studies is desirable because this will enable more timely access of patients to new cost-effective treatments.

Conclusions: Clinical trials evaluating medicines, medical devices, and procedures now commonly assess the economic value of these interventions. Methods for designing, conducting, and reporting economic analyses alongside randomized controlled trials will continue to develop and improve over time, reflecting changes in knowledge and the evolving needs of decision makers.

S06/3

FARMAKOEKONOMIKA BIOLOŠKIH LIJEKOVA

PHARMACOECONOMICS OF BIOLOGICAL DRUGS

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The development of biological medicinal products has transformed the treatment of a number of serious chronic and disabling diseases. However, patient access to these life-changing therapies could be limited due to their high cost. Since patent protection and data exclusivity for a number of biologicals are near expiration or already have expired, regulatory pathways have been established to allow the development and approval of biosimilars. A biosimilar is defined as a biological medicine highly similar to another already approved (reference) biological medicine with no clinically meaningful differences in their safety, quality and efficacy. Development of a biological product is different from the process applied to a small molecule drug. A small molecule drug can be fully defined structurally and, therefore, a generic equivalent can be reproduced with an identical chemical structure via a defined chemical synthesis. Biologicals are usually larger, complex proteins produced in living cells which are impossible to be fully characterized. Therefore, biosimilars cannot be considered generic equivalents to reference product and comprehensive comparability studies with the reference product should be performed. The European Medicines Agency (EMA) has approved more than 20 biosimilars, including biosimilars of the monoclonal antibodies (mAbs) infliximab, etanercept, adalimumab, rituximab and trastuzumab. Biosimilars generally have lower costs compared to the reference product. Therefore, they introduce price competition into the market, which leads to reduced treatment price and higher access to therapy.

S06/4

FARMAKOEKONOMIKA NA BOLNIČKOJ RAZINI I DOPRINOS KLINIČKOG FARMAKOLOGA

PHARMACOECONOMICS ON THE HOSPITAL LEVEL AND A CLINICAL PHARMACOLOGIST CONTRIBUTION

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The role of pharmaconomic and clinical pharmacologist in hospital policy at the hospital level depends on the health system in which the hospital is. In healthcare systems where the hospitals are self-sufficient or even profitable, they formulate their own list of medicines, whereby traditional decisions have been made to compare the clinical efficacy of drugs, safety, interaction, pharmacokinetics, pharmacology and procurement price without taking into account the impact of drug use on overall health expenses. Pharmacoeconomics is now being used in almost all hospitals when it comes to compiling medical records where they are in addition to the relationship between efficiency and price, the most important parameters yet the effectiveness and safety of medicines. The correlation between the use of pharmacoeconomic methods and decision-making on the use of medicines in hospitals is positive, and it is now an unavoidable method of compilation of hospital formularys. In systems like ours, pharmacoeconomics at the hospital level is reduced to the use of the cheapest drugs available from the national drug list (HZZO Drugs List in Croatia). While compiling this list, dominant studies have been budget impact analysis, and not cost-effectiveness analysis as the most widely used pharmacoeconomic studies, although there are contradictory opinions. The Hospital Drug Comittee and the Clinical Pharmacologist have the option of choosing the cheapest drug from the list and in the hospital to convince doctors and patients that it is as effective and safe as the most expensive (in our case, the originator). This is now the most pronounced for the selection of biological drugs because of the introduction of more so-called biosimilars in therapy.

S06/5

MOGUĆNOSTI PRIMJENE BLOCKCHAIN TEHNOLOGIJE U MEDICINI I FARMAKOEKONOMICI

OPPORTUNITIES FOR USE OF BLOCKCHAIN TECHNOLOGY IN MEDICINE AND PHARMACOECONOMICS

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Introduction: Blockchain technology is a decentralized database that stores a registry of assets and transactions across a peer-to-peer computer network, which is secured through cryptography, and over time, its history gets locked in blocks of data that are cryptographically linked together, secured and immutable.

Materials and methods: Internet and literature search were performed in order to evaluate potential use cases of blockchain technology in medicine, pharmacology and pharmacoeconomics.

Results: So far, there have been use cases of this technology for cryptocurrencies, digital contracts, financial and public records and property ownership. It is expected that future uses will expand into medicine, science, education, intellectual property and supply chain management. Likely applications in the field of medicine could include electronic health records, health insurance, biomedical and health outcomes research, drug supply, procurement processes and medical education. Utilization of blockchain is not without its weaknesses and currently, this technology is still immature and lacks public or even expert knowledge. Presently, there are issues with scalability, security of smart contracts and user adoption, which all make it difficult to have a clear strategic vision of the technology's true future potential.

Conclusions: Nevertheless, with capital investments into blockchain technology projected to reach 400 million US$ in 2019 alone, health professionals should be aware of the transformative potential that this technology offers for health care organizations and medical practice.

S07 *LIDIJA BACH ROJECKY*: SREDIŠNJI UČINCI BOTULINUM TOKSINA TIPA A: PRETKLINIČKI NALAZI I NJIHOVO ZNAČENJE ZA KLINIČKU PRAKSU / CENTRAL EFFECTS OF BOTULINUM TOXIN TYPE A: EVIDENCE FROM THE LABORATORY AND THEIR IMPACT ON CLINIC

S07/1
NOVA SAZNANJA O SREDIŠNJIM UČINCIMA BT-A

UPDATE ON CENTRAL EFFECTS OF BT-A

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Introduction: Years of investigation of botulinum toxin A (BT-A) action on pain provided important insight into its mechanism of action, but uncovered some of the unresolved issues, as well. In numerous behavioral experiments, it was shown that BT-A reduces pain hypersensitivity after retrograde axonal transport into the central nervous system from the peripheral site of application.

Materials and methods: In this review, some persuasive evidence for BT-A central site of action on pain coming from behavioral as well as immunohistochemical experiments will be summarized.

Results: 1. Bilateral effect of unilateral BT-A application has been demonstrated in different models of bilateral pain; 2. BT-A reduces different types of experimental pain if applied intrathecally, but in contrast to the peripheral application, the antinociceptive effect is achieved faster and in lower doses; 3. The presence of the cISNAP-25 was observed in the dorsal horn of the spinal cord following different routes of peripheral application; 4. Interaction with endogenous spinal opioid and GABA neurotransmitter system as well as glia cells is involved in BT-A's action; 5. Recent experiments demonstrated that BT-A reaches different CNS structures following application in cerebral ventricles, but this did not affect its action on pain of peripheral origin.

Conclusion: Based on a set of data coming from more than 15-years of research, it can be concluded that the main site of BT-A action on pain is situated at the level of the sensory regions of brainstem or spinal segment, associated with the toxin-injected area.

S07/2

PREDLOŽENI MEHANIZMI DJELOVANJA BT-A NA PATOLOŠKU BOL

PROPOSED MECHANISMS OF BT-A ACTION ON PATHOLOGICAL PAIN

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Introduction: Novel preclinical evidences have demonstrated that axonal transport from periphery through sensory neurons to the central nervous system is a prerequisite for beneficial effects of botulinum toxin type A (BT-A) in painful disorders, although the mechanism of action at central synapses remains unclear. Some observations suggest that it might interfere with complex processes of central sensitization at spinal level, including those causing attenuations of inhibitory influences in dorsal horn of the spinal cord.

Materials and Methods: Experiments were performed on male Wistar rats using pathophysiologically different types of pain. Behavior indicating pain was followed in each experiment and biochemical analyses were performed to detect changes in neuronal and glial cells' activation, neurotransmitters and receptors in spinal cord and brain tissue.

Results: Opioid and GABAA antagonists, applied systemically and intrathecally, dose-dependently abolished the antinociceptive effect of peripheral BT-A in all tested models, while no effect was observed following their supraspinal application. The effect of antagonists was short-lasting. BT-A reduced neuronal activation in dorsal horn, which was abolished by both, opioid and GABAA antagonist. BT-A reduced pain after peripheral and intrathecal application, but not after application in cisterna magna or cerebral ventricles, despite of its enzymatic activity in brain regions involved in nociception.

Conclusion: BT-A has segmental antinociceptive effect at the spinal level, which involves an indirect activation of endogenous opioid and GABA-ergic systems. These findings might guide clinical investigations of potentially useful additive or synergistic effects with conventional analgesics and direct clinical trials for novel routes of application, like intrathecal.

S07/3
UTJECAJ BT-A NA KLINIČKU PRAKSU

BT-A ITS IMPACT ON CLINICAL PRACTICE

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Botulinum toxin (BTX), produced by Clostridium botulinum is a potent toxin with therapeutic effects based on its specific synaptic physiology. Since its development for clinical use in 1980s BTX, the most potent biological toxin known to man, has become a useful drug in a range of clinical conditions, especially in the management of muscle over-activity. One of the most notable contributions of BTX therapy is in the field of movement disorders associated with muscle over-activity such as dystonia. According to evidence-based review of the safety and efficacy of BTX in the treatment of movement disorders and classified literature based on AAN criteria (Class I-IV) BTX should be offered as a treatment option for the treatment of cervical dystonia, blapharospasm, hemifacial spasm, adductor laryngeal dystonia, focal upper and lower limb dystonia. BTX is used as therapy in oromandibular dystonia, task-specific dystonia, tremor, ticks, as well as in some uncommon movement disorders as spinal myoclonus, painful/painless legs moving toes and hands. Over the past 10 years, the use of botulinum neurotoxin type A (BTX-A) has revolutionized the treatment of intractable symptoms associated the overactive bladder. More and more clinical data showed that BTX-A also reduce some chronic pain condition not associated with muscular disorders like chronic migraine, neuropathic pain etc. Further research is needed to evaluate different BTX formulations where currently there is insufficient and/or conflicting clinical data. In addition, central effects of BTX should be assessed not only as potential site of therapeutic action but also still not recognized side effects.

S07/4

NOVI REKOMBINANTNI BOTULINUM TOKSIN TIPA A1 UČINKOVIT JE U ŽIVOTINJSKIM MODELIMA AKUTNE I KRONIČNE BOLI

A NOVEL, RECOMBINANT BOTULINUM NEUROTOXIN TYPE A1 SHOWS EFFICACY IN RODENT MODELS OF ACUTE AND CHRONIC PAIN

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Introduction: There is growing evidence that natural botulinum neurotoxin (BoNT) reduces pain by inhibiting release of pain mediators. Here, we evaluated efficacy of a recombinant BoNT type A1 (rBoNT/A1) in models of acute, ultraviolet-B (UVB)-induced pain and chronic chemotherapy and osteoarthritis associated pain in rats.

Materials and methods: UVB pain was induced by exposing plantar surface of the hindpaw to fluorescent light, followed by von Frey tests up to 72h post-irradiation. Chemotherapy pain was induced by injection of oxaliplatin, followed by von Frey test up to 18-days post-injection. Osteoarthritis pain was induced by intra-articular injection of monoiodoacetate (MIA) into the knee joint, followed by weight bearing tests up to 28 post-injection. BoNT/A1 (10, 20, 30 pg/animal) or its vehicle, gelatine phosphate buffer (GPB) were administered via intraplantar route either 3 days before UVB or 5 days after administration of oxaliplatin. In osteoarthritis study BoNT/A1 was administered via intraarticular route 3 days following MIA injection.

Results: BoNT/A1 resulted in dose-dependent reversal of the deficit in mechanical sensitivity 48h post-UVB. BoNT/A1 (20, 30 pg/animal) also reduced oxaliplatin-mediated allodynia 8- and 12-days post-injection and at 10 pg/animal on day 18. BoNT/A1 (20, 30 pg/animal) resulted in long-lasting and near complete reversal of mechanical hyperalgesia, while 10 pg/animal was without effect.

Conclusions: rBoNT/A1 shows robust and long-lasting effects reducing allodynia and hyperalgesia in a rat models acute and chronic pain. Thus, it represents a useful tool, comparator compound for evaluation of analgesic activity of novel, recombinant modified neurotoxins.

S08/1
PREVENTIVNO FARMAKOGENETIČKO TESTIRANJE U EUROPI: PROJEKT U-PGX

PRE-EMPTIVE PHARMACOGENETIC TESTING ACROSS EUROPE: THE U-PGX PROJECT

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Introduction: The Horizon2020 project Ubiquitous Pharmacogenomics (U-PGx; www.upgx.eu; grant agreement H2020 No 668353) is the largest EU multicentre project on implementation of pre-emptive pharmacogenomic testing in the clinical practice.

Materials and methods: Within the U-PGx project, a bloc-randomized multi-centre clinical trial PREPARE was set up to establish if implementation of PGx-guided drug prescribing for a panel of drug-pharmacogene pairs reduces drug-genotype associated adverse drug reactions (ADRs) in comparison to patients receiving standard of care treatment, improves the quality of life and brings economic advantage for the healthcare systems in seven European countries, including Slovenia.

Results: A panel of pharmacogene-drug pairs with major influence on interpatient variability in drug response was first established. Respective KASPar assays were designed for 44 common functional variants within the 13 pharmacogenes and validated on SNPline (LCG Group) genotyping platforms. Evidence based treatment guidelines curated by the Dutch Pharmacogenetics Working Group (DPWG; https://www.pharmgkb.org/page/dpwg) were translated into seven EU languages and multilingual electronic or paper based clinical decision support tools were introduced at all implementation sites. Seven EU countries have been block-randomized to start with either PGx-guided prescribing (study arm) or standard-of-care (control arm) for 18 months. The main inclusion criterion is the first prescription of a drug with DPWG guideline (index drug) in the routine care. So far, over 5000 patients were included in the PREPARE study across Europe.

Conclusions: Within the U-PGx project and the PREPARE study genotyping methodology and infrastructure for panel-based pre-emptive pharmacogenomic testing were successfully implemented in seven EU countries, including Slovenia.

S08/2
FARMAKOGENOMIKA I INTERAKCIJE LIJEKOVA

PHARMACOGENOMICS AND DRUG INTERACTIONS

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Pharmacogenomics (PGx) is the study of how persons' unique genetic makeup influences their response to drugs. Polypharmacy represents a significant risk for drug-drug, drug-disease and drug-gene interactions and has become a significant source of health care utilization and costs. The availability of genomic testing has grown, but its clinical application is still in the early stages. Although majority of current pharmacogenomics decision support tools provide assessment only of single drug-gene interactions utility of pharmacogenomics for preventing potential side effects of polypharmacy has been proven in many cases. Particular attention in pharmacogenomics has been devoted to cytochrome P450 (CYP) enzymes, which are involved in the metabolism of 70%–90% of all prescribed drugs. The most common CYP enzymes involved in drug metabolism are CYP2D6, CYP2C9, CYP2C19, CYP3A4/5. With these enzymes, there may be many spectrums of genotypes resulting in poor to ultrarapid metabolizers. The importance of polymorphic drug transporters in determining drug disposition is increasingly appreciated. Two major families of drug transporters relevant for drug-drug-gene interactions are ABC and SLC. There are well documented data for drug interactions on the level of ABCB1, ABCC2, ABCG2, SLCO1B1. To develop comprehensive pharmacogenomics decision support for medication risk assessment in people with polypharmacy that simultaneously accounts for multiple drug and gene effects, proposed is approach that addressed two aims: (1) development of comprehensive knowledge repository of actionable pharmacogenes; (2) introduction of scoring approaches reflecting potential adverse effect risk levels of complex medication regimens accounting for pharmacogenomic polymorphisms of multiple drug metabolizing pathways and drug transporters.

S08/3

LIJEČENJE TIOPURINSKIM LIJEKOVIMA BOLESNIKA S UPALNIM BOLESTIMA CRIJEVA - PREDIKCIJA TEMELJENA NA GENOTIPU I FENOTIPU TPMT

THIOPURINE THERAPY IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE – TPMT GENOTYPE AND PHENOTYPE-BASED PREDICTIONS

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Introduction: Azathioprine or 6-mercaptopurine are not effective in one-third of patients, and up to one-fifth of patients have adverse reactions. This variability in drug response is explained by TPMT gene variants and by variable formation of active metabolites. Since genetic variability shows marked interindividual and interethnic differences, the aim of this study was to examine thiopurine metabolites (6-methyl mercaptopurine, 6-MMP and 6-tioguanine, 6-TG) in Croatian IBD patients with their dose prescribed based on preemptive TPMT genotyping.

Materials and methods: Thiopurine metabolites (6-MMP and 6-TG) were analyzed in blood samples of 204 patients aged 2-73 years. TPMT *2, *3B, *C genotyping was performed by methods based on RealTime PCR. Concentrations of thiopurine metabolite were determined using in-house HPLC-DAD method and metabolite ratio (6-MMP/6-TG) was calculated.

Results: Mean dose of azathioprine adjusted according to TPMT genotype was 129.7 mg (40-250 mg). Medians of 6-MMP and 6-TG concentrations were 869 and 213 pmol/8X108E, respectively, and medians of 6-MMP and 6-TG concentrations corrected for dose were 6.34 and 1.61 pmol/8X108E/mg, respectively. Median of metabolic ratio (6-MMP/6-TG) was 4.37. Among patients, 9.2% were carriers of *3A allele. A statistically significant correlation was confirmed between metabolite concentration ($p<0.01$) and dose-corrected concentration ($p<0.01$) according to TPMP variants. Concentration of 6-MMP was significantly lower and of 6-TG higher in subjects who were *3A allelle carriers.

Conclusion: The obtained results show that TPMT polymorphisms exert an influence on pharmacokinetics of thiopurine drugs. Besides preemptive TPMT genotyping, metabolite concentration and ratio serve as additional tools for individualization of thiopurine therapy.

S08/4

IMPLEMENTACIJA PODATAKA O FARMAKOGENOMICI U INFORMACIJE O LIJEKU

IMPLEMENTATATION OF PHARMACOGENOMIC INFORMATION IN PRODUCT INFORMATION

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Knowledge about influence of genes variants in drug response is increasing and consequently enables transition from population-based treatment to individualized therapy. Product information (PI), approved during marketing authorisation procedure, is the basis of information for healthcare professionals on how to use the medicinal product (MP) safely and effectively and therefore inclusion pharmacogenomic information (PGX) in PI is of paramount importance. This presentation reviews implementation of PGX in PI worldwide with special focus on European union. Relevant regulatory guidelines and work of Pharmacogenomic Working Party will be also presented. Results of our own study analysing implementation of pharmacogenomics in PI of MP approved through national procedures in Croatia will also be included in presentation. In conclusion although, problems in implementation are anticipated, especially in view of different healthcare systems in EU, PI should aim to be specific in term when testing is indicated and how to interpret results of pharmacogenomic testing.

S08/5

FARMAKOGENOMIKA I DIGITALNO ZDRAVSTVO

PHARMACOGENOMICS AND DIGITAL HEALTH

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Introduction: This talk will present digital and electronic tools that can facilitate a wide spread implementation of pharmacogenomics, within the international and the Croatian context. We will focus on three aspects: 1.) we will present currently available information technology (IT) solutions that support pharmacogenomic service delivery, 2.) we will present the challenges and the potential of using digital technologies to simplify and scale up pharmacogenomic analysis workflow within the Croatian public healthcare system, and 3.) we will present the potential benefits of including pharmacogenetic test results into the Croatian electronic health record.

Materials and methods: Market research was conducted to identify currently available IT solutions to support pharmacogenomic service delivery and interviews with members of a Croatian pharmacogenetic laboratory were performed to identify obstacles and opportunities for digitization.

Results and conclusions: Several IT solutions for pharmacogenomics are currently available worldwide, but these come with limitations related to data privacy, storage, reliability, repeatability, and validity of generated reports. In the context of Croatian healthcare, we propose to improve the use of existing expertise and resources by including digital solutions in the process of pharmacogenetic report creation and dissemination. Finally, we describe the benefits and opportunities of including pharmacogenetic reports into the electronic health record, where relevant genetic information would be readily available to all medical personnel.

S09 *MARTINA SMOLIĆ*: SVIJET BEZ HCV DO 2030: MIT ILI STVARNOST? / WORLD WITHOUT HCV BY 2030: MYTH OR REALITY?

S09/1

NOVE SPOZNAJE O PATOGENEZI I LIJEČENJU HEPATITISA C

UPDATE ON HEPATITIS C PATHOGENESIS AND TREATMENT

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In the US, there are about 4 million cases (2%) of HCV infection, but worldwide, there are an estimated 200 million cases. In the US, because of an opioid epidemic, the number of new cases has tripled to 60,000 new cases/yr. Unfortunately, 85% of acute exposures do not clear the virus and develop chronic hepatitis. This can lead to cirrhosis and liver failure, the most common indication for liver transplantation in the US. Once cirrhosis has appeared, the risk for development of hepatocellular carcinoma increases dramatically. Fortunately, direct-acting agents that inhibit viral replication are highly effective. The remaining challenges include dealing with the opioid epidemic, providing addiction treatment and rehabilitation to decrease the infection rate. Hepatitis B and C co-infection is common. Because HCV naturally suppresses HBV replication, treatment of HCV alone can result in a flare of HBV. Patients with HCV should be screened for HBV, and if present, simultaneous treatment for both viruses may be recommended. Zepatier and Mavyret can be used in cases of renal insufficiency. Hepatic failure can occur in patients who have hepatic decompensation. Harvoni and Epclusa can be used in these patients. Access to anti-HCV meds can be a challenge. In the US, direct-acting anti-HCV meds require prior approval by insurance companies. Without insurance, the cost of direct-acting anti-HCV would be prohibitive and greatly limit access. Prices have decreased, but still can present a financial burden. In spite of these challenges, treatment of HCV has made a quantum leap forward in the past decade.

S09/2

INTERAKCIJE DIREKTNODJELUJUĆIH PROTUVIRUSNIH LIJEKOVA ZA LIJEČENJE HEPATITISA C

DIRECT ACTING ANTI-HCV DRUGS: DRUG-DRUG INTERACTIONS

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Direct-acting antiviral agents (DAAs) significantly transformed and improved treatment of hepatitis C virus (HCV) infection due to higher response rates and reduced toxicities. HCV infected patients take combination of DAA drugs to treat infection, often take multiple comedications to manage other co-morbidities or adverse events. Drug–drug interactions (DDI) associated with this polypharmacy, thus became one of challenges in treatment of these patients. The careful assessment of the regular out-patient medication and subsequent evaluation of potential DDIs with a DAAs are absolutely crucial to ensure drug safety in all treated patients and role of clinical pharmacist become important in multidisciplinary care team . Web-based DDIs tools like http://www.hep-druginteractions.org represent the best way for an assessment of potential DDIs. However, although this web resource includes a huge number of drugs and regular update, some of drugs probably are not covered. An overview of conducted drug–drug interaction studies and a list of contraindicated medications is not enough for the clinical management of these drug–drug interactions. Knowledge of pharmacogenomics, pharmacokinetic profiles, concentration–effect relationships, as well as data from „real life“, especially for new DAA are the keys for the interpretation of these data. Also, insight into how to manage these interactions (e.g., dose adjustments, safe alternatives and therapeutic drug monitoring) is of equal importance. This review provides a practical overview of the safe and effective management of these clinical challenges with the last update.

S09/3

UČINCI DIREKTNODJELUJUĆIH PROTUVIRUSNIH LIJEKOVA NA ENDOKRINE MANIFESTACIJE HCV INFEKCIJE

EFFECTS OF DAA TREATMENT ON ENDOCRINE MANIFESTATIONS OF HCV INFECTION

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Chronic hepatitis C is a systemic disease inducing metabolic alterations leading to extrahepatic consequences. In particular, hepatitis C virus (HCV) infection seems to increase the risk of insulin resistance (IR) and incident type 2 diabetes mellitus in predisposed individuals, independently of liver disease stage. Also, interferon and ribavirin treatment of HCV hepatitis may be less successful in the presence of IR and the effect of IR on the new direct-acting antiviral treatment is unclear. Moreover, clinical trials in HCV-positive patients have reported improvement in glucose metabolism after antiviral treatment; recent studies have suggested that this metabolic amelioration might have a clinical impact on type 2 diabetes mellitus-related complications. These observations raise the question as to whether the HCV eradication may also have an impact on the future morbidity and mortality due to type 2 diabetes mellitus. Autoimmune thyroid diseases are among the most frequent endocrine disorders in HCV infected patients IFN-α therapy is a well-known risk for the development of autoimmune thyroid diseases and thyroid dysfunctions,however effects of DAA therapy seems to have quite the opposite effect. As a consequence of the overall slower liver disease progression and increased viral clearance in women due to protective effectsof estrogen and estradiol , the disease burden from HCV infection is found predominantly in men. This seems to be less important in the direct-acting antiviral era, when response rates for HCV therapy have increased so substantially that baseline demographic factors seem to have less of an effect on overall rates of cure.

S09/4

NAPREDAK U RAZVOJU IMUNOPROFILAKSE HCV-A

PROGRESS TOWARDS HCV IMMUNE PROPHYLAXIS

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One of the major causes of liver diseases that may progress to liver cirrhosis and hepatocellular carcinoma is Hepatitis C (HCV) infection. Immunopathogenesis of HCV-induced hepatitis is affected by host immune factors along with the actions of HCV proteins that promote viral persistence and immune system dysregulation. Although vaccines for hepatitis A and hepatitis B exist , development of a hepatitis C vaccine has presented a challenge and still a vaccine capable of protecting against HCV, is not available but several vaccines are currently under evaluation. A vaccine against HCV is required to prevent transmission, regardless of risk factors, and significantly reduce the global burden of HCV-associated diseases. Several elements hamper the development of a vaccine against HCV due to viral factors such as HCV genomic diversity, the cell to cell spread of HCV, the high viral mutation rate, and the development of infectious lipoviral particles. Although highly effective vaccines could prevent infection altogether, immune responses that increase the rate of HCV clearance and prevent chronic infection may be sufficient to reduce disease burden. Despite development challenges, a prophylactic vaccine is necessary for HCV global control.

S09/5

MOLEKULARNE I KLINIČKE OSNOVE REZISTENCIJE DIREKTNODJELUJUĆIH PROTUVIRUSNIH LIJEKOVA ZA LIJEČENJE HEPATITISA C

MOLECULAR AND CLINICAL BASES OF ANTI-HCV DRUG RESISTANCE

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With excellent efficacy of direct-acting antivirals (DAA) now available, treatment failure is rare, however, when it occurs, it is mostly due to relapse. On treatment failure is very rare. Baseline resistance-associated amino acid (aa) substitutions (RASs) are common, but do not frequently result in treatment failure. Therefore, detection of baseline mutations is not generally predictive of response. In addition, the frequency of resistant mutation data is often underestimated because virus <500 IU/ml cannot be deep sequenced, and therefore, is not included in resistance data. Mutations resulting in resistance have been identified, and involve NS5A and protease inhibitors. Resistance to NS5B polymerase inhibitors is rare. To minimize the development of resistance mutations, it is recommended to limit exposure to reliable compliant patients because missed doses increase the risk of development of resistance mutations. Use of multiple agent combinations with different mechanisms of action including NS5B inhibitors, increases the barriers to resistance and reduce the risk of development of resistance. In recently published real-life studies on DAA failures, RASs prevalence was remarkably high. These findings advocate for HCV resistance testing at failure, in order to optimize the second-line therapeutic options and to overcome treatment failure.

S10/1

AKTUALNI IZAZOVI SIGURNE PRIMJENE LIJEKOVA – PERSPEKTIVA PRAC-A

CURRENT CHALLENGES OF DRUG SAFETY – PRAC PERSPECTIVE

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The Pharmacovigilance Risk Assessment Committee (PRAC) is the European Medicines Agency's (EMA) committee responsible for assessing and monitoring the safety of human medicines. The PRAC was established in line with the pharmacovigilance legislation, which came into effect in 2012 to help strengthen the safety monitoring of medicines across Europe. The PRAC is responsible for assessing all aspects of risk management of human medicines, including the detection, assessment, minimisation and communication of the risk of adverse reactions, while taking the therapeutic effect of the medicine into account, design and evaluation of post-authorisation safety studies and pharmacovigilance audit. Presentation will describe the most important safety issues discussed by PRAC in last 2 years. It will also tackle challenges especially failure to implement regulatory risk minimization measures crucial for positive benefit risk ration of medicinal products. PRAC established Strategy on Measuring the Impact of Pharmacovigilance Activities. The PRAC strategy aims to systematically measures patient-relevant health outcomes of major regulatory interventions, shifting the focus of pharmacovigilance to activities and regulatory tools that make a difference in daily healthcare. The presentation will also describe future challenges in assessment of advanced therapy medicinal products (ATMPs).

S10/2
(NE)UČINKOVITOST MJERA MINIMIZACIJE RIZIKA

(IN)EFFECTIVENESS OF RISK MINIMISATION MEASURES

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Risk minimisation measures (RMMs) are interventions intended to prevent or minimise the occurrence of adverse drug reactions associated with medicinal products or to minimise their severity and impact on the patient if they occur. Routine risk minimisation measures are in place for every medicinal product, while additional ones are implemented when routine RMMs are deemed insufficient. Examples of additional risk minimisation measures include educational materials, controlled distribution systems or pregnancy prevention programmes. When in place for a specific medicinal product, effectiveness of RMMs has to be evaluated to establish whether RMMs are effective or not and whether modifications are needed to fulfil their objectives. Depending on the measured indicators, if evaluated as ineffective, RMMs could be strengthen or reduced/simplified. In certain instances, depending on the indication for use of the medicinal product and the availability of other therapeutic options, ineffectiveness of RMMs can lead to a stricter monitoring of the medicine's use or even to a withdrawal of the product from the market. In recent years, the number of EU wide assessment procedures (called referrals) triggered by the ineffectiveness of RMMs has increased. The Pharmacovigilance Risk Assessment Committee (PRAC) of the European Medicines Agency's (EMA) is responsible for conducting safety referral procedures. In several cases, PRAC concluded that the medicinal products need to be withdrawn from the EU market, while in others stricter conditions for the use of products were recommended. The presentation will focus on recent cases of ineffectiveness of RMMs for medicinal products**,** PRAC evaluation and subsequent impact on everyday clinical practice.

S10/3

FARMAKOVIGILANCIJA U EDUKACIJI ZDRAVSTVENIH RADNIKA

PHARMACOVIGILANCE AS A PART OF CONTINUOUS HEALTH WORKERS EDUCATION

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Adverse drug reactions (ADR) are among the leading causes of mortality and morbidity responsible for causing additional complications and longer hospital stays. Education in pharmacovigilance (PV) is of paramount importance for building knowledge of and raising awareness about ADRs among healthcare professionals and implementation of risk minimization measures. In Europe, a set of 252 learning outcomes for clinical pharmacology and therapeutics (CPT) education were published to harmonize medical education. The key PV aspects are covered in the description of the CPT learning outcomes. World Health Organization (WHO) developed WHO PV Core Curriculum for University Teaching. WHO PV Core Curriculum identifies competencies students need to develop and what key aspects to be taught. According to WHO PV Core Curriculum, the importance of PV can be taught from the first year of education. The clinical aspects of PV can be considered as logical extension of general subjects such as pharmacology and pharmacotherapy. The actual recognition, management and prevention of ADRs therefore requires broad knowledge and skills from pharmacology, pharmacokinetics, pathophysiology, pharmacotherapy, drug development, pharmaceutical regulations, epidemiology and general science. HALMED participates in pharmacists and medical students' education and works closely with healthcare associations. This presentation will describe HALMED's experience gained in graduate/postgraduate education in pharmacovigilance and continuous professional development of healthcare professionals during the last ten years. In addition, it will describe implementation of key PV elements according to WHO PV Core Curriculum for University Teaching, in curriculum of medical, pharmacy and nursing schools in Croatia.

S11 *GORDANA ŽUPAN, KRISTINA PILIPOVIĆ*: PATOFIZIOLOŠKE I FARMAKOLOŠKE PRETKLINIČKE STUDIJE POREMEĆAJA I OŠTEĆENJA SREDIŠNJEG ŽIVČANOG SUSTAVA / PATHOPHYSIOLOGICAL AND PHARMACOLOGICAL PRECLINICAL STUDIES OF CENTRAL NERVOUS SYSTEM DISTURBANCES AND DAMAGE

S11/1

IDENTIFIKACIJA MOLEKULA VAZNIH ZA NEUROREGENERACIJU KORIŠTENJEM *IN VITRO* MODELA I PROTEOMIKE

IDENTIFYING MOLECULES IMPORTANT FOR NEUROREGENERATION USING IN VITRO MODELS AND PROTEOMICS

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Introduction: One of the major challenges of modern neurobiology concerns the inability of the adult mammalian central nervous system (CNS) to regenerate and repair itself after injury. Unlike the situation in adult mammals, lower vertebrates, such as fish and amphibians can regenerate significant portion of their CNS. Moreover, even higher vertebrates, including mammals, can regenerate CNS during their embryonal development. It is poorly understood why this potential is lost with evolution and development and why it becomes very limited in adult mammals.

Materials and methods: A preferred model to study and reveal the cellular and molecular basis of neuroregeneration is neonatal opossum (Monodelphis domestica). Opossums are marsupials that are born at very immature stage with unique possibility to successfully regenerate spinal cord after injury in the first two weeks of their life and thus offer an exceptional opportunity to study neuronal regeneration. We have analyzed the proteoms of the spinal tissue of the opossums of different age, looking for the molecules associated with neuroregeneration.

Results: We have identified more than 4700 proteins to be differentially present in the opossum spinal tissue that can and cannot regenerate. Among those, we found many proteins related to different neurodegenerative diseases. We have developed several in vitro preparations (for example opossum spinal primary cultures) to study function of the selected candidate proteins.

Conclusion: The results of the project could make substantial contribution to our understanding of neuronal regeneration in mammals, but also provide candidate targets for future novel therapeutic interventions for neurodegenerative disorders.

S11/2

TRAUMATSKA OZLJEDA MOZGA: PATOFIZIOLOGIJA I NEUROPROTEKTIVNA TERAPIJA

TRAUMATIC BRAIN INJURY: PATHOPHYSIOLOGY AND NEUROPROTECTIVE THERAPY

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Traumatic brain injury (TBI) is a major cause of death and long-term disability, especially in previously healthy young people. It is also associated with increased posttraumatic incidence of different diseases and disturbances such as neurodegenerative, psychiatric, cognitive and sleep disorders, systemic metabolic and neuroendocrine dysregulations, sexual dysfunction, etc., many of them lifelong. The most frequent causes of single TBI are falls and motor vehicle accidents while the repetitive form is more often associated with contact sports, domestic spousal violence, child abuse or blast injuries. Because of all the above mentioned, TBI presents a significant public health problem all over the world. It is a complex continuous disease process that includes different events with subsequent brain tissue damage. Although numerous preclinical and clinical studies were done in order to clarify the pathophysiology of the brain trauma, all the mechanisms involved in the brain structures' damage still remain unknown. Today's therapy of TBI patients is supportive and symptomatic. Clinically effective pharmacological neuroprotective therapy for TBI has not yet been established. Therefore, development of novel pharmacological treatment options is of the highest priority. Today, special attention is on multifunctional drugs that affect different processes of brain damage in TBI. In this talk, an overview of current knowledge on the pathophysiology of single and repetitive mild TBI as well as on different neuroprotective therapeutic strategies, including our recent results in this field, will be presented.

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S11/3

MULTIMODALNA *IN VIVO* SNIMANJA MIŠJEG MOZGA KAO OSNOVA PRETKLINIČKIH ISTRAŽIVANJA LIJEČENJA MOŽDANOG UDARA

MULTIMODAL IN VIVO IMAGING OF MOUSE BRAIN IN PRECLINICAL STUDIES OF STROKE THERAPIES

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Introduction: Stroke is the second leading cause of death and third cause of disability worldwide. Although, therapies in form of recanalization are in use, possible neurorestorative options are not available. In order to define the critical elements to design or validate medical interventions, we created a multimodal imaging platform to longitudinally monitor the molecular events in the mouse brain following ischemic brain injury.

Materials and methods: Stroke was induced by 60 minutes middle cerebral artery occlusion followed by reperfusion. The animals were monitored during 28 days by multiple imaging sessions, functional evaluation and subsequent histological brain analysis. Magnetic resonance imaging (MRI) with 7T preclinical scanner (Bruker) was used to volumetrically asses the ischemic lesion, bioluminescence imaging (BLI) by optical imager (Perkin Elmer) to monitor gene activity using luciferase reporters, and Tlr2, Casp3 and 7, and Gap43 as molecular markers.

Results: BLI molecular activity included neuroinflammaton by Tlr2 gene, neurorepair by Gap43 gene and apoptosis by an innovative caged DEVD-luciferin approach developed in our laboratory. Combining BLI and MRI allowed the standardization of the measurements according to the size of ischemic lesion, revealing significant increase of repair and apoptosis elements in the tested Tlr2-deficient mice with modified neruoinflammation.

Conclusions: BLI combined with MRI as a multimodal approach allows the assessment of the elements of brain repair after ischemic lesion in the mouse. Tlr2-deficient mice indicated that modified neuroinflammation enhanced the neurorepair and survival rate, but was accompanied by increased apoptosis and tissue loss, in the chronic phase of ischemic stroke.

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S11/4

NEUROPROTEKCIJA I LIJEČENJE AKUTNOG ISHEMIJSKOG UDARA

NEUROPROTECTION AND THE TREATMENT OF ACUTE ISCHEMIC STROKE

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Acute ischemic stroke is one of the major causes of mortality and long-term disability in both developed and developing countries of the world. Intravenous tissue plasminogen activator and mechanical thrombectomy are basic standards of treatment for acute ischemic stroke although not ideal for various restrictions and complications, limiting to 10% or less the percentage of patients treated within the appropriate time window. Despite various limitations, the concept of neuroprotection as potentially valuable adjunct therapy for acute ischemic stroke is still the subject of extensive research. It is based on extention of the window for recanalization therapy or on prevention of neuronal death caused either by ischemic brain injury or by reperfusion injury after successful therapy. The main targets for decreasing or preventing reperfusion injury include decreasing reactive oxygen species, suppressing microglia, preventing hyperglycemia etc. In the past decades, the efficacy and safety of numerous neuroprotective agents were shown in various animal stroke models. However, in clinical trials, promising pre-clinical studies have not been translated into positive outcomes. The combination of thrombolysis with pharmacological and non-pharmacological neuroprotective approaches, the clinical studies pertaining to neuroprotection, as well as the different preclinical neuroprotective therapies, their presumed mechanisms of action and their future applications in stroke patients will be discussed.

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S12 *MELITA ŠALKOVIĆ PETRIŠIĆ, JELENA OSMANOVIĆ BARILAR*: MODELIRANJE NEURODEGENERATIVNIH POREMEĆAJA I INZULINSKE REZISTENCIJE: TERAPIJSKE MOGUĆNOSTI / MODELLING OF NEURODEGENERATIVE DISORDERS AND INSULIN RESISTANCE: THERAPEUTIC OPTIONS

S12/1

UČINCI LIRAGLUTIDA I ORALNE GALAKTOZE U ŠTAKORSKOM MODELU SPORADIČNE ALZHEIMEROVE BOLESTI

THE EFFECTS OF LIRAGLUTIDE AND ORAL GALACTOSE IN A RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Introduction: Glucose hypometabolism and insulin resistance in the brain are well-established features of sporadic Alzheimer disease (sAD) and drugs aiming to improve these alterations are currently under investigation. We explored whether treatment with glucagon-like peptid-1 (GLP-1) agonist liraglutide and oral galactose (GLP-1 secretion stimulator) has therapeutic effects on metabolic and cognitive deficit in sAD model, streptozotocin intracerebroventricularly-treated (STZ-icv) rats.

Materials and methods: Adult male Wistar rats were STZ-icv injected (1-3 mg/kg) and subjected after 2 months to 1-month intraperitoneal liraglutide (150 and 300 mg/kg) or after 1 and 4 months to 2-month oral galactose treatment (200 mg/kg). Memory was assessed by Morris Water Maze and Passive avoidance tests, brain glucose metabolism by fluorodeoxyglucose PET scan, plasma GLP-1 activity by ELISA and neuroinflammation by immunohistochemistry.

Results: Chronic liraglutide (high dose) and oral galactose (initiated 1 month after STZ-icv) treatments attenuated the neuroinflammatory reaction and restored the cognitive deficit caused by STZ-icv, associated with increased plasma GLP-1 activity and normalization of brain glucose metabolism. Oral galactose treatment had no effect on cognition and GLP-1 level when initiated 4 months after STZ-icv.

Conclusions: The dose-dependent effect of liraglutide and sAD stage-dependent effect of oral galactose on normalization of cognitive deficit seems to be mediated by GLP-1 increase and possibly by normalization of cerebral glucose hypometabolism in STZ-icv rat sAD model.

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S12/2

METABOLIČKO PROFILIRANJE I PRIMJENA ORALNE GALAKTOZE U TRANSGENIČNOM TG2576 MIŠJEM MODELU ALZHEIMEROVE BOLESTI

METABOLIC PROFILING AND ORAL GALACTOSE TREATMENT IN TRANSGENIC TG2576 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder associated with metabolic dysfunction in the brain. Our recent research demonstrated that chronic oral galactose treatment prevented/normalized early cognitive decline in a rat model of sporadic AD. Here we explored the effect of 2-month oral galactose treatment on cognition and brain metabolism in familial AD model, 5- and 9-month old transgenic Tg2576 mice.

Materials and methods: Cognition and behavior were tested by Morris Water Maze, Open Field and Elevated Plus Maze tests and metabolic status in plasma, cerebrospinal fluid and hippocampus was assessed by means of intraperitoneal glucose tolerance test and fluorodeoxyglucose PET scan. The level of insulin and glucagon-like peptide-1(GLP-1) was measured by ELISA.

Results: Oral galactose normalized alterations in cerebral glucose metabolism in both age groups of Tg2576 mice. However, while in cognitively unimpaired younger mice it additionally normalized plasma insulin and reduced grooming behavior, in older ones it caused further decrement in plasma insulin and worsening of glucose tolerance abnormalities without cognitive improvement. GLP-1 level was unaltered in all groups.

Conclusions: The results indicate that glucose/insulin- (not GLP-1) related metabolic changes precede cognitive impairment in Tg2576 mice model of familial AD with therapeutic potential of oral galactose seen on improvement of cerebral glucose metabolism only but not on cognition, underlying the differences in metabolic pathology between the two AD forms.

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S12/3

GUBITAK TOLERANCIJE U NEURODEGENERACIJI: KOLIKO JE KORISTAN METFORMIN?

LOSS OF TOLERENCE IN NEURODEGENERATION: HOW USEFUL IS METFORMIN?

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The biology of neurodegenerative disease is complex and still no causative treatments are available. Major challenges ahead are to understand the specific vulnerabilities of cell subtypes, the impact on the brain network and the role of aging. Our aim is to identify molecular mechanisms that tolerate gene defects and mitochondrial dysfunction in Parkinson's disease that could be hijacked for therapies. We use primary cells from patients and healthy individuals to model neurodegenerative diseases and employ reprogramming and gene editing techniques. We differentiate neurons via neural progenitors and perform deep phenotyping using a wide range of physiological, genetic and biochemical readouts. In monogenetic PD the trigger is a faulty gene product that initiates biological compensation (tolerance) allowing neurons to initially survive and thrive. Mitochondria play a crucial and central role in the tolerance process and the diabetes drug Metformin and similar compounds could enhance this compensation mechanism, pushing back tipping points and extending quality of life. Metformin acts primarily at the mitochondria by slowing oxidative respiration, initiating mitochondrial signalling and modulation of energy metabolism. Hence, Metformin influences pathways associated with biological renewal, damage compensation, metabolic restriction and longevity. It is important to first pin down the exact disease mechanisms at both a molecular and systems level to understand why such diseases develop later in life. Only then, the therapeutic potential of metformin can be properly assessed, controversies addressed and translation into the clinics can be justified.

S12/4

GENI POVEZANI S DIJABETESOM I MOTORNO-BIHEVIORALNI FENOTIPOVI: LEKCIJE IZ MIŠJIH I STANIČNIH MODELA

DIABETES-RELATED GENES AND MOTOR-BEHAVIOURAL PHENOTYPES: LESSONS FROM MOUSE AND CELLULAR MODELS"

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The advances in medicine, together with lifestyle modifications, led to a rising life expectancy. Unfortunately, however, ageing is accompanied by an alarming boost of age-associated chronic pathologies, including neurodegenerative and metabolic diseases. Interestingly, a non-negligible interplay between alterations of glucose homeostasis and brain dysfunction has clearly emerged. In particular, epidemiological studies have pointed out a possible association between Type 2 Diabetes (T2D) and neurodegenerative disorders, including Parkinson’s (PD) and Alzheimer’s Diseases. Insulin resistance, one of the major hallmarks for T2D, has a detrimental influence on PD, negatively impacting neurologic phenotype, accelerating its progression and worsening cognitive impairment. The PED/PEA-15 protein represents a possible candidate linking T2D and PD, because it is increased in subjects with T2D and is highly expressed in the brain. To test this hypothesis, we have analyzed the neurological and neurochemical phenotype of transgenic mice overexpressing PED/PEA-15 (tgPED). These mice develop impaired glucose tolerance and insulin resistance, accompanied by neurological features resembling paralleled by 48% reduction of dopamine levels in the striatum. Thus the tgPED mice may represent a genetic animal model of neurological disease linked to T2D. Another example is given by a mouse strain carrying heterozygous hypomorphism of the Prep1, a gene whose overexpression impairs insulin action and glucose metabolism. In these mice, Prep1 deficiency alters olfactory morpho-functional integrity and olfaction-mediated eating behavior by affecting BDNF-TrkB signaling. Prep1 could, therefore, play a crucial role in behavioral dysfunctions associated to impaired responsiveness to BDNF and control food intake.

S13 *PREDRAG SIKIRIĆ*: BPC 157 / BPC 157

S13/1
BPC 157: PROŠLOST, SADAŠNJOST, BUDUĆNOST

SUMMARY OF BPC 157 STORY: PAST, PRESENT, FUTURE

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We reviewed again the significance of the stable gastric pentadecapeptide BPC 157 as a likely mediator of Robert's stomach cytoprotection/adaptive cytoprotection and organoprotection and as novel mediator of Selye's stress coping response to reestablish homeostasis. Specific points of BPC 157 therapy and the original concept of Robert's cytoprotection/adaptive cytoprotection/organoprotection are discussed, including the beneficial effects of BPC 157. First, BPC 157 protects stomach cells and maintains gastric integrity against various noxious agents (Robert's killing cell by contact) and is continuously present in the gastric mucosa and gastric juice. Additionally, BPC 157 protects against the adverse effects of alcohol and nonsteroidal anti-inflammatory drugs on the gastric epithelium and other epithelia, that is, skin, liver, pancreas, heart (organoprotection), and brain, thereby suggesting its use in wound healing. Additionally, BPC 157 counteracts gastric endothelial injury that precedes and induces damage to the gastric epithelium and generalizes "gastric endothelial protection" to protection of the endothelium of other vessels (thrombosis, prolonged bleeding, and thrombocytopenia). BPC 157 also has an effect on blood vessels, resulting in vessel recruitment that circumvents vessel occlusion and the development of additional shunting and rapid bypass loops to rapidly reestablish the integrity of blood flow (ischemic/reperfusion colitis, duodenal lesions, cecal perforation, and inferior vena caval occlusion). Lastly, BPC 157 counteracts tumor cachexia, muscle wasting, and increases in pro-inflammatory/procachectic cytokines, such as interleukin-6 and tumor necrosis factor-α, and significantly corrects deranged muscle proliferation and myogenesis through changes in the expression of FoxO3a, p-AKT, p-mTOR, and P-GSK-3β (mitigating cancer cachexia).

S13/2

PATOLOGIJA I MOLEKULARNI POGLED: PROŠLOST, SADAŠNJOST, BUDUĆNOST

PATHOLOGY AND MOLECULAR VIEW: PAST, PRESENT, FUTURE

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Commonly, the angiogenic growth factors signify healing. However, gastrointestinal ulceration is still poorly understood particularly with respect to a general pharmacological/pathophysiological role of various angiogenic growth factors implemented in growth factors wound healing concept. Thereby, we focused on the stable gastric pentadecapeptide BPC 157, a peptide given always alone vs. standard peptidergic angiogenic growth factors (EGF, FGF, VEGF), and numerous carriers. Further, we reviewed how the gastrointestinal tract healing could be generally perceived (i) in terms of angiogenic growth factors, and/or (ii) through the healing of extragastrointestinal tissues healing, such as tendon, ligament, muscle and bone, and vice versa. Respected were the beneficial effects obtained with free peptides or peptides with different carriers; EGF, FGF, VEGF, and BPC 157, their presentation along with injuries, and a healing commonality, providing their implementation in both gastrointestinal ulcer healing and tendon, ligament, muscle and bone healing. Only BPC 157 was consistently effective in all of the models of acute/chronic injury of esophagus, stomach, duodenum and lower gastrointestinal tract, intraperitoneally, per-orally or locally. Unlike bFGF-, EGF-, VEGF-gastrointestinal tract studies demonstrating improved healing, most of the studies on tendon, muscle and bone injuries provide evidence of their (increased) presentation along with the various procedures used to produce beneficial effects, compared to fewer studies in vitro, while in vivo healing has a limited number of studies, commonly limited to local application, diverse healing evidence with diverse carriers and delivery systems. Contrary to this, BPC 157 - using same regimens like in gastrointestinal healing studies - improves tendon, ligament and bone healing, accurately implementing its own angiogenic effect in the healing. Thus, we claim that just BPC 157 represents in practice a pharmacological and pathophysiological role of various peptidergic growth factors.

S13/3
BPC 157 PERSPEKTIVE

BPC 157 PERSPECTIVES

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Cancer cachexia, one of metabolic syndromes caused by cancer, is a devastating and miserable condition encountered in more than 50% of terminal cancer patients presenting with significant weight loss associated with skeletal muscle atrophy and fat loss. Though cachexia may account for up to 20% of cancer deaths, and urgent unmet medical need in cancer treatment, the significant treatment is still lacking. Therefore, understanding the underlying molecular mechanisms is essential for anticipating therapeutic approaches. Since the primary events driving cachexia are mediated via either the central nervous system related- or inflammation related-anorexia, hypoanabolism, and hypercatabolism, therapy usually targets nutritional support to compensate reduced food intake along with some anti-inflammatory agents to cover specific inflammation-related metabolic derangement, and encourages exercise to supplement reduced physical activity, but all proven to be not so effective so far. Therefore, combination therapies such as a standard multi-modal package including anorexic agent, megestrol acetate, and anti-inflammatory agent coupled with the development of potential novel therapeutics promise a new era in rescuing patients from cancer cachexia. Thus, we propose the potential application of BPC157, one of active cytoprotective agent isolated from gastric juices for cancer cachexia. Before clinical trial, we introduced the evidence showing BPC157 rescued from cancer cachexia supported with explored mode of actions.

S13/4
BPC 157 I KARDIOLOGIJA

BPC 157 AND CARDIOLOGY

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BPC 157 counteracts various cardiac disturbances after bupivacaine, KCl-overdose, digitalis-overdose, hypoxia and reoxygenation, succinylcholine, neuroleptics and prokinetics, and doxorubicine. Hypothesis is that BPC 157 (since formed constitutively in the gastric mucosa, stable in human gastric juice, along with significance of NO-synthase and the basal formation of NO in stomach mucosa, greater than that seen in other tissues) exhibits a general, effective competing with L-arginine and its analogues. This has some physiologic importance (NO-generation), in (i) gastric mucosa and mucosal protection, following alcohol lesions, in cytoprotection course, NO-generation, and blood pressure regulation; (ii) alcohol acute/chronic intoxication, and withdrawal; (iii) cardiovascular disturbances, chronic heart failure, pulmonary hypertension, and arrhythmias; (iv) disturbances after hypokalemia and hyperkalemia, and potassium-cell membrane dysfunction; and finally, in (v) complex healing failure, proved by the fistulas healing, colocutaneous and esophagocutaneous. However, how this advantage of modulating NO-system (i. e., particular effect on eNOS gene), may be practically translated into an enhanced clinical performance remains to be determined. Also, this may be that BPC 157 may have a particular effect on vasculature. The rapid activation of the bypassing loop occurs in the rat with the occluded inferior caval vein (and thereby, resolved Virchow), much like in the rats with ischemic/reperfusion colitis, duodenal venous congestion lesions, perforated cecum, bile duct ligation-induced liver cirrhosis and portal hypertension. Accordingly, BPC 157 interacts with several molecular pathways. In particular, BPC 157 increased expression and internalization of VEGFR2, the activation of the VEGFR2-Akt-eNOS signaling pathway without need of other known ligands or shear stress.

S13/5
BPC 157 I KIRURGIJA

BPC 157 AND SURGERY

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Stable gastric pentadecapeptide BPC 157 is an anti-ulcer peptidergic agent, safe in inflammatory bowel disease clinical trials and wound healing, stable in human gastric juice and has no reported toxicity. We focused on BPC 157 as a therapy in peridontitis, esophagus, stomach, duodenum, intestine, liver and pancreas lesions. Particularly, it has a prominent effect on alcohol-lesions (i.e., acute, chronic) and NSAIDs-lesions (interestingly, BPC 157 both prevents and reverses adjuvant arthritis). In rat esophagitis and failed function of both lower esophageal sphincter (LES) and pyloric sphincters (PS), BPC 157 increased pressure in both sphincters till normal and reduced esophagitis. However, in healthy rats, it may decrease (PS) or increase (LES) the pressure in sphincters. It has strong angiogenic potential, it acts protectively on endothelium, prevents and reverses thrombus formation after abdominal aorta anastomosis, affects many central disturbances (i.e., dopamine and 5-HT system), the NO-system (either L-arginine and L-NAME effects), endothelin, acts as a free radical scavenger (counteracting CCl4-, paracetamol-, diclofenac-injuries) and exhibits neuroprotective properties. BPC 157 successfully heals the intestinal anastomosis, internal and external fistulas in rats, as well as interacting with the NO-system. Interestingly, the fistula closure was achieved even when the BPC 157 therapy was postponed for one month. In short-bowel syndrome escalating throughout 4 weeks, the constant weight gain above preoperative values started immediately with peroral and parental BPC 157 therapy and the villus height, crypth depth and muscle thickness (inner (circular) muscular layer) additionally increased. Thus, BPC 157 may improve gastrointestinal tract therapy.

S14 *NELA PIVAC I DUBRAVKA ŠVOB ŠTRAC*: TERANOSTIČKI PRISTUP POSTTRAUMATSKOM STRESNOM POREMEĆAJU / THERANOSTIC APPROACH TO PTSD)

S14/1
UVODNA RIJEČ: TERANOSTIČKI PRISTUP PTSP-U

INTRODUCTION: THERANOSTIC APPROACH TO PTSD

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The symposium "Theranostic approach to PTSD", organized by Nela Pivac and Dubravka Svob Strac (Croatia) will present 5 lectures related to translational targets for the development of PTSD pharmacotherapies (Seth Norrholm, USA), neuroimaging biomarkers for PTSD (Tanja Jovanovic, USA), metabolomics in PTSD (Gordana Nedic Erjavec and Matea Nikolac Perkovic, Croatia) and glycomics in PTSD (Dubravka Svob Strac, Croatia). Theranostic approach to PTSD involves an approach that combines diagnosis, prognosis, and therapy for PTSD. This symposium will present novel data related to "omics" approach (i.e. novel metabolomic and glycomic findings in PTSD) and novel findings related to PTSD pharmacotherapy and neuroimaging data. There are only a few studies describing metabolomics and altered metabolites in PTSD, and for now only one preliminary study evaluated selected 9 N-glycan structures in plasma, which included a small number of subjects exposed to trauma and subjects with PTSD. In addition, neuroimaging findings in PTSD are still mixed, so novel neuroimaging data might reveal signaling pathways and structures altered in PTSD, while translational approach might help in detecting targets for the development of the novel pharmacotherapy of PTSD. The goal of the future research should be on the integration of novel "omics" data with neuroimaging data, using translational approach, in the enlarged and narrowly defined groups of subjects with PTSD. These findings might provide new molecular targets and might improve theranostic approach to PTSD.

This research was supported by the Croatian Science Foundation (IP-2014-09-4289).

S14/2

TRANSLACIJSKI CILJEVI ZA RAZVOJ FARMAKOTERAPIJE U PTSP-U

TRANSLATIONAL TARGETS FOR THE DEVELOPMENT OF PTSD PHARMACOTHERAPIES

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Introduction: The classical conditioning paradigm of fear learning has resulted in a number of experimental variations for the explanation of posttraumatic stress disorder (PTSD) etiology. These paradigms include extinction learning and recall, fear inhibition, fear generalization, and conditioned avoidance. As such, each of these paradigms have significant applications for understanding the development, maintenance, treatment, and relapse of the fear-related features of PTSD. Importantly, these methods also provide a platform by which to evaluate the effectiveness and therapeutic targets of pharmacological interventions.

Materials and Methods: The presentation will describe each of these conditioning-based paradigms (extinction, fear inhibition, fear generalization) with reference to the clinical applications, provide case examples from patients with severe PTSD symptoms, and review recent pharmacological successes. The neurobiological models of conditioning and extinction in animals, psychiatrically healthy humans, and PTSD patients will be reviewed, and the current balance of evidence suggesting a number of biological, behavioral, neuropharmacological, and cognitive mechanisms/moderators of the conditioning and extinction process in experimental and clinical contexts will be discussed.

Results: While some of these paradigms have been studied extensively in PTSD patients (e.g., conditioning, extinction, and inhibition), other paradigms have been involved in limited studies with clinical populations (e.g., avoidance and generalization). The primary moderators include genetic (for example, BDNF, COMT, 5-HTTLPR), hormonal (for example, estradiol), behavioral, and cognitive mechanisms.

Conclusions: Fear conditioning and extinction paradigms offer valuable methods to study PTSD and its treatments in clinical applications. However, there is a need for more study in areas of avoidance and generalization in PTSD.

S14/3
NEUROSLIKOVNI BIOMARKERI ZA PTSP

NEUROIMAGING BIOMARKERS FOR PTSD

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Introduction: Posttraumatic stress disorder (PTSD) occurs in 10-20% of people who experience an extremely traumatic event. It is still unclear what factors may contribute to risk for developing PTSD. The underlying neurobiological contributors to PTSD have focused largely on the function of the amygdala, a brain region that is central to recognizing and coordinating responses to emotionally salient stimuli, including fear conditioned cues and fearful facial expressions. Individuals with PTSD show amygdala hyper-reactivity to negative emotional stimuli, relative to control groups, and this hyper-reactivity has been proposed as a causal contributor to the disorder, however few prospective studies have been conducted to determine whether amygdala hyperactivity with a risk factor or a consequence of the disorder.

Materials and Methods: Our work has included chronic PTSD (n=100) and acute trauma survivors recruited from Emergency Departments using functional magnetic resonance imaging (fMRI) with fearful and neutral facial expressions. We also examined prefrontal cortex inhibition using a go/nogo fMRI task.

Results: The results of our research show that those with PTSD show greater amygdala activation and less prefrontal activation. In addition, higher amygdala activation and lower PFC activation immediately after trauma predicted higher PTSD symptoms six months later.

Conclusions: These data support the literature indicating that amygdala activity may play a predisposing role in PTSD development.

S14/4

METABOLOMIKA U PTSP-U: UVOD I PRELIMINARNI NALAZI

METABOLOMICS IN PTSD: INTRODUCTION AND PRELIMINARY FINDINGS

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Introduction: Posttraumatic stress disorder (PTSD) is a severe mental disorder that develops after experiencing or witnessing traumatic or terrifying event. The exact biological factors, that contribute to vulnerability or resilience for developing PTSD after traumatic exposure, are still not well understood. Metabolomics is a new fast developing scientific discipline which allows us to study the final products of all biochemical pathways driven by genetic regulation and influenced by the environment. In the case of PTSD, one of the most important goals nowadays is to identify reliable, peripheral, non-invasive and easily accessible biomarkers that will potentiate the development of new diagnostic tests in order to early recognize, better diagnose, treat or even prevent possible development of PTSD. In metabolomics, usually this type of study starts with untargeted and non-hypothesis driven approach.

Materials and methods: The aim of this study was to apply LC-MS and GC-MS based untargeted metabolomics approach to investigate a metabolic profile of patients with combat related PTSD, collected within the project „GlycoGenPTSD" supported by Croatian Science Foundation (IP-2014-09-4289). Study included plasma samples from PTSD patients (N=50) and matching controls (N=50).

Results: After appropriate statistical analysis, a trend of increased levels of different types of glycerophospholipids and decreased levels of different types of carnitines and bile acids was found in PTSD subjects when compared to healthy controls.

Conclusions: This preliminary study proposes metabolomics as a promising novel approach for a better understanding of PTSD etiology, but further research has to be carried out to validate current results.

S14/5
GLIKOMIKA U PTSP-U

GLYCOMICS IN PTSD

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Introduction: Protein glycosylation represents most common post-translational modification, which can significantly change biological role of different proteins, as well as alter variety of signalling pathways. Glycosylation pattern drastically changes with aging and in various diseases. However, so far there is only one small study that investigated N-glycome in patients with post-traumatic stress disorder (PTSD) versus low stressed group. Therefore, the aim of our study was to determine glycomic differences between patients with PTSD and control subjects.

Materials and methods: Participants enrolled in the study were unrelated male war veterans with PTSD and healthy subjects of Croatian origin. Discovery cohort consisted of 233 subjects, while replication cohort involved 310 individuals. Plasma glycans were determined using ultra-high performance liquid chromatography that distinguished 39 N-glycan species. Differences in distribution of N-glycans between PTSD and control subjects were analyzed using Student t-test on values corrected for the effect of age and multiple testing.

Results: There were total of 19 altered N-glycans in plasma of patients with PTSD in discovery cohort, whereas six of them were replicated in the second cohort. Four N-glycans were significantly higher, and two N-glycans were significantly lower in PTSD patients in comparison to control subjects.

Conclusions: Specific alternations of plasma N-glycans in patients with PTSD suggest the role of N-glycans in PTSD pathogenesis. Changes in plasma protein glycosylation observed in PTSD are also similar to alternations seen in different inflammatory and pathophysiological states.

This research was supported by the Croatian Science Foundation, project No. IP-2014-09-4289.

S15 *MLADEN BOBAN*: KARDIOMETABOLIČKI UČINCI ALKOHOLA I VINA / CARDIOMETABOLIC EFFECTS OF ALCOHOL AND WINE

S15/1
MEHANIZMI ANTIOKSIDACIJSKIH I PROTUUPALNIH UČINAKA VINA

MECHANISMS OF ANTIOXIDANT AND ANTINFLAMMATORY EFFECTS OF WINE

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Inflammation and oxidative stress are implicated in the pathogeneses of many chronic diseases including diabetes and diabetic complications, hypertension and cardiovascular diseases, neurodegenerative diseases, cancer, and aging. Acting in concert, inflammation and oxidative stress accentuate each other inducing progressive damage to the biological systems.

Numerous foodstuffs and their ingredients are regarded as having beneficial effects on human health due to their anti-inflammatory and antioxidant properties. One of the most examined foodstuffs in this context is wine, an important component of the Mediterranean diet. However, the exact mechanisms, sites of action, and relative contribution of different biological responses associated with moderate wine consumption of the observed anti-inflammatory and antioxidant effects are far from clear. The common perception that wine phenolics acting as chemical antioxidants is primarily the underlying mechanism responsible for the mentioned effects of wine is oversimplified and insufficient.

Here, we discuss several other mechanisms that may contribute to the overall antioxidant and anti-inflammatory properties of wine. These include: the role of wine phenolics acting locally in the gastrointestinal tract, the specific metabolic response to the non-phenolic constituents of wine, and the para-hormesis concept and role of wine phenolics in activating the cellular antioxidant defense system by mimicking effects of endogenously produced oxidants.

This work was supported by the Croatian Science Foundation research grant No. 8652.

S15/2

KONZUMIRANJE BIJELOG VINA I OPORAVAK SRCA NAKON INFARKTA: MIOKARDIJALNA PERSPEKTIVA

CONSUMPTION OF WHITE WINE AND CARDIAC REPAIR FOLLOWING INFARCTION: MYOCARDIAL PERSPECTIVE

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Introduction: Effects of white wine consumption on the expression of inflammatory markers/mediators (MMP-2, MMP-9, NF-κB p65 and TGF-β1) in myocardial tissue following experimentally induced permanent myocardial ischemia was investigated. Furthermore, the expression of immunoreactivity for myeloperoxidase (MPO) and cluster of differentiation 68 (CD68) was performed to observe the nature and the speed of inflammatory infiltration.

Materials and methods: Male Sprague-Dawley rats were given either a combination of white wine (with low and high phenolic content) and water or water only, for 28 days. After coronary ligation, animals were left to survive for 24 hours. Three to five representative areas: infarct/ischemic, peri-infarct/border zone and control/non-ischemic zones were analysed for expression of immunoreactivity by measuring threshold area % of signal density.

Results: For MMP-9, significantly smaller expression was found in all three zones of wine drinking animals ($p<0.001$). There was no difference in MMP-2 immunoreactivity between the two groups, except in peri-infarct zones. The same pattern of expression was found for the NF-κB p65 signal, while no differences between experimental groups was observed for TGF-β1. Significantly smaller expression for both MPO and CD68 was found in all three peri-infarct zones of wine drinking animals ($p<0.001$).

Conclusion: White wine consumption decreases expression of three investigated inflammatory markers/mediators in the peri-infarct zone, suggesting its significant modulatory effect. For MMP-9 and MMP-2, expression was similar to the effect of post-ischemic reperfusion. Standard white wine consumption caused attenuation of expression of MPO but not of CD68.

S15/3

UČINCI KONZUMIRANJA VINA NA HEMODINAMIKU I NAPETOST ARTERIJA

THE EFFECTS OF WINE CONSUMPTION ON HEMODYNAMICS AND ARTERIAL STIFFNESS

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Each heartbeat generates a pulse wave that travels from the heart through aorta along the arterial tree. Non-invasive devices for pulse wave analysis were developed from the concept that there is hemodynamic information contained in the shape of the arterial pressure pulse, which can be used to supplement the conventional measurement of blood pressure. Although the importance of arterial stiffness as a marker of vascular health has been recognized since the 19th century, European Society of Hypertension/Cardiology guidelines recently included carotid-femoral pulse wave velocity as the indicator of arterial stiffness in the cardiovascular risk assessment. Therefore, therapeutic strategies, which are able to improve arterial stiffness, are needed. According to strong epidemiological evidence, moderate consumption of wine has beneficial effects on human health, particularly on cardiovascular system. Apart from the alcohol itself, polyphenolics are considered mostly responsible for the biological effects of wine, although mechanisms underlying their bioactivity remain largely unknown. Most of published studies investigating the effects of wine consumption on arterial stiffness are focused on acute beverage consumption and hardly comparable regarding differences in study design and protocol. The effects of moderate wine consumption on blood pressure, arterial stiffness and pulse wave reflections in apparently healthy subjects and in patients with type II diabetes mellitus after consumption period of three weeks will be presented and accordingly discussed. The consumption trials were approved by the Ethics Committee, School of Medicine University of Split.

The studies were supported by the Croatian Science Foundation (Project no. 8652).

S15/4

VINO I PRETILOST: ŽIVOTINJSKI MODEL

WINE AND OBESITY: ANIMAL MODEL

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It is still controversial whether alcohol intake represents a risk factor for weight gain and obesity. Based on recent reviews, it seems that only heavy drinking is positively related with the weight gain. Also, it appears that the type of alcoholic beverage is an important element in modifying the effect of alcohol consumption on the weight gain with wine being regarded as an alcoholic beverage with more favorable effects. Among proposed explanations for this is possible role of wine phenolics on food intake, gastrointestinal energy harvest and/or nutrients metabolism. In order to evaluate this assumption, we compared effects of 4 weeks consumption of standard (W) and macerated white wine (MW) on weight gain in rats. One and three months old Sprague-Dawley male rats were used. Each age group was subdivided into: water-only-drinking controls (C), W and MW drinking animals. Daily wine and total liquid consumption, food intake and body weight were measured, and energy intake and feed efficiency index were calculated. Results showed that in both age categories wine-drinking animals gained less weight in comparison to C, regardless of wines' polyphenols content. Wine consumption was associated with decreased food intake implying that additional calories provided by wine partially compensate for calories from other foodstuff. Although our results are indicative of the major role of non-phenolic constituents of the wines, probably ethanol, the present study cannot exclude the modifying role of wine phenolics on weight gain as the animals consuming MW had lower total energy intake in comparison to other groups.

This work was supported by the Croatian Science Foundation research grant No. 8652.

OKRUGLI STOLOVI / ROUND TABLES:

OS01 BRANIMIR ANIĆ, IVETA MERČEP

SPONZORIRANI OKRUGLI STOL - MERCK SHARP & DOHME: PERSPEKTIVA LIJEČNIKA I PACIJENATA U LIJEČENJU BIOLOŠKOM TERAPIJOM - SIGURNOST, ISHODI LIJEČENJA I TROŠAK ZAMJENE TERAPIJE

SPONSORED ROUND TABLE MERCK SHARP & DOHME: DOCTORS AND PATIENTS PERSPECTIVE IN BIOLOGICS TREATMENT - SAFETY, TREATMENT OUTCOMES AND COST OF THERAPY SWITCHING

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Biological medicines have changed the course of treatment for many diseases. Those are medicinal products that has an active substance which is derived from living cells. Medicines considered as biologicals are all types of prophylactic vaccines, immunological drugs (immunosera, immunoglobulins); blood or plasma derived medicines (albumin, coagulation factors); biotechnological medicines; and advanced therapy products (gene therapy, stem cells, tissue engineering). Various fields of medicine are covered with biopharmaceuticals including rheumatology (rheumatioid arthritis), dermatology (psoriasis), gastroenterology (Crohn's disease) etc. Biosimilar approach implies that for a biosimilar product, comprehensive comparability with original is fully demonstrated. Biosmilar is almost an identical copy of original biotechnological medicinal product, with which it is highly similar (in terms of quality characteristics, biological activity, safety and efficacy), but not fully identical (biosimilar is not generic). However, biotechnological medicines have the potential of evoking an immune response. Immunogenicity is the potential of the therapeutic protein/biotechnological medicine to induce an immune response (to itself and to related proteins) and the consequence of that response or to induce immunologically related adverse clinical events. Taking into account the known effects of immunogenicity, and complexity of mechanism of action of therapeutic proteins, perhaps we are not yet fully aware of unprecitable nature of biotechnological medicines and all complications of their use, especially those that can develop after long-term therapy. The increased competition resulting from biosimilars entering the market affects not just the price of the respective biosimilars referenced product, but also the price of whole product class (1). It can have almost as large an impact on the total market price as it has on

the biosimilar/referenced product price and therofore more and more patients have the opportunity to get the treatment with biologicals.

OS02 VLADIMIR TRKULJA, NADA ČIKEŠ:

HRVATSKI PROGRAM ZA EUCP KVALIFIKACIJU: INFLACIJA SPECIJALISTIČKIH STUDIJA ILI KLJUČ ZA ODRŽANJE STRUKE U SKLADU S EUROPSKIM STANDARDIMA? ZAMISAO, PREPREKE I TRENUTNO STANJE

CROATIAN PROGRAM FOR EUCP QUALIFICATION: INFLATION OF SPECIALIST STUDIES OR KEY FOR MAINTAINING A PROFESSION IN ACCORDANCE WITH EUROPEAN STANDARDS? IDEA, OBSTACLES AND CURRENT STATE

POSEBAN GOST – **MOJCA KRŽAN**, PREDSJEDNICA EPHAR-A (SPECIAL GUEST – **MOJCA KRŽAN**, THE PRESIDENT OF EPHAR*):*

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1 University of Zagreb School of Medicine

Introduction: European Certified Pharmacologist (EuCP) is a project by the Federation of the European pharmacological societies (EPHAR) motivated by the fact that pharmacology is a profession and a scientific discipline that incorporates a wide range of knowledge and skills, from (bio)chemistry, biology, classical pharmacological methods, elements of pharmaceutics, toxicology, physiology to clinical, legislative and epidemiological expertise and scientific reasoning. Appreciating that an individual may achieve in-depth competencies in only some of these areas, a structured insight into other aspects is viewed as need to achieve higher standards in academic/commercial research/teaching, legislative aspects and all what is meant under rational use of drugs and drug policies. National societies are to develop training programs as they see fit, but these need to be approved by EPHAR.

Materials and Methods: A brief overview of developments related to the EuCP in Croatia.

Results: HDF supported the initiative from the beginning with the idea of a program similar to that in Austria, where “pharmacologist” is a recognized profession, a medical specialty (distinct from “clinical pharmacologist”) based on a 5-year specialty training program and a board exam. HDF presented the initiative and a program outline to relevant academic/research institutions. Obstacles: (i) administrative: a) “pharmacologist” is not a recognized profession (HDF started a process); b) “pharmacology” is not yet expected to be recognized as a specialty (seems insurmountable); c) available framework - a 2-year specialist study?; (ii) mental: no interest by relevant ministries, limited by potential providers.

Conclusion: We do not need yet another pro-forma specialist study, we need a modern profession. Conclusion. We need an excellent postgraduate specilist study program and modern profession.

OS03 PREDSTAVNICI HALMED-A I FARMACEUTSKE INDUSTRIJE / REPRESENTATIVES OF HALMED AND PHARMACEUTICAL INDUSTRY

OKRUGLI STOL AGENCIJE ZA LIJEKOVE I MEDICINSKE PROIZVODE REPUBLIKE HRVATSKE (HALMED); NOVOSTI NA PODRUČJU REGULATIVE LIJEKOVA: PITANJA I ODGOVORI

ROUND TABLE OF AGENCY FOR MEDICINAL PRODUCTS AND MEDICAL DEVICES OF CROATA (HALMED); NEW REGULATORY ISSUES: QUESTIONS AND ANSWERS

Lovrek Romcevic M[1], Boric Bilusic A[1], Davosir Klaric Z[1], Ikic Komesar J[1], Ilic Martinac A[1], Mihalic V[1], Osrecki V[1], Uzeirbegovic S[1], Koscak S[2, 3], Srkoc Z[4]

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4 Innovative Pharmaceutical Initiative (iF!), Zagreb, Croatia

Regulation of medicines is an ever-evolving field, and an open dialogue between the regulatory authorities and pharmaceutical industry is crucial for maintaining efficient and sustainable system. The round table will gather representatives of the Agency for Medicinal Products and Medical Devices of Croatia (HALMED) and representatives of innovative and generic pharmaceutical companies present on Croatian market. Recent events as well as future initiatives in the field of regulation of medicines will be discussed. The topics will cover issues concerning pharmaceutical industry and issues with direct impact on patients and healthcare professionals, such as introduction of safety features on packages of medicines and initiatives towards electronic product information for EU medicines. HALMED's contribution to the European regulatory network through active involvement in the assessment of medicines as well as regional collaboration with non-EU countries will also be discussed. Planned events within the Croatian EU Presidency in 2020 in the field of regulation of medicines will also be presented.

PP01

NOVI OBLICI ZNANSTVENIH PREVARA POVEZANI S RAZVOJEM INFORMACIJSKIH TEHNOLOGIJA

NEW FORMS OF SCIENTIFIC MISCONDUCT ASSOCIATED WITH ADVANCEMENT OF INFORMATION TECHNOLOGIES

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New information technologies have made significant changes to all the ascetics of our lives. In scientific research, this is evident from the efficient and fast retrieval of data, the mathematical design of complex research, and the rapid publication of results. In the fundamental sciences and the medical sciences, the public presentation of discoveries is particularly important phase of research. Scientific publication is also an important element of prestige and motivation of scientist. Much like doping in sport competitions, some researchers tend to resort to the various forms of scientific misconduct from fabrication, falsification to plagiarism. New information theologies have brought about new and often innovative forms of scientific dishonesty. Most „inventive“ are the various manipulations of the email addresses of the reviewers. On the other hand there rapid growths of so called predatory journals even predatory conferences. Those are journals and conferences that, by try false promises, to make as much money as possible from scientists who strive to publish their results more easily. Because of that some researchers have lost potentially valuable results. Main targets are young researchers from scientifically less developed centers. How to identify such predators is a main goal of this presentation.

R01
RADIONICA AGENCIJE ZA LIJEKOVE I MEDICINSKE PROIZVODE REPUBLIKE HRVATSKE (HALMED): MJERE MINIMIZACIJE RIZIKA

WORKSHOP OF THE AGENCY FOR MEDICINAL PRODUCTS AND MEDICAL DEVICES OF CROATIA (HALMED): RISK MINIMISATION MEASURES (RMM)

Mirosevic Skvrce N[1], Margan Koletic Z[1], Krnic D[1]

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Risk Minimisation Measures (RMM) are additional measures introduced in order to prevent or reduce the possibility of adverse reactions associated with exposure to medicine or to reduce their extent. RMM are introduced in certain medicines that are bearing risks and therefore may not be authorised without these measures. One of the recent examples of safety issue, which required the introduction of RMMs, is medication errors associated with the use of methotrexate. Methotrexate (MTX) is authorised in the European Union since the 1960s for multiple oncology and non-oncology indications. In chemotherapy MTX acts by competitive inhibition of the enzyme dihydrofolate reductase subsequently interfering with DNA and RNA synthesis, DNA repair and cellular replication. Although MTX was originally developed as a chemotherapeutic agent, in low doses it is also commonly used in the treatment of certain autoimmune diseases. However, each group of indications has a different administration schedule. For the treatment of neoplasms, various administration schedules are in place, including daily dosage. In contrast, for the treatment of autoimmune diseases, methotrexate is administrated weekly. Serious cases of overdose, sometimes fatal, have been reported in patients inadvertently receiving the product daily instead of weekly for indications that require weekly dosing. Despite additional risk minimisation measures having been put in place by several Member States, reports continue to be received. Aim of the workshop will be for participants to suggest appropriate RMMs for the presented case.

SPONZORIRANO PREDAVANJE ROCHE / ROCHE SPONSORED LECTURE

SP01
PERSONALIZIRANA MEDICINA - PRAVI PUT KA BOLJIM ISHODIMA

PERSONALIZED MEDICINE - RIGHT PATH TOWARD BETTER OUTCOMES

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Cancer diagnosis and treatment are evolving rapidly due to key advances in scientific research and clinical care, the development of more accurate diagnostic tools, and more targeted andeffective therapeutic options. At the centre of this transformation is the understanding of cancer at the molecular level, including the identification of genomic alterations that drive tumour progression, leading to a deeper understanding of the disease and how to treat it. It is now understood that not only the location of the tumour, but also its genomic status, have substantial implications for the effective management of cancer. Cancer is a highly diverse disease, with each patient's particular cancer often different at the DNA level from others with a similar diagnosis. This means that to understand each patient's cancer and ensure that the most appropriate treatments are selected, there is a growing need to identify genomic status in order to select the best treatment. Over the past decade, personalized, targeted treatments based on a patient's genomic profile have improved response rates, progressive-free survival (PFS) and overall survival (OS) compared with treatments that are not personalized. Accordingly, there is a substantial unmet need for a comprehensive and efficient approach to identify clinically relevant alterations for each and every patient with advancing disease in order to effectively maximise the chance that each patient receives the best therapy for their disease.

POSTER SEKCIJE / POSTERS SESSIONS

POSTER SEKCIJA S ORGANIZIRANOM DISKUSIJOM I /
POSTER SESSION I WITH ORGANIZED DISCUSSION I:

KLINIČKA FARMAKOLOGIJA 1 / CLINICAL PHARMACOLOGY 1 (PI-C1: 1-8)
KLINIČKA FARMAKOLOGIJA 2 / CLINICAL PHARMACOLOGY 2 (PI-C2: 1-8)
OSTALE TEME 1 / OTHER TOPICS 1 (PI-O1: 1-6)

ČETVRTAK, 26. RUJNA 2019. / THURSDAY, 26 SEPTEMBER 2019

14:10 – 15:10

PI-C1-1

THE ROLE OF PHARMACOGENETICS IN PRECISION MEDICINE: REINTRODUCTION OF ANTICOAGULANT THERAPY FOLLOWING INTRACRANIAL HEMORRHAGE

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INTRODUCTION: Intracranial haemorrhage (ICH) is the most serious adverse event of oral anticoagulants and reintroducing anticoagulation following ICH requires an individual approach. Direct oral anticoagulants (DOACs) have reduced ICH risks versus warfarin and represent optimal treatment. Pharmacogenetics enables personalised therapy approach as genetic information for interindividual variability in drug metabolism and response is provided.

PATIENTS AND METHODS: A 76-year male patient with ICH history on dalteparin was referred due to progression of deep venous thrombosis, with concomitant therapy (phenobarbital, diazepam, metoprolol and ramipril). To avoid possible drug-drug interactions pharmaco-genotyping (CYP2C9*2,*3; CYP2C19*2,*17; CYP2D6*2,*3,*4,*5,*6,*41; CYP3A4*22; MDR1 1236C>T, 3435C>T; ABCG2 421C>A and UGT2B7 -161C>T) was performed by TaqMan real-time PCR system. According to pharmacogenetic data (CYP2C9, CYP2D6 and CYP3A4 intermediate metabolizer, MDR1 intermediate transport function), age, concomitant medication and bleeding risk, instead of the standard dose (150mg BID), a lower dose of dabigatran was introduced (110mg BID). Dabigatran concentration (Ctrough and Cpeak) and coagulation tests (PT, aPTT, TT, Fibrinogen) were measured one week after dabigatran introduction.

RESULTS: Optimal plasma dabigatran trough and peak concentrations (66ng/mL, 176ng/mL) were confirmed. Coagulation parameters were 94% PT, 33.3s aPTT, 137.4s TT, 3.1g/L Fibrinogen (Ctrough) and 59% PT, 46.4s aPTT, >150.0s TT, 2.8g/L Fibrinogen (Cpeak). The further clinical course was without bleeding complications.

CONCLUSIONS: This case is an example of a personalised therapy approach with pharmacogenetics that provided information for the optimal choice of the proper medication at the proper dose. Pharmacogenetic profile of metabolic enzyme CYP3A4 and transporter P-glycoprotein (MDR1/ ABCB1) may enable individual choice between DOACs.

PI-C1-2
PHARMACOGENOMICS OF IMMUNOSUPPRESSIVE DRUGS

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Introduction: Standard immunosuppressive therapy in kidney transplantation is combination of mycophenolic acid, cyclosporine, tacrolimus, sirolimus or everolimus and corticosteroids that function on multiple pathways of the immune response. Immunosuppressants have narrow therapeutic window and exhibits an interindividual pharmacokinetic variability that affects the dose required to reach target concentration in blood. Genetic variability in some of the genes that affect absorption, distribution, metabolism and elimination ("pharmacogenes") can significantly influence an individual's response to the immunosuppressants and consequently the effectiveness of treatment and possible adverse drug events. The aim of the present study was to investigate the frequency of potentially actionable pharmacogenetics findings in the Croatian renal transplant recipients.

Materials and methods: Study included 158 post renal transplantation patients treated with immunosuppressants. Genotyping of ABCB1 (3435C>T), ABCC2 (-24C>T), ABCG2 (421C>A), SLCO1B1 (521T>C), CYP3A4*22, CYP3A5*1 and UGT1A9 (-2152C>T) was performed by TaqMan real time PCR for discovery of clinically actionable variants.

Results: At least one clinically actionable variant was found in 68 of 150 patients (45%). The frequencies of variant/minor alleles in the observed group were: ABCB1 (45%), ABCC2 (28%), SLCO1B1 (27%), ABCG2 (12%), CYP3A5 (12%), UGT1A9 (3%), CYP3A4 (1%).

Conclusion: In the present study, we estimated the burden of pharmacogenetic variants in kidney transplant recipients that deserve different personalized treatment approach for optimized treatment. Implementation of pharmacogenetic testing to guide drug prescribing has potential to improve response and prevent adverse events.

PI-C1-3

CORRELATION BETWEEN POLYMORPHISM OF ABCG2, ABCB1 AND SLCO1B1 GENES AND SIDE EFFECTS OF ROSUVASTATIN

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INTRODUCTION: Statins are very efficient drugs for treatment of hypercholesterolemia but can cause side effects like myopathy, rhabdomyolysis and hepatotoxicity. Interactions of rosuvastatin with many different drugs are well documented. Recent data showed a strong association between myotoxicity of rosuvastatin and variants in the SLCO1B1 gene which encodes OATP1B1, one of the key hepatocellular uptake transporters providing extraction of statins from portal venous blood into the liver. However, data about genetic risk factors for developing rosuvastatin induced hepatotoxicity and myotoxicity are missing.

MATERIALS AND METHODS: We analyzed 30 patients who developed hepatotoxicity or myotoxicity or both caused by rosuvastin (10-20-40 mg/day). The patients were analyzed for SLCO1B1 388 A>G and 521 T>C, ABCB1 2677 G>T/A and 3435 C>T. Genotyping was performed by real time PCR method with ready made kits of TaqMan®Drug Metabolism. Genotyping Assays (Applied Biosystems, Ca, USA)

RESULTS: Nine patients developed hepatoxicity and 4 among them were the homozygote carrier for both low activity SLCO1B1 genotypes (521 C/C and 388 G/G) had worst clinical presentation. All patients were carriers of at least one polymorphism in SLCO1B1 gen. Five patients with hepatotoxicity had also increased CK levels. Sixteen patients developed myotoxicity and among them 2 patients developed rhabdomyolysis.

CONCLUSION: Our preliminary data indicate associations between miotoxicity and hepatotoxicity caused by rosuvastatin and SLCO1B1 polymorphysms. ABCB1 and ABCG2 allele variants increases susceptibility to rosuvastatin toxicity.

PI-C1-4

PHARMACOGENETICS OF LONG ACTING RISPERIDONE - THE PREDICTIVE ROLE OF ABCB1, ABCG2, CYP2D6, CYP3A4, AND CYP3A5 POLYMORPHISMS

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3 Department of Pharmacology, School of Medicine University of Zagreb, Zagreb, Croatia.
4 Department of Laboratory Diagnostics, University Hospital Centre Zagreb, Zagreb, Croatia.

Introduction: Risperidone and its active metabolite 9-OH-risperidone are metabolised by enzymes CYP2D6 and CYP3A, and both are substrates of drug transporters ABCB1 and ABCG2. We investigated the role of genetic variations of ABCB1, ABCG2, CYP2D6, CYP3A4, and CYP3A5 in long-acting risperidone treatment.

Materials and methods: Study included 101 schizophrenia patient treated with long-acting risperidone. Genotyping of ABCB1, ABCG2, CYP2D6, CYP3A4, CYP3A5 and CYP2D6 was performed by TaqMan real time PCR and long-range PCR. Serum steady-state concentrations of risperidone and 9 OH risperidone were measured by HPLC-DAD on the 5th and 14th day following risperidone injection.

Results: CYP2D6 EM/UM phenotype was independently associated with lower dose corrected risperidone (GMR=0.67, 95%CI 0.52-0.86; P=0.002) and risperidone+9 OH risperidone levels (0.76, 0.61-0.95; P=0.015), and risperidone/9 OH risperidone ratio (0.80, 0.69-0.92; P=0.002). ABCG2 421C>A variant allele carriage was independently associated with lower risperidone+9 OH risperidone levels (0.75, 0.56-0.99; P=0.046). The effect of ABCG2 variant allele on dose-corrected risperidone+9 OH risperidone levels in CYP2D6 EM/UM subjects appeared more pronounced than in overall cohort (0.60, 0.42-0.85 vs. 0.75, 0.56-0.99), with no effect in other CYP2D6 phenotypes (1.11, 0.69-1.80). It was also associated with lower risperidone (0.66;0.44-0.98) and 9-OH risperidone (0.64;0.45-0.90) levels. ABCB1 and CYP3A variants, age, and concomitant use of CYP inhibitors had no effects.

Conclusions: CYP2D6 phenotype and ABCG2 genotype had a significant influence on risperidone concentrations in treatment with long-acting risperidone. The effect of CYP2D6 EM/UM phenotype was more pronounced in ABCG2 421C>A variant allele carriers. This study

points out for the first time importance of interaction between metabolic enzyme and drug transporter phenotype/genotype CYP2D6*ABCG2 in risperidone treatment.

PI-C1-5

WHAT TO GENOTYPE TO PREDICT FLUOROPYRIMIDINES TOXICITY – POSSIBLE ROLE OF DPYD C.496A>G VARIANT

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Introduction: Dihydropyrimidine dehydrogenase (DPD), the main fluoropyrimidines (FLs) catabolic enzyme, is coded by a highly polymorphic DPYD gene. Certain DPYD polymorphisms are linked to decreased DPD activity, associated with a greater risk of severe toxicity. Dose reductions are recommended for such carriers. FLs are foundation agents in numerous cancer treatments but may cause severe adverse events (SAE) resulting in pharmacogenetic DPYD investigations. We report 12 cases of heterozygous carriers for DPYD c.496A>G with FLs-related toxicity.
Materials and Methods: We reviewed records for 12 cancer patients with FLs-toxicity and DPYD genotyping (DPYD*2, DPYD*13, c.496A>G, c.1236G>A, c.2846A>T) who were heterozygous carriers for only DPYD c.496A>G variant. Genotyping was performed by TaqMan real-time PCR method.

Results: From 12 patients, 9 suffered from colorectal cancer, and 1 each from gastric, breast and pancreatic cancer. Therapy included FLs (5-fluorouracil or capecitabine), alone or in combination. All patients developed SAE associated with FLs-toxicity: febrile neutropenia (N=8), mucositis (N=6), diarrhoea (N=2) and hand-foot syndrome (N=2). SAE lead to mortality in 2 patients. Due to severe clinical conditions, FLs treatment was stopped in 3 patients and doses reduced in 7 patients. This resulted with a better safety profile and treatment was continued.

Conclusion: The correlation between DPYD c.496A>G variant and decreased DPD activity is still controversial. This case series indicates a possible association of DPYD c.496A>G variant with FLs-toxicity emphasizing the importance of further research and reconsidering inclusion of this variant into the DPYD genotyping panel in order to minimise severe FLs-toxicity and aid clinicians in FLs dosing.

PI-C1-6
GENETIC AND GLYCOMIC INTERACTIONS IN POSTTRAUMATIC STRESS DISORDER

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Introduction: Detection of specific and validated biomarkers of posttraumatic stress disorder (PTSD) is hindered by complexity of PTSD symptoms. Glycosylation affects majority of proteins and can change their biological properties. Although not genetically determined, there are several genes encoding the enzymes involved in metabolism of glycans, that might potentially contribute to the overall glycosylation pattern. Some of the most prominent single nucleotide polymorphisms (SNPs) related to N-glycosilation are rs11621121, rs7953249, rs1257220 and rs3760776. The aim of this study was to determine the influence of the selected SNPs on N-glycans in patients with PTSD and controls.

Materials and methods: Plasma N-glycome was determined using ultra-high performance liquid chromatography in 233 male war veterans with PTSD and age- and sex-matched healthy control. Genotyping was done in the same subjects using real-time PCR. Replication was done in additional cohort consisted of 310 war veterans and controls. The differences in frequency and glycosylation pattern between different genotype carriers were calculated using chi-square test and ANOVA.

Results: The frequency of the rs11621121, rs7953249, rs1257220 genotypes was significantly different between PTSD and control subjects. Out of 39 plasma N-glycan peaks (GP), 4 were significantly different in both cohorts in controls, and 1 GP differed in PTSD, depending on rs3760776 genotype.

Conclusions: Our study showed that glycosylation of plasma proteins, in addition to several tested genes involved in this process, could have a role in development of PTSD and could represent a potential biomarker for PTSD.

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PI-C1-7

THE ACCURACY OF SELF-PERCIEVED BREAST CANCER (BC) RISK ASSESMENT DOES NOT AFFECT ATTITUDES TOWARDS BC CHEMOPREVENTION

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Introduction: The aim of this study was to assess knowledge about BC risk factors and attitudes about BC chemoprevention among women without diagnosed BC and to investigate whether the accuracy of self-percieved BC risk affects the willingness to the BC chemoprevention.

Materials and Methods: A random sample of 164 women without BC who were referred to an ultrasound or mammographic examination at the Health Center Osijek were included in the study. An anonymous questionnaire about BC risk factors and chemoprevention was administered. Participants reported self-perceived 5-year and lifelong risk of BC using a 5-point Likert scale. Participants' BC risk was objectively estimated using The Breast Cancer Risk Assessment Tool (BCRAT).

Results: Participants were divided into 3 groups based on the accuracy of their 5-year risk self-assessment; 110 participants have accurately stimated their risk, 37 underestimated and 13 overestimated the risk. There was no significant difference ($\chi 2$ test) between the groups in knowledge about BC risk factors. The best known risk factor in all groups was having a 1st degree relative diagnosed with BC ($\chi 2$ test, $P>0,99$). Participants in an overestimated risk group have more relatives with BC, except 1st degree relative with BC ($\chi 2$ test, $P=0,006$). Participants in the underestimated risk group were older (Kruskal Wallis test, $P<0,001$), postmenopausal ($\chi 2$ test, $P=0,001$), had more chronic illnesses ($\chi 2$ test, $P=0,01$) for which were taking therapy daily (Kruskal Wallis test, $P=0,004$).

Conclusion: Raising awareness of chemopreventive options for BC risk reduction may facilitate informed decision about preventative chemotherapy utilization among women with high risk of developing BC.

PI-C1-8

RATES OF CYP3A4, CYP3A5 AND UGT1A4 SINGLE NUCLEOTIDE POLYMORFISMS IN CROATIAN BREST CANCER PATIENTS AND ITS LINKAGE TO ANASTROZOLE INDUCED CHANGES OF BONE MINERAL DENSITY

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Introduction: Breast cancer (BC) is the most common malignant disease in females taking 26 % of all cancer sites. Third generation aromatase inhibitors like anastrozole are becoming more important in treating BC because of their efficacy and better overall safety in the adjuvant treatment. Single nucleotide polymorphism (SNP) in genes encoding drug metabolizing enzymes could have an important role in individual responses to anastrozole therapy including drug efficacy and side effects.

Aim: To explore rates of three SNPs (CYP3A4*1B, CYP3A5*3, UGT1A4*2) important in anastrozole metabolism in population of Croatian BC patients, and its possible correlation to anastrozole induced side effects.

Materials and methods: 126 BC patients were included in the study of which 82 were postmenopausal patients with ER positive BC treated with anastrozole and 44 were postmenopausal ER positive patients before hormonal adjuvant therapy. DNA for SNPs was genotyped by TaqMan RT-PCR and BMD was measured by DXA.

Results: Homozygotes for the wild type A allele of CYP3A5*3 were not detected, moreover mutant G allele homozygotes were predominant with 88%. Wild type homozygotes of CYP3A4*1B were predominant with 94%, and mutant homozygotes were not detected. CYP3A4*1B and CYP3A5*3 SNPs were in 84.3% linkage disequilibrium and 95.1% in group treated with anastrozol and without treatment. Possible association of BMD changes induced by anastrozole therapy with prevalence of the three explored SNPs was not demonstrated.

Conclusion: Even though the mutant CYP3A5*3 SNP was predominant, which may result in poor anastrozole metabolism, no significant differences in BMD between the groups were confirmed.

PI-C2-1
ALTERED PHOSPHATIDYLCHOLINE LEVELS IN PTSD

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Introduction: Metabolomics is a scientific study of small molecules, called metabolites. Together with genomics, transcriptomics and proteomics, metabolomics is a part of "omics" technologies. It provides insights into complex alterations of key metabolites and metabolic pathways in various diseases and might be useful tool in future diagnosis and treatment. The aim of this study was to determine specific metabolites as potential biomarkers that would differentiate subjects with posttraumatic stress disorder (PTSD) from healthy controls.

Materials and Methods: Liquid chromatography coupled with mass spectrometry (LC/MS-QTOF) was used to determine metabolites in plasma samples from 50 PTSD subjects and 50 healthy controls. Prior to untargeted analysis on LC-MS, samples were prepared using protein precipitation in cold methanol:ethanol (1:1). The analysis was performed in positive and negative ionization. After the data treatment, tandem mass spectrometry (LC-MS/MS) was performed for statistically significant compounds ($p<0.05$) in order to identify them.

Results: Identified metabolites belong to the compound group of glycerophosphocholines. The levels of phosphatidylcholines, PC(16:0/0:0), PC(16:2/0:0), PC(17:0/0:0), PC(18:1/0:0), PC(19:0/0:0), PC(20:4/0:0), PC(22:4/0:0) and PC(24:0/0:0) were significantly increased among PTSD patients compared to control group. These phosphatidylcholines are included in a several metabolic pathways, such as lipid metabolism, membrane-mediated signaling and enzyme activation.

Conclusion: Altered levels of phosphatidylcholines indicate increased inflammation and association with several metabolic disorders that are characteristic for PTSD. Increased levels of phosphatidylcholines has been previously associated with several brain disorders. Therefore, in order to validate the altered phosphatidylcholine levels in PTSD, targeted analysis will be conducted on a larger number of samples.

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PI-C2-2
MELATONIN AND SLEEP QUALITY IN PATIENTS WITH ORAL CANCER

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Introduction: Low levels of melatonin (MLT) have been registered in patients with breast, prostate, lung, stomach and colon cancer. One of explanations for this was decreased sleep quality and increased fatigue in cancer patients.

Although there is a significant improvement in oral cancer (OC) treatment in the last few years, a 5-year survival rate of these patients amounts to 60%. The aim of this research was to measure MLT in unstimulated whole saliva (UWS), stimulated whole saliva (SWS) and serum. Furthermore, the aim was to assess sleep quality using the Pittsburgh Sleep Quality Index (PSQI).

Materials and methods: Altogether, 34 patients with OC and 33 sex and age matched healthy control subjects were included in this study. All samples were tested using ELISA commercially available kits.

Results: Melatonin levels in UWS were significantly higher (P<0.001) in the OC group [median: 3.1 (95% CI: 2.3-4.5)] compared to the control group [median: 0.7 (95% CI: 0.44-1.52)]. Melatonin levels in the SWS were significantly higher (P<0,001) in the experimental group [median: 1.7 (95% CI: 0.9-2.6)] compared to the control group [median: 0.6 (95% CI: 0.1-1.0)]. Serum MLT levels were significantly higher in patients with OC compared to the reference values for serum MLT [median 13,01 (95% CI: 10,08 - 15,14)]. Sleep quality was significantly lower in patients with OC (P = 0.0001, U=249.50).

Conclusion: Salivary and serum MLT could present a potential diagnostic marker for OC. To our knowledge, this is the first study measuring MLT in OC patients.

PI-C2-3
BISPHOSPHONATE-RELATED OSTEONECROSIS OF THE JAW – CASE REPORT

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Bisphosphonates are drugs used widely to manage various diseases that lead to bone disorder. In recent years the use of these drugs has increased, as have their complications. Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a severe complication that occurs in patients on bisphosphonate therapy, especially after dento-alveolar surgery procedures, such as tooth extraction. In this report we present two cases of severe BRONJ. First is 69 years old male patient with diagnosed multiple myeloma 9 years ago, treated with intravenous pamidronate for 2.5 years and intravenous zoledronic acid for 6 years. When examined he had a complaint of bony exposure on the right posterior mandible which is persistent 2 months after extraction of tooth 47. Radiographic examination showed the decrease in bone density on the right mandibular alveolar process and resorptive area with irregular shape. Second case is 56 years old female with breast cancer, treated with intravenous pamidronate for last 3 years. When presented in clinic her major complaint was orocutaneous fistula on the left cheek and exposed bone with suppurative discharge in the left posterior mandible, persistent after extraction of the teeth 36 and 37. Radiographic examination showed the large resorptive area with irregular shape and sequestrum on the crestal area. Therapy of BRONJ requires cessation of bisphosphonates, and often takes combined conservative and surgical therapy. These case reports demonstrate the importance of caution of physicians when prescribing bisphosphonates. Prevention of BRONJ can be easily achieved with better communication between physicians who prescribe bisphosphonates and dentists.

PI-C2-4

THE ASSOCIATION OF SALIVARY PARAMETERS WITH THE SEVERITY OF OBSTRUCTIVE SLEEP APNEA

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Introduction: Salivary parameters may offer a noninvasive and easy sampling alternative in diagnosis and monitoring high risk behaviour and disease progresion. The aim of this study is to establish association of salivary flow rate, salivary pH, salivary calcium, phosphate and magnesium levels with the severity of obstructive sleep apnea (OSA).

Materials and methods: The study involved 138 subject who were grouped according to the severity of OSA: 10 subject served as controls, 78 subjects had mild to moderate OSA and 50 had severe OSA. Unstimulated saliva was collected from all subjects and they had completed a questionnaire for evaluating the subjective assessment of dry mouth. Salivary pH was measured immediately after collection with pH tester of ±0.01accuracy. Ca, P, Mg levels were measured with inductively coupled plasma mass spectrometry (ICP-MS).

Results: There was significant correlation between salivary flow rate and the severity of OSA. The subject with severe OSA had higher salivary flow rate than subjects with mild to moderate OSA (3,97±2,59 vs 4,95±2,83, p=0,046). Furthermore, the prevalence of subjective assessment of dry mouth upon awakening was higher in subjects with severe OSA than in subjects with mild to moderate OSA (p=0,023). There were no significant differences in salivary calcium, phosphate and magnesium levels between subject groups.

Conlusion: Based on the study results indicate that severe OSA subjects had higher salivary flow rate compared to mild and moderate OSA subjects. However, subjective assessment of dry mouth upon awakening appears to be significantly associated with severe OSA.

PI-C2-5

EFFECTS OF TOPICAL HYPERICUM PERFORATUM TREATMENT ON WOUND HEALING IN CHEMICAL AND MECHANICAL SKIN DAMAGE MODELS

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Introduction: Hypericum perforatum topical preparations are used in traditional medicine for the treatment of wounds, abrasions and burns. Their use in those indications could be justified with Hypericums' anti-inflammatory, antimicrobial and astringent effects, as well as its stimulating effects on tissue growth and keratinocyte differentiation. Further, several clinical trials had shown its potential in wound healing. The aim of the study was to determine the effectiveness of ointment containing 20% Hypericum perforatum oil on promoting skin recovery in different human skin damage models on healthy volunteers.

Materials and methods: Skin damage was induced on four sites on volunteers' forearms (N = 28). Two sites were irritated chemically using sodium laurylsulphate under 24-hour occlusion. The stratum corneum on the other two sites were removed by tape-stripping to induce mechanical damage. Two damaged skin areas were treated with Hypericum ointment while the remaining were treated with placebo for nine days. Skin recovery were assessed measuring skins' transepidermal water loss, hydration and erythema values.

Results: Induced skin damage lead to symmetrical increase in measured parameters in comparison to baseline in both tested models. Treatment with Hypericum oil improved skin parameters in comparison to their values after irritation in both models. However, there were no differences in outcomes when compared with placebo treatment in either model.

Conclusions: Tested Hypericum ointment in used dose did not improve skin recovery in chemical and mechanical skin damage models.

PI-C2-6

INCIDENCE OF POSITIVE RESULTS OF HYPERSENSITIVITY TESTING TO LOCAL ANESTHETICS – SINGLE CENTRE EXPERIENCE

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Introduction: Local anesthetics (LAs) (amide or ester group) block nerve conduction and are used for local or regional anesthesia. Allergic reactions to LAs are uncommon and mainly described after dental surgery. LAs are weakly antigenic due to their low molecular weight (<300 Da). Amongst allergic reactions; delayed cutaneous reactions (type IV) such as eczema are the most frequent, while immediate (type I) are rare. Clinical manifestations range from cutaneous, neurological, cardiovascular or respiratory signs to severe reactions including life-threatening anaphylactic shock. Most adverse effects that are not related to the LAs itself: neurological symptoms (vasovagal syncope, panic attack or spasmophilia crisis) are frequently reported. So we amid this study to see the incidence of positive results of hypersensitivity testing among patient with suspected allergic reactions to LA.

Materials and methods: Skin testing (prick and intradermal with incremental concentrations) followed by provocation challenge is the best way to establish the diagnosis. This is performed by injection of incremental doses of LAs with the patient monitored for 30 minutes to one hour after each administration.

Results: In this study we will show the incidence of positive results of hypersensitivity testing to LAs from 1.7.2018. until 1.7.2019. among patients with suspected allergic reaction to LAs, performed in the Unit for Clinical Pharmacology, University Hospital Centre Zagreb.

Conclusions: Allergy to LAs is rare. In most cases, the signs reported by patients are related to psychomotor responses to trauma or anesthetic technique, or to epinephrine added to LAs. An allergic investigation is mandatory to support or more often to rule out the diagnosis of allergy to LAs, from the skin tests to the challenge test.

PI-C2-7
SAFETY OF ANTIDEPRESSANTS DURING PREGNANCY

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Introduction: Adequate treatment of depression is very important during pregnancy. Selective serotonin reuptake inhibitors (SSRIs): citalopram, escitalopram, fluoxetine, fluvoxamine and sertraline, with the exception of paroxetine, are the current treatment of choice for depressive disorders in pregnant women. All SSRIs have category C safety profile for use in pregnancy, with only paroxetine belonging to the Food and Drug Administration's (FDA's) D group. Other antidepressants that also have FDA's category C include: venlafaxine, duloxetine, bupropion, mirtazapine and amitriptyline.

Materials and methods: We performed a mini-review of currently available data on UpToDate, Micromedex and Drugs.com databases, regarding the safety of use of various antidepressants during pregnancy.

Results: The teratogenic risks of various SSRIs have been more widely studied in comparison to other antidepressants. However, maprotiline, a tetracyclic antidepressant, which was patented in 1966, has FDA's pregnancy category B, although the scientific literature advises it should be prescribed during pregnancy only when clearly needed. Furthermore, agomelatine, a drug with a different mechanism of action in comparison to other available antidepressants is rated by the Australian Drug Evaluation Committee as category B1.

Conclusions: Even though SSRIs, except paroxetine, currently remain the most commonly used antidepressants in pregnant women, maprotiline and agomelatine appear to be antidepressants with the best safety profile for use during pregnancy.

PI-C2-8
DILEMMAS IN THE USE OF NEW ORAL ANTICOAGULANTS

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Introduction: Thromboembolic disease is a major leading cause of mortality and morbidity in industrialized countries. Warfarin has been the main oral anticoagulant in clinical use since its discovery in 1954. Currently the management of patients is challenging due to the avalilability of new drugs, non-vitamin K oral anticoagulants (NOACs), with proven efficacy and security compared to traditional warfarin therapy. NOACs are characterized by a predictable pharmakokinetic profile for which blood monitornig is not routinely needed. Due to perceptions of better or comparable efficacy and safety profile in regard to warfarin, NOACs usage has been increasing at a rapid rate over recent years.

Materials and Methods: Systematic reviews of scientific literature.

Results: The perceptions is that patients treated with NOACs do not need frequent coagulation monitoring, do not have dietary restrictions, minimal drug interactions and have lesser risk of bleeding, especially intracranial bleeding. However there are certain dilemmas in the use of NOACs which include the use in chronic kidney failure, in patients with mechanical valves, the issue of expensive antidotes and question of whether drug level monitoring may be necessary for these drugs. Answers to some questions will be presented in this paper.

Conclusions: Although they are already widely prescibed, there are still a number of dillemas concerning use of NOACs.

PI-O1-1

COMPARISON OF BIOSIMILAR SOMATROPIN UTILIZATION IN 2018 IN CROATIA WITH REGARDS TO OTHER EUROPEAN COUNTRIES

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Introduction: Somatropin, also known as human recombinant growth hormone (rGH), is a peptide that stimulates cellular growth, reproduction and regeneration. Its primary indications for treatment include growth disorders in children and growth hormone deficiency in adults. Somatropin is typically administered subcutaneously and dosing is individualized for each patient according to their weight. There are several somatropin products available on the European market, including biosimilar: Omnitrope; referenced medicinal products: Genotropin and Humatrope and non-referenced medicinal products: Norditropin, Saizen, NutropinAq, Zomacton and Maxomat. In Croatia, only Genotropin, Norditropin and Omnitrope are reimbursed by the Croatian Health Insurance Fund.

Materials and methods: The utilization of defined daily doses (DDDs) of rGH in Croatia was analyzed and compared with trends in other European countries.

Results: In 2018, a total of 240.365,00 DDDs of somatropin was prescribed in Croatia, of which 72.500,00 DDDs of Genotropin (30.2%), 125.438,00 DDDs of Norditropin (52.2%) and 42.428,00 DDDs of Omnitrope (17.7%). Croatia with 17.7% biosimilar somatropin utilization ranks below the European Union average of 23%, together with the Netherlands (17%), Belgium (16%), Italy (18%), Spain (18%) and Germany (19%). In contrast, Poland (99%), Denmark (73%) and Bulgary (51%) are the leading European countries when it comes to prescribing of biosimilar somatropin, while Norway (3%), Slovenia (5%), Hungary (7%) and Portugal (8%) have the lowest biosimilar human growth hormone utilization on the continent.

Conclusion: Prescribing of rGH could be improved in Croatia as well as in other European countries and biosimilar prescribing should be encouraged as a cost saving measure.

PI-O1-2

COMPARISON OF BIOSIMILAR FILGRASTIM UTILIZATION IN 2018 IN CROATIA WITH REGARDS TO OTHER EUROPEAN COUNTRIES

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Introduction: Filgrastim, granulocyte-colony stimulating factor is glycoprotein that stimulates bone marrow to produce granulocytes and stemcells and release them into the blood. It is used prophylactically in patients with certain cancers in order to accelerate recovery from neutropenia, allowing administration of higher intensity regimens.

Objectives: There are several filgrastim products available on European market with referenced products: Neupogen and Granulokine as well as biosimilars: Filgrastim Hexal, Tevagastrim, Grastofil, Neukine, Nivestim, Ratiograstim and Zarzio. The objective was to evaluate filgrastim biosimilars reimbursment by Croatian Health Insurance Institute (CHII).

Materials and Methods: The utilization o defined daily doses (DDDs) of filgrastim in Croatia was analysed and compared with trends in European countries.

Results: Filgrastim biosimilars reimbursed by CHII include: Tevagastrim, Nivestim, Zarzio, Grastofil and Accofil. Moreover, CHII also reimburses second generation G-CSFs including pegfilgrastim (Neulasta) and lipegfilgrastim (Lonquex). In 2018, a total of 3.985,00 DDDs of filgrastim were prescribed in Croatia, of which 582 DDDs of Accofil (14.6%), 113 DDDs of Nivestim (2.9%), 569 DDDs of Tevagastrim (14.34%), 16 DDDs of Zarzio (0.4%) and 2685 DDDs of Neulasta (67.4%). In contrast, in 2018, at Clinical Hospital Centre Zagreb, the largest Croatian university hospital, 27132 DDDs of filgrastim were prescribed, of which 2083 were Nivestim (7.68%), 326 were Tevagastrim (1.2%), 8710 DDDs were Zarzio (32.11%), 10337 DDDs were Neulasta (38.1%), 5657 were Lonquex (20.85%) and 18 DDDs were Leukine (sargramostim; 0.07%).

Conclusions: In Europe, an average biosimilar filgrastim market share in 2017 was 30%, thus Croatia with 32.17% (CHCZ 41%) biosimilar filgrastim utilization, ranks slightly above average in that regard. European countries with the largest biosimilar filgrastim utilization include Greece (90%), Hungary (80%), Spain(74%), Romania (70%) and UK (68%).

PI-O1-3

ARE PHARMACOLOGICAL INTERVENTIONS COMPLETELY DESCRIBED IN CLINICAL TRIALS ON DRUG-DRUG INTERACTION FROM CLINICALTRIALS.GOV?

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Introduction: Complete and transparent description of interventions is a prerequisite to enable the adequate interpretation of drug-drug interactions (DDIs). The aim was to evaluate the completeness of drug intervention data for trials on DDIs registered in ClinicalTrials.gov and published in journal articles.

Materials and methods: In this cross-sectional study, trials were included if they 1) primarily investigated DDIs, 2) had a National Clinical Trial (NCT) identifier, 3) were completed interventional trials by October 2015, and 4) had up to two drugs within the Intervention registration element in ClinicalTrials.gov. The quality of intervention description in the registry and matching publications was assessed using 12 items from the Template for Intervention Description and Replication (TIDieR) checklist.

Results: A total of 642 eligible trials with 1180 drug interventions were analyzed. Most poorly described TIDieR items in ClinicalTrials.gov were intervention provider (0.3%), adherence strategies (0.8%), manufacturer (1.8%), location (12.1%), procedure (16.2%), brand name (28.3%) and dosage form (38.4%). Regarding 51 trials with protocol reported both in ClinicalTrials.gov and publication, less than half of interventions had clear and congruent description of procedure, dosage form and route of administration in both sources.

Conclusions: Despite DDIs being an important cause of morbidity and mortality related to drug use, DDI trials did not sufficiently report important elements of interventions. To ensure patient safety in clinical practice regarding concomitant medication use, registration and publishing requirements for essential data on drug interventions should be expanded and based on the TIDieR checklist.

PI-O1-4

ACCORDANCE OF REGISTERED DRUG PACKS WITH HELICOBACTER PYLORI TREATMENT REGIMENS

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Introduction: In the light of increasing antibiotic resisance several authors have raised questions about accordance of drug packs of antibiotics with recommended duration of treatment in the guidelines. These have been investigated for most frequently consumed antibiotics in primary care. Considering rising prevalence of Helicobacter pylori infection, complexity of treatment regimens and a variety of treatment regimens proposed, the aim of this study was to investigate how many of the proposed regimens could be matched perfectly with drugs registered in Croatia.

Material and methods: We considered treatment regimens for H. pylori infection proposed by the Maastricht V/Florence Consensus Report. Shortest recommended treatment duration of 10 days and longest recommended treatment duration of 14 days were considered. Drugs were identified in Croatian drug database. Metronidazole 500 mg was considered interchangeable with 400 mg metronidazole. Furthermore, doxycycline replaced tetracycline as it is not registered in Croatia. Patient adherence to the treatment was assumed to be ideal.

Results: Drug packs registered in Croatia more frequently matched 10-day treatment regimens than 14-day. Sequential therapies could not be matched for 10-day duration. Rifabutin and furazolidone-based treatments were not matched due to limited availability of drugs in Croatia. None of the 14-day treatment regimens was matched.

Conclusions: This study outlines poor accordance of registered antibiotics for treatment of H. pylori that result in excess units of antibiotics. Antibiotics stocked at home may be used inappropriately in the future what contributes to the emergence of antibiotic resistance.

PI-O1-5

EVALUATION OF TEACHING QUALITY OF THE FULLY ONLINE COURSE ON RATIONAL PRESCRIBING AIMED AT FINAL YEAR STUDENTS OF THE UNIVERSITY OF ZAGREB SCHOOL OF MEDICINE

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Introduction: In June 2019, 309 final year medical students of the University of Zagreb School of Medicine took a fully online course in rational prescribing of medicines developed in collaboration with the University of Michigan Medical School (Ann Arbor; USA).

Materials and methods: The course comprised 90 multimedia, problem based, therapeutics cases as well as 24 timed, formative, weekly quizzes, covering disciplines such as: pain treatment, neurology, psychiatry, metabolism, diabetes, infective diseases, gastroenterology, ENT, cardiology, clinical pharmacology, pharmacogenetics, rheumatology, transfusiology as well as obstetrics & gynaecology. After the course, a validated, online questionnaire with a 5-point Likert scale (1 - I strongly disagree to 5 - I strongly agree) on teaching quality was distributed to students, with a response rate of 24%.

Results: Most respondents, with a score of 4.85 ± 0.39 (mean ± SD), strongly agreed that this course empowered them to generally perform better in their curricular and extracurricular activities. The overall mean teaching quality grade awarded on a scale from 1 (poor) to 5 (excellent) was 4.32 ± 0.82. Furthermore, with a score of 4.13 ± 0.85, students were very satisfied with how well the online teaching technology of the course performed and they also considered the difficulty of online teaching materials to be just about right (3.47 ± 0.53).

Conclusion: Once developed, online teaching courses allow for significant savings in space, staff and teaching materials costs, while providing the same course quality, accessibility as well as simulation of virtual patients where prescribing errors students inevitably make do not result in irreversible damage in real life.

PI-O1-6

PHARMACOVIGILANCE KNOWLEDGE AND ATTITUDES: A SURVEY AMONG FINAL YEAR MEDICAL STUDENTS

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Introduction: Pharmacovigilance has a very important role in patient safety and despite adverse drug reaction (ADR) reporting being a legal obligation for medical doctors in Croatia, underreporting remains an issue, although significant progress has been made. Our aim was to determine medical students' knowledge about ADR reporting and analyse their attitudes towards pharmacovigilance.

Materials and methods: A computer-based survey was conducted in June 2019 among all final year medical students at the University of Zagreb School of Medicine. Knowledge regarding ADR reporting was tested using dichotomous (yes/no) and open-ended questions, while attitudes were evaluated using Likert scales.

Results: A total of 212 students completed the questionnaire (response rate 69%). Only 57% of students knew how to report an ADR and 88% were unfamiliar with the details required for reporting. The mean score for knowledge questions was 68.5%. There was no difference in the number of correct answers between students who believed to have adequate knowledge and those who did not (Wilcoxon rank sum test, $p = 0.62$). However, 78% of students thought that medical doctors remain the most important professionals involved in ADR reporting and 82% planned to report ADRs. Most students chose practice assignment as the best way to learn about pharmacovigilance.

Conclusions: The majority of students recognized the importance of ADR reporting, although their knowledge and skills could be significantly improved, perhaps by introducing more problem-based teaching assignments into the school's curriculum.

POSTER SEKCIJA S ORGANIZIRANOM DISKUSIJOM II /
POSTER SESSION II WITH ORGANIZED DISCUSSION II:

TEMELJNA FARMAKOLOGIJA 1 / BASIC PHARMACOLOGY 1 (PII-B1: 1-10)
TEMELJNA FARMAKOLOGIJA 2 / BASIC PHARMACOLOGY 2 (PII-B2: 1-9)
TEMELJNA FARMAKOLOGIJA 3 / BASIC PHARMACOLOGY 3 (PII-B3: 1-6)
OSTALE TEME 2 / OTHER TOPICS 2 (PII-O2: 1-5)

PETAK, 27. RUJNA 2019. / FRIDAY, 27 SEPTEMBER 2019

13:55 – 14:55

PII-B1-1

EFFECTS OF VGLUT3 POSITIVE CELLS OF THE MEDIAN RAPHE REGION ON SOCIAL BEHAVIOUR

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Introduction: Median raphe region (MRR) influences various behaviours, e.g.: locomotion, anxiety, social behaviour and memory. MRR is known as a serotoninergic nucleus, although numerous other neurone types, such as the vesicular glutamate transporter 3 (VGluT3) positive ones are more abundant here. Our goal was to investigate their role in behaviour.

Materials and methods: We used VGluT3-Cre mice and pharmacogenetics (DREADDs). The following behavioural tests were conducted: in open field (OF) locomotion was measured. In sociability, social interaction (SI) and resident intruder (RI) test different aspects of social behaviour were investigated. Anxiety was assessed on elevated plus maze (EPM). Memory was tested with Y-maze and social discrimination (SD) tests. The animals were injected with the synthetic ligand clozapine-N-oxide before each test, except for SD.

Results: In OF the inhibitory group moved less. In sociability the excitatory group spent less time with the conspecific. In SI the inhibitory group was more social; moreover, friendly social behaviour was increased. In RI the inhibitory group had lower frequency of friendly social behaviour. On EPM the excitatory group spent more time in the open arm and showed higher frequency of risk assessment behaviour. In Y-maze and SD there were no differences between the groups. However, in SD the inhibitory group spent more time with the conspecifics.

Conclusions: We found that the excitation of MRR VGluT3+ neurones decreases anxiety and social interest. Their inhibition decreases locomotion but increases social interest and affects social behaviour. Their activity has long-lasting effect on behaviour.

PII-B1-2

THE ROLE OF THE DOPAMINERGIC CELLS IN THE MEDIAN RAPHE IN THE BEHAVIOR OF THE MALE MICE

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Introduction: According to previous studies the median raphe region (MRR) is known to contribute significantly to social behavior. Apart from serotonin, there are some dopaminergic neurons in this region. Dopamine is connected to the reward system and locomotion, but very little is known about its role in the MRR. This experiment was designed to clarify this question.

Materials and Methods: We used pharmacogenetic technology in mice containing Cre enzyme under the promoter of the dopamine transporter (DAT). With the help of adenoassociated virus, artificial receptors DREADD (both stimulatory and inhibitory as well as control mCherry) were injected into the MRR. A few weeks after the virus surgery, the animals were injected with the artificial ligand (clozapine-N-oxide) 30 min before the designed experiments: locomotion (open field/OF), social behavior (sociability, social interaction/SI), anxiety (elevated plus maze/EPM) and short-term memory (y-maze).

Results: Manipulation of the dopaminergic cells of MRR had no effect on locomotion (OF, closed arm entries in EPM, total arm entries in y-maze). Stimulation of DAT+ cells of MRR decreased social interest (sociability and SI, detectable even 24h later) and increased aggression with a tendency of reduced anxiety and better short-term memory.

Conclusion: Stimulation of dopaminergic neurons showed the opposite of what was for discovered for the whole MRR in the previous studies. Nevertheless, these findings support specific role of MRR dopaminergic cells in social behaviour, anxiety and memory formation.

PII-B1-3
COGNITIVE AND DEPRESSIVE-LIKE SYMPTOMS IN TRIPLE TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder, which is usually accompanied by depressive symptoms in approximately 30-40% of patients. We focused on this comorbidity.

Materials and Methods: In our study, triple-transgenic mouse model of AD (3xTg-AD) was used, that expresses human mutated presenilin-1, amyloid precursor and tau protein. This is considered as the most relevant in vivo model of typical behavioural dysfunctions of AD. The histological hallmarks appear around 6 month of age. To explore the progression of symptoms we compared 4- and 8-month-old male mice (Bl/6 controls and 3xTg-AD). A behavioural test battery was used to examine anxiety- (open field (OF), light-dark box (LD)) and depression-like symptoms (splash, forced swim test (FST)) as well as cognitive decline (Morris Water Maze test (MWM)).

Results: In the MWM, the latency of finding the platform was higher for 3xTg-AD mice compare to controls, both in 4-month and 8-month-old cohorts. In the OF test, both age groups of 3xTg-AD mice moved significantly less, than controls. In the splash test, both the 4-month-old and the 8-month-old 3xTg-AD mice spent significantly less time with grooming, than the age-matched control. There was not significant genotype difference in FST and LD tests.

Conclusion: We can conclude that the 3xTg-AD mice learn significantly slower from the age of 4 months. We could not detect robust anxiety- and depressive-like behavior at either studied age-groups. Moreover, 3xTg-AD mice did not show significant progression in cognitive decline or depressive-like symptoms between the 4- and 8-months.

PII-B1-4

ACUTE CHANGES OF ACETHYLCHOLINESTERASE ACTIVITY AND TAU PROTEIN PHOSPHORYLATION IN RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Introduction: Intracerebroventricular (icv) administration of streptozotocin (STZ) generates a rat model of sporadic Alzheimer's disease. In contrast to the long term effects of STZ-icv treatment, the acute neurochemical impairments have not been investigated thoroughly enough. We aimed to explore acute (within 24 hours post icv injection) cholinergic and tau protein changes in STZ-icv rat model.

Materials and Methods: Rat (strain Wistar) were injected icv with STZ (1,5 mg/kg) or vehicle-citrate buffer (controls). The animals were sacrificed 15 min, 1, 6 and 24 hours following the STZ-icv treatment. Acetylcholinesterase (AChE) activity in hippocampus (HPC) and parietal cortex (PC) was measured spectrophotometrically by Ellman's method. Protein expression of total (T-tau) and phosphorylated (PHF13) tau protein in HPC and PC was measured by SDS-PAGE electrophoresis and Western blot analysis. Data were analysed by Mann-Whitney U test ($p<0.05$).

Results: The expression of PHF13 was found significantly increased (+27%) in PC 15 minutes after STZ-icv injection while no significant changes were found in tau phosphorylation in HPC as well as in the expression of T-tau. The AChE activity in rat PC was significantly increased after 15 min (+35%), decreased after 6 hours (-18%) and again increased 24 h (+17%) after STZ-icv treatment. Similar to tau phosphorilation, there were no significant changes in AChE activity in HPC.

Conclusion: The results indicate that within 24 hours following the STZ- icv injection the changes in tau phosphorylation and AChE activity occur in time- and region-dependent manner in the rat brain. In contrast to STZ-icv mice model changes in rats are more pronounced in PC than in the HPC.

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PII-B1-5

TIME RESPONSE OF ORAL GALACTOSE ON METABOLIC EFFECTS IN COGNITIVELY NORMAL RATS

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Introduction: Brain insulin resistance is the pathophysiological core of sporadic Alzheimer's disease (sAD). Our previous research pointed to the therapeutic potential of oral galactose in an intracerebroventricular-streptozotocin (STZ-icv) rat model of early sAD, which affected brain glucose hypometabolism and induced metabolic changes. We explored a time-course of peripheral and central effects following a single oral galactose dose in healthy rats used as controls to the STZ-icv model.

Materials and Methods: Three-month old male Wistar rats received 0.05M citric buffer icv (2 µL/ventricle). A month later, plasma, cerebrospinal fluid (CSF) and hippocampal samples were taken before, 15, 30, 60 or 120 minutes after 200 mg/kg galactose given by oral gavage. We measured glucose, galactose and total glucagon-like peptid-1 (GLP-1) levels (spectrophotometry, ELISA) in plasma/CSF and hippocampal GLP-1 receptor levels (Western blot).

Results: Galactose levels showed a significant increase both in plasma and CSF after 15 min (+257%; +137%, $p<0.05$) but were normalized at later points (decline below control found only in CSF at 120 min /-58%, $p<0.05$/). Increase in glucose levels was significant only in CSF at 15 and 30 min (+40%; +31%, $p<0.05$), while similar but lower tendency in plasma remained insignificant (+14%). Total GLP-1 levels showed similar but insignificant acute tendencies of increase in plasma and CSF (+63%), followed by significant increase in hippocampal GLP-1R expression at 15 and 30 min (+72%; +15%, $p<0.05$) with subsequent normalization.

Conclusion: A single dose of oral galactose does not disrupt glucose and GLP-1 homeostasis but acutely activates GLP-1R signalization in healthy rats.

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ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

PII-B1-6

ACUTE ORAL GALACTOSE TREATMENT DECREASES OXIDATIVE STRESS IN RAT BRAIN AND PLASMA IN TIME-DEPENDENT MANNER

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Introduction: Oral galactose treatment has been shown to prevent and reverse cognitive decline in rat model of sporadic Alzheimer's disease induced by intracerebrovetricular administration of streptozotocin (STZ-icv). Oxidative stress has been extensively studied as an etiopathogenetic factor of Alzheimer's disease, but the effect of acute oral galactose on oxidative stress parameters has never been examined in this context.

Materials and Methods: One month after intracerebroventricular vehicle administration Wistar rats were given oral galactose (200mg/kg) and sacrificed 30, 60 or 120 minutes after (C0;C30;C60;C120). Spacial distribution of reductive potential (RP) and peroxidase activity (PA) was analyzed by passive diffusion slice print blotting followed by redox permanganometry or 3,3'-Diaminobenzidine precipitation. Total antioxidant capacity (TAC) was measured by I2/KI redox couple oxidation reduction potential (ORP) and dot blot permanganometry. Reduced glutathione and protein thiols were analyzed with 5,5'-Dithiobis-(2-Nitrobenzoic Acid). Lipid peroxidation was estimated by thiobarbituric acid reactive substances assay and superoxide dismutase activity (SODa) with 1,2,3-trihydroxybenzene autooxidation.

Results: Oral galactose treatment increased RP and PA in the brain with highest values measured in C60. TAC was unaltered in plasma and increased in hippocampus (HPC) in C60 (-2,47% vs C0;$p<0,05$; -1,87% vs C30;$p<0,05$) . No changes in concentration of reduced glutathione and protein thiols were observed in plasma or HPC. Lipid peroxidation products were significantly decreased in plasma in C30 (-14%vsC0; $p<0,01$), C60 (-11%vsC0; $p<0,01$) and C120 (-16%vsC0; $p<0,05$). Galactose increases SODa with highest values in C120 (-12%vsC0; $p<0,05$).

Conclusion: Acute oral galactose treatment decreases parameters of oxidative stress in healthy animals in time dependent manner.

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Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

PII-B1-7

DIFFERENCES AMONG SUBLINES OF WZ-5HT RATS IN THE EMERGENCE TEST, AUTOGROOMING AND REARING BEHAVIOUR DURING THE OPEN FIELD TEST

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In several models of anxiety-like behaviour sublines of WZ-5HT rats with extremely low levels of platelet serotonin (low-5HT subline) showed less anxious behaviour than rats with extremely high platelet serotonin (high-5HT subline). To further investigate phenotype differences among WZ-5HT sublines in the domain of anxiety, we compared high-5HT (n=9) with low-5HT male rats (n=15) for a total number of grooming and rearing episodes during 10 minutes of the open field test and for the incidence to appear on rims of the open-top home cage during 5 minutes of the emergence test. Rats were tested in three subsequent sessions. In comparison to high-5HT rats, low-5HT rats had a significantly lower number of grooming episodes during all open field test sessions ($p<0,05$ unpaired t-test, $p<0,001$ and $p<0,0001$ Mann-Whitney tests) and a significantly higher number of rearing episodes in last two open field test sessions ($p<0,05$ and $p<0,0001$, unpaired t-tests). Regarding emergence test, low-5HT rats started to appear on the edges of cages during the first test session (33 %) and continued to emerge during next two sessions (87 % and 100 %, $p<0,0001$ and $p<0,001$ compared to high-5HT rats, Fisher's exact tests). At the same time, high-5HT rats just started to appear during the last test session (0 %, 0 % and 55,5 % for 1st, 2nd and 3rd session, respectively). Results presented here suggest that low-5HT rats had less anxious behaviour and more impulsive, disinhibited temperament as compared to high-5HT rats characterized by timidity, cautious and inhibited temperament.

PII-B1-8
BILIRUBIN IN LOW CONCENTRATIONS PROTECTS ASTROCYTES FROM CELL DEATH

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Introduction: Bilirubin exerts anti-oxidant, anti-inflammatory, and anti thrombogenic activity. Recent studies show that serum bilirubin levels inversely correlate with the incidence of cardiovascular diseases. On the other hand, higher concentrations of bilirubin are neurotoxic, and data regarding the influence of bilirubin on astroglial cells, which are crucial for the maintenance of brain homeostasis, are scarce. In the present work, we aim to elucidate the effect of different concentrations of bilirubin on programmed death of astrocytes, as well as the influence of bilirubin on staurosporine-induced cell death.

Materials and Methods: We used primary cultures of cortical astrocytes, prepared from new-born Wistar rats. The cells were exposed to different concentrations of bilirubin in the presence or absence of 1 μM staurosporine. Different forms of cell death were measured using flow cytometric analysis.

Results: Bilirubin has no effect on apoptosis and necroptosis in concentrations up to 50 nM, while 100 nM bilirubin reduces cell viability. The concomitant exposure of the cells to 1 μM staurosporine and bilirubin leads to biphasic response; whereas 10 nM bilirubin reduces staurosporine induced cell death, 100 nM bilirubin increases the proportion of death cells.

Conclusion: In this study, we show that low concentrations of bilirubin protect astrocytes against staurosporine-induced apoptosis and overall cell death, whereas high concentrations are toxic. It is important to notice that the free bilirubin (unbound and unconjugated) is present in human serum in concentrations around 10 nM, which opens up the perspective that bilirubin is important also for astrocyte redox status homeostasis.

PII-B1-9
THE POSSIBILITY FOR DEVELOPMENT OF NON-TRANSGENIC RAT TAUOPATHY MODEL BY APPLICATION OF TAU OLIGOMERS INTO THE ENTORHINAL CORTEX

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Introduction: Alzheimer's disease (AD) is the most common secondary tauopathy characterized by progressive loss of cognitive functions and behavioral impairment. The hyperphosphorylation and aggregation of tau proteins follows a distinct pattern with the first changes seen in the locus coeruleus and entorhinal cortex from where they spread to the hippocampus and other cortical regions. We aimed to explore if intracerebral injection of tau oligomers and tau fibrils will induce trans-synaptic spread of pathological tau proteins, and will those changes be associated with cognitive impairment.

Materials and Methods: Four month old male Wistar rats (n = 96) were stereotaxically injected into the entorhinal cortex with tau oligomers, tau fibrils, and phosphate-buffered saline. Animals were analyzed 4, 8 and 11 months post-injection. Cognitive performance was tested using T-maze, novel object recognition and object-location test. To specifically detect tau protein changes and perform staging of tau pathology, we used specific anti-tau antibodies. Proteins isolated from the entorhinal cortex and hippocampus were analyzed by immunoblotting.

Results: The results obtained suggest that stereotaxic injection of tau oligomers or tau fibrils into the lateral entorhinal cortex induces phosphorylation of Ser202/Thr205 tau epitope. Using antibody which recognizes human tau, we found a signal present in the brainstem and transentorhinal region. Rewarded learning in the T-maze showed slower learning curve with more incorrect choices in rats injected with tau fibrils.

Conclusion: Understanding of the role of tau oligomers and tau fibrils in this rat model of neurodegeneration has a great potential for revealing mechanisms underlying development and progression of AD in humans.

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PII-B1-10
EXPRESSION OF ACTIVATED MICROGLIA CELLS MARKERS IBA1, CD68 AND HLA-DR IN THE HIPPOCAMPAL FORMATION OF PATIENTS WITH ALZHEIMER'S DISEASE

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Numerous studies have documented that uncontrolled immune response in the brain contributes to the progression of Alzheimer's disease (AD). Although microglia cells should be protective, pathological changes cause aberrant and stronger microglial activation. Uncontrolled microglial response leads to the constant production of pro-inflammatory factors, severe inflammation and oxidative stress which becomes detrimental for neighboring neurons. Therefore, the main goal of this study was to analyze the expression of microglia cells markers Iba1, CD68 and HLA-DR in the different areas of the hippocampal formation (granular cell layer of dentate gyrus, hilus, CA2/3, CA1, subiculum and white matter) of patients with Alzheimer's disease and control samples. Markers of microglial activation are detected with immunohistochemistry method. Different areas of hippocampal formation are tested for the expression of microglia cell markers and compared with numbers of amyloid plaques and neurofibrillary tangles in the same hippocampal regions. The research has shown that the expression of microglia cell markers differs in the AD brains and controls. There was a positive correlation between the expression of activated microglia cells markers CD68 and HLA-DR with the AD and their higher expression in the areas of hippocampal formation most affected by AD pathology. Iba1 was negatively correlated with AD. These results support the inflammatory hypothesis of AD and suggest that the aberrant microglial response could be essential for the development and progression of degenerative changes in.

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PII-B2-1

LOCALIZATION OF BOTULINUM TOXIN TYPE A IN SENSORY AND MOTOR REGIONS OF THE BRAIN AFTER INTRA-TMJ INJECTIONS IN RATS

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Introduction: Botulinum toxin type A is a neurotoxin capable of inhibiting acetylcholine, and other neurotransmitters from nerve terminals. Action of BoNT-A on pain and inflammation was suggested and clinical effects on neuropathic pain and other chronic states have been reported. For these reasons, it has been hypothesized that the analgesic action of BoNT-A is mainly centrally-mediated and can reach upper brain levels through retrograde axonal transport. AIM: To localize BoNT-A into upper regions of the brain and prove the transport of the neurotoxin into central levels after peripheral applications.

Materials and Methods: Intra-articular injection of BoNT-A was applied into the left TMJ of Wistar rats in a dose of 7U/Kg. Saline was injected as a control. After 48h all animals were sacrificed, perfused and fixed for the dissection of the brainstem. Samples were cut on a freezing microtome into coronal sections and immunofluorescence of cleaved-anti-Snap-25 was performed. Inmunostained sections were visualized and analyzed with an epifluorescent microscope.

Results: No stained cl-sp25 was found in control group samples. Stained cl-sp25 was found in the Trigeminal nucleus caudalis of all animals in both BoNT-A groups. In addition, cl-sp25 was found into the Facial nucleus and the Trigeminal motor nucleus in both BoNT-A groups.

Conclusion: Our results demonstrate the ability of BoNT-A to reach upper levels in the CNS, strengthening the idea of a central effect of the neurotoxin. Furthermore, the presence of BoNT-A specifically into the TNC points for a possible analgesic use of BoNT-A on orofacial neuropathic conditions and other painful chronic-orofacial states.

PII-B2-2

ANTINOCICEPTIVE EFFECT OF BOTULINUM TOXIN TYPE A IN CARRAGEENAN-INDUCED ARTHRITIS IN RATS

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Introduction: Joint inflammation and associated pain is a challenge to treat due to the side effects and reduced effectiveness of available drugs. Botulinum toxin type A (BoNT/A) might be an interesting therapeutic option because of its long-lasting effect after single local injection. Although the beneficial effect of BoNT/A in a specific patient population with joint pain has been observed in several clinical trials, the mechanism of action is still not fully elucidated.

Materials and Methods: Here we investigated the antinociceptive and anti-inflammatory effect of BoNT/A (Dysport®) on carrageenan-induced knee joint arthritis in Wistar rats. BoNT/A was applied intraarticularly (i.a., 20 U/kg) 5 days before or intrathecally (i.t., 4 U/kg) 1 day before carrageenan injection (2%, 50 µl). BoNT/A effects on mechanical hyperalgesia, extravasation, and edema in the inflamed knee joint were measured 4 h after carrageenan injection. Cleaved SNAP-25 (cl-SNAP-25) was assessed immunohistochemically in the dorsal horn of the spinal cord (DHSC) after BoNT/A i.a. and i.t. injections.

Results: BoNT-A reduced mechanical hyperalgesia only after i.t. application. However, BoNT-A did not affect either joint plasma extravasation or edema regardless of the site of application. clSNAP-25 was detected in the lumbar DHSC tissue after both, i.a. and i.t. application.

Conclusion: These results provide evidence that BoNT/A has no effect on local inflammation while reducing associated pain. Moreover, after injection in the joint, BoNT/A is transported to the CNS as demonstrated by cl-SNAP-25 immunoreactivity in the spinal cord.

PII-B2-3

MICROGLIA POLARISATION IN THE RAT THALAMUS AND CEREBELLUM AFTER TRAUMATIC BRAIN INJURY

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Introduction: Neuropathophysiological cascade following traumatic brain injury (TBI) includes many complex processes and despite numerous preclinical and clinical studies, there is still no neuroprotective pharmacotherapeutic approach that could significantly improve clinical outcomes in patients. Neuroinflammation is considered to be one of the key processes in the TBI pathobiology. Therefore, better knowledge of microglial roles and activities could be very important for the development of new therapeutic strategies. The aim of this study was to determine the microglial/macrophages activation and possible signs of phenotypic "M1/M2" polarization in thalamus and cerebellum, within the first week after experimental TBI in the rat.

Materials and methods: TBI of moderate severity was induced over the left parietal cortex using lateral fluid percussion injury (LFPI) and sham-operated animals were used as a control group. Rats were sacrificed 1, 3 or 7 days following TBI or sham procedure and their brains were prepared for immunohistological analyses. Double-staining of the brain slices was performed using primary antibody against ionized calcium binding adaptor molecule 1 (Iba1), in combination with anti-CD86, anti-CD206 or anti-Mac-2.

Results: Microglia/macrophages with different morphology, observed in different time points in both thalamus and cerebellum, showed some colocalization with CD86 and CD206, but not with Mac-2 in the thalamus, while in cerebellum we did not detect any colocalization.

Conclusion: Our preliminary results suggest that usage of "M1/M2" phenotypic polarization of microglia, originally described in in vitro studies, seems to have limited potential in experimental in vivo TBI research, at least with markers used in this study.

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PII-B2-4
SINGLE MODERATE TRAUMATIC BRAIN INJURY IN MICE CAUSES MISSLOCALIZATION AND PHOSPHORYLATION OF TRANSACTIVE RESPONSE BINDING PROTEIN 43 IN THE PARIETAL CORTEX

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Introduction: Transactive response DNA-binding protein 43 (TDP-43) is a ubiquitous protein that controls gene expression and it is mainly located in the nuclei. In some neurodegenerative diseases it was found that TDP-43 translocates to the cytoplasm where it forms phosphorylated, ubiquitinated and proteolytically cleaved deposits which may cause neurotoxicity. Aim of this preliminary study was to investigate the changes in TDP-43 subcellular position, degradation, and phosphorylation in the mouse parietal cortex after a single traumatic brain injury (TBI).

Materials and Methods: Moderate severity TBI was induced over the left parietal cortex in adult male mice using the lateral fluid percussion injury apparatus. Animals were sacrificed 1, 3 or 14 days after the trauma and their brains were prepared for Western blot or immunohistological analyses. Animals of the control group were sacrificed 1 day after the sham procedure.

Results: In the injured mice, an increase in the cortical expression of the cytoplasmic TDP-43 was detected on the first and the third day, and decrease in the nuclear TDP-43 expression on the third day after TBI. Phosphorylated form of 35 kDa cleavage product of TDP-43 was also observed in the injured animals' cortices. Co-labeling of TDP-43 with neuronal and microglial markers revealed cytoplasmic mislocalization of this protein in these cell types.

Conclusion: Our preliminary results showed that a single moderate TBI causes changes in the TDP-43 expression in the mouse parietal cortex.

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PII-B2-5

TDP-43 EXPRESSION IN THE SPINAL CORD AND MOTOR ABILITY SIX MONTHS AFTER REPETITIVE TRAUMATIC BRAIN INJURY IN MICE

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Introduction: Repetitive mild traumatic brain injury (mTBI) is a risk factor for the development of amyotrophic lateral sclerosis in which TAR DNA-binding protein 43 (TDP-43) disregulation has been identified. The aim of this research was to investigate the long term effects of rmTBI on the expression of TDP-43 in the spinal cord as well as on the motor performance in mice.

Materials and Methods: Repetitive mTBI was induced by the weight drop method (Kane et al. 2012), twice a day during 5 consecutive days in C57BL/6 mice. Sham injured animals were subjected to the same procedures without receiving any impact. Mice were exposed to the accelerating rotarod test for three days before the first injury, and their postinjury motor function was tested every 30 days, up to 6 months after the last injury when they were sacrificied. Their spinal cords were dissected and prepared for the Western blot analyses. Some animals were perfused transcardially and their spinal cords were prepared for the immunofluorescent stainings.

Results: In the spinal cords of injured mice increased cytoplasmatic expression of TDP-43 was detected. It was more pronounced in neurons of injured mice in comparison with animals of the control group. The motor function of injured mice was not not impaired at any time point investigated.

Conclusion: Our results suggest that repetitive mTBI in mice induces TDP-43 dysregulation in the spinal cord at 6 months after the last brain trauma but has no influence on the motor performance in our experimental conditions.

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PII-B2-6

THE EFFECTS OF ROSUVASTATIN, SIMVASTATIN AND FENOFIBRATE ON BRAIN SUPEROXIDE DISMUTASE ACTIVITY IN RATS

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Introduction: Superoxide dismutase (SOD) is key enzyme that metabolize superoxide. Non-clinical investigations have evidenced that fenofibrate either increase, decrease or have no effect on SOD activity. The results of experiments with statins, have also shown different effects on SOD activity. Because of these opposite literature data, we decided to investigate the influence of simvastatin (SIMV), rosuvastatin (ROSU) and fenofibrate (FENO) on brain SOD activity in rats that is first biomarker of oxidative stress.

Material and Methods: Male Wistar rats were divided in six control groups (saline), two experimental groups on SIMV treatment (10 and 50 mg/kg/day), two experimental groups on ROSU (5 and 10 mg/kg/day) and remaining groups on FENO treatment (30 and 50 mg/kg/day) for 3 weeks. Brain SOD activity was measured with sensitive spectrophotometric assay. Data were analyzed using two-group t-test. Results are expressed as the means, SDs and 95% confidence intervals for the difference between means.

Results: ROSU 5 mg and SIMV in both doses caused significant decrease ($p<0.05$) of SOD activity by 33%, 20% and 40% vs. control (4,1 ± 0,31vs. 6,1 ± 0,50, 3,9 ± 0,71 vs. 4,9 ± 0,58; and 3,7 ± 0,80 vs. 6,2 ± 0,40). Higher doses of ROSU and FENO caused significant increase of SOD activity by 56% and 52 % vs. control (8,9 ± 1,63 vs. 5,7 ± 0,62 and 8,2 ± 0.82 vs. 5,4 ± 0,87).

Conclusion: Our results indicate that statin therapy has variable effects on oxidant balance while fenofibrate treatment increased capacity of the antioxidant system.

PII-B2-7

THE NEUROPROTECTIVE EFFECT OF HYPNOTIC ZOLPIDEM AGAINST GLUTAMATE-INDUCED APOPTOTIC DEATH IS MEDIATED VIA THE PI3K/AKT SIGNALLING PATHWAY

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Introduction: Excitotoxicity is a form of neuronal death induced by increased glutamate signaling. It is involved in ischemia-induced brain damage, traumatic brain injury and various neurodegenerative diseases. Prolonged activation of ionotropic glutamate receptors results in substantial calcium overload that ends in oxidative stress-related apoptotic death. We studied neuroprotective potential of zolpidem, a drug that posses structural similarity to antioxidant melatonin and exerts its central effects acting at GABAA receptor complex.

Materials and methods: Effects of zolpidem were studied in the culture of P19 neurons. Neuronal death was induced by exposure to 300 μM glutamate for 3 hours. 24 h later we monitored changes in neuronal viability, generation of reactive oxygen species (ROS) and caspase-3/7 activity. Protein expression of transcription factor p53 and phosphorylated Akt was determined by western blot method, whereas gene expression analysis of Bax, Bcl-2 and p53 was assessed by the semiquantitative RT-PCR method.

Results: Zolpidem prevented glutamate-induced neuronal death. It attenuated enhancement of ROS generation, increase in p53 and Bax expression and caspase-3/7 activity, and induced prominent over-activation of Akt kinase. The pro-survival effect and pAkt induction were prevented in the presence of wortmannin, an inhibitor of phosphatidylinositol-3-kinase (PI3K). Interestingly, flumazenil, a GABAA receptor antagonist, did not affect zolpidem-mediated neuroprotection, whereas PK11195, a drug that modulates mitochondrial translocator protein 18 kDa (TSPO) and F0F1-ATPase, prevented effect of zolpidem.

Conclusion: The obtained results suggest promising therapeutic potential of zolpidem against excitotoxic insults and highlight importance of mitochondria and Akt signalling as valuable therapeutic targets against glutamate-mediated neurodegeneration.

PII-B2-8

EFFECTS OF CAFFEINE ON DEVELOPMENT OF OXIDATIVE STRESS AND CRYSTALLIZATION ON IN VITRO MODEL OF OXALATE UROLITHIASIS

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Introduction: Urolithiasis is a common disease characterized by formation of solid deposits in the urinary tract. Most of the stones generated inside urinary tract are predominantly composed of calcium oxalate which can produced free radicals.

Aim: To study the effects of caffeine on formation of crystals and it's antioxidative effects in an in vitro model of urolithiasis.

Materials and methods: LLC-PK1 cells were used as in vitro model of urolithiasis. Oxidative stress was induced by exposing cells to crystals of calcium oxalate. Cells were treated with different concentrations of caffeine. Effects of caffeine on crystallization and oxidative stress was estimated by crystals counts and total glutathione concentrations, respectively.

Results: Increasing the concentration proportionally, caffeine stops the crystallization of calcium oxalate in in vitro model of proximal kidney tubules urolithiasis. Number of calcium oxalate crystals was decreasing in correlation with increasing concentrations of caffeine up to 60% and with the length of treatment with caffeine. Concentrations of total glutathione change up to 1,8% when caffeine is added to cells treated with calcium oxalate crystals suggesting amelioration of oxidative stress.

Conclusion: Results suggest that caffeine can lower crystallization and has a positive effect by lowering oxidative stress in our in vitro models of urolithiasis. More studies are needed to further evaluate caffeine's role in formation of kidney stones.

PII-B2-9
SPLIT LUCIFERASE NANOLUC AS A REPORTER FOR THE DETECTION OF HUMAN TAU PROTEIN OLIGOMERIZATION

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One of the main neuropathological hallmarks of Alzheimer's disease are neurofibrillary tangles (NTFs), large aggregates of microtubule-binding protein tau that form within the affected neurons. The formation of NTFs is preceded by early-stage tau oligomers, however the molecular pathways that initiate tau aggregation are unclear. To better understand early steps in tau pathology, we constructed a tool for studying tau oligomerization in living cells, based on the luminescent reporter NanoBiT, in which protein-protein interaction results in the complementation of the luciferase NanoLuc, and consequently in generation of luminescence. Since molecular pathways of protein aggregation are largely evolutionarily conserved, we selected a simple cell model, yeast Saccharomyces cerevisiae. We separately fused two luciferase subunits to the human tau protein C-terminus. Expression of tau constructs was verified using western blot. Cells expressing tau separately fused to two luciferase subunits exhibited an increased luminescence, as compared to controls, indicating the formation of tau oligomers. In order to test whether tau-NanoBiT reporter is able to detect sarkosyl-insoluble tau aggregates, we measured tau-NanoBiT luminescence in pho85Δ, rim1Δ and sod2Δ mutants that were previously reported to have increased levels of sarkosyl-insoluble tau. Our preliminary data showed that the tau-NanoBiT luminescence was elevated in the sod2Δ mutant, while the signal in pho85Δ, and rim1Δ mutants was similar to the wild-type levels. In conclusion, we constructed a luminescent reporter for human tau protein oligomerization in living yeast cells. Our future experiments will address whether tau-NanoBiT reporter correlates with the levels of sarkosyl-insoluble tau aggregates.

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PII-B3-1

ANTIOXIDATIVE PROPERTIES OF HYDOLATES AND THEIR POTENTIALLY PROTECTIVE EFFECT ON LIPID PEROXIDATION

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Introduction: Hydrolates, also known as a hydrosols or aromatic waters, are the byproducts in production of natural essential oils. After removing essential oil, the remaining 90% of the water is considered hydrolate. They are popularly used directly on the skin or as an aqueous phase in creams. Therefore, the aim of the study was to determine antioxidant capacity of different hydolates and their potentially protective effect on lipid peroxidation, using different in vitro models.

Materials and Methods: Commercially available hydrolates from rose, immortelle, German chamomile, Roman chamomile, lavender, sage, and geranium were obtained for this study. Spectrophotometric (Trolox equivalent antioxidant capacity (TEAC)) and spectrofluorometric (Oxygen radical absorbance capacity (ORAC)) methods were used to determine total antioxidant capacity (AOX) of different hydolates. In order to investigate potentially protective effect of tested hydolates on lipid peroxidation, two hydolates with highest level of antioxidant capacity were applied with linolenic acid (LNA) and exposed to ultraviolet (UV) radiation, as a model of oxidative stress. Lipid peroxides were measured by thiobarbituric acid (TBA) assay and quantified by spectrophotometry.

Results: Rose hydrolate showed the highest AOX level measured by TEAC method, and lavender hydrolate showed the highest AOX level measured by ORAC method. Therefore, these two hydrolates were applied with LNA and exposed to UV. While lavender hydrolate showed mild antioxidant effect on lipid peroxidation, rose hydrolate induced an increase of lipid peroxides when compared to control.

Conclusions: Further studies are warranted to investigate antioxidant effect of hydolates ex vivo and in vivo after topical application.

PII-B3-2

ASSESSMENT OF HEPATOSTEATOGENIC EFFECTS IN CELL CULTURE MODEL OF DRUG INDUCED FATTY LIVER DISEASE

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Introduction: Toxicity represents one of the major obstacles to drug development and reasons for drug withdrawal from the market, with emphasis on drug-induced liver injury (DILI), as being one of the most often toxicity incidences. Therefore, the pharmaceutical industry is still in need to predict hepatotoxicity in vitro. So far, various hepatic cell models have been developed in order to better understand drug metabolism and toxicity. In our study, we focused on a specific form of DILI- drug induced fatty liver disease (DIFLD). The aim of this study was to assess the effect of two known hepatotoxic drugs amiodarone and tamoxifen on cell survival and formation of lipid vesicles in HuH7 cell line.

Materials and Methods: HuH7 cells were incubated with 5 μM to 20 μM concentrations of amiodarone and tamoxifen, respectively. Cell viability was estimated after 24h, 48h and 72h of exposure to the drugs by MTT assay. Changes in cell shape, formation of lipid droplets and the extent of steatosis were evaluated by fluorescent microscopy after Oil-Red-O and DAPI staining, respectively.

Results: The decrease in cell survival with increasing concentrations of drugs was observed by MTT assay, with slightly greater decrease demonstrated in amiodarone-treated cells. Appearance of the cells also changed: increased number of lipid vesicles, positioned on the inner side of the cell membrane, however displacement of the nucleus was not detected.

Conclusion: Consistent with other studies, our results also confirmed the steatogenic and apoptogenic effect of amiodarone and tamoxifen. In order to better understand and possibly prevent hepatotoxic effect of these drugs, further research is required.

PII-B3-3

THE ACTIVITY OF GARLIC EXTRACTS (ALLIUM SATIVUM) TO THE EPITHELIAL DAMAGE CAUSED BY SODIUM TAUROCHOLATE IN A CELL CULTURE MODEL OF ULCER DISEASE

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Introduction: Peptic ulcer is a chronic disease affecting up to 10% of the world's population. A cellular model of ulcer disease can be established in the human gastric cell line (AGS) using bile salts such as sodium taurocholate (NaT). Hence, the aim was to establish a cell culture model of ulcer disease, measure the toxic effect of NaT and to determine possible inhibition of NaT caused oxidative stress by antioxidant treatment of garlic extracts.

Materials and Methods: AGS cell line was used to develop the cell culture model of ulcer disease by NaT exposure. To prevent oxidative stress, AGS cells were co-treated with different concentrations of garlic extracts (GE). The extent of cytotoxicity and apoptosis was evaluated by cell counting and MTT, respectively. The extent of GE's gastroprotective effect was evaluated by expression of superoxide dismutase (SOD) by RT PCR.

Results: Sodium taurocholate caused a significant reduction in the viability of AGS. It was observed that cells treated with NaT and co-treated with GE showed significant increase in viability, compared to the cells treated solely with NaT. SOD expression had shown positive correlation with antioxidant treatment.

Conclusion: Garlic extracts co-treatment demonstrated gastroprotective effect on AGS cell culture model of ulcer disease. However, more experiments are needed to elucidate effect of garlic extracts in gastric ulcer disease.

PII-B3-4

EXPRESSION OF FERRITIN LIGHT AND HEAVY CHAINS IN RAT LIVER: SEX AND AGE DIFFERENCES

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Introduction: The ferritin complex consists of two protein chains structured in a 24 subunit nanocage for iron storage. Very early research on liver in experimental animals found sex differences in favor of females in the amount of iron, which was later associated with sex differences in the ferritin amount in the liver as an iron storage organ.

Materials and Methods: We used specific antibodies for two ferritin subunits followed by immunochemical methods for sex and age differences in the expression of light and heavy subunits of ferritin in the liver of three-month and two-year old Wistar rats together with gonadectomized three-month animals for steroid hormone influence determination. Distribution of ferritin subunits inside the organ was obtained by immunohistochemistry, while differences in the expression of individual subunits were determined by western analysis.

Results: The results of immunochemical analyses confirmed sex differences in both ferritin subunits and these remained with age, while the amount of ferritin in liver increased with age. Gonadectomy showed a positive effect of estrogen on the expression of both chains and a negative of testosterone. Both ferritin subunits displayed an overlapping distribution in liver cells, which was most pronounced in hepatocytes around the central veins.

Conclusion: We can conclude that both ferritin subunits were expressed in the liver and showed sex differences influenced by steroid hormones and age differences which may be correlated with an enlarged amount of stored iron and thus with a great threat of increased oxidative stress crucial to the pathophysiological mechanisms of the aging process.

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PII-B3-5

ANIMAL GASTROINTESTINAL FUNCTIONAL TESTS AS USEFUL TOOL IN PRECLINICAL DRUG DEVELOPMENT

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Introduction: Oral drug application represents the most convenient delivery route for the majority of medications used in various therapeutic areas. The physiology and function of the gastrointestinal tract are shown to be crucial factors influencing drug absorption and bioavailability. In the present study, clinically approved tests of gastric function: acid secretion, gastric emptying and intestinal permeability were validated on CD rats treated with selected clinically relevant drugs.

Materials and Methods: All experiments were performed on male 6-8 weeks old CD(SD) rats (n = 6-8). Acute pyloric ligation model was used to evaluate an inhibitory effect of oral omeprazole pretreatment on gastric acid secretion. Intestinal barrier permeability was assessed by determining the ability of fluorescein isothiocyanate-dextran (FITC-dextran) to cross from the lumen into circulation in indomethacin-induced intestinal injury model. Evaluation of gastric emptying was performed through timecourse of paracetamol absorption following oral administration.

Results: The analysis of gastric juice acidity showed a significant reduction in gastric acid output following pyloric ligation in the group receiving omeprazole therapy (20 mg/kg). Intestinal permeability was higher in animals subjected to indomethacin-induced intestinal injury (30 mg/kg). In contrast to oral metoclopramide treatment (30 mg/kg) which accelerated gastric emptying (GE), paracetamol plasma levels were significantly lower in loperamide treated group suggesting a delayed GE.

Conclusion: The implementation of validated gastric function tests into preclinical pharmacology models could be a valuable tool for the development of oral drugs and optimization of their delivery systems.

PII-B3-6
CRKL EXPRESSION IN THE DEVELOPING HUMAN KIDNEYS

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Introduction: The CRK like proto-oncogene, adaptor protein (CRKL) has been previously linked to many signaling pathways in a wide variety of normal and diseased tissues. It is downstream of Dab1 in the reelin signaling pathway which has diverse functions in the development of many organs including kidneys. The encoded protein regulates intracellular signaling transduction from multiple growth factors, which are essential for the normal development of the kidneys. The objective of this study was to explore the spatio-temporal expression pattern of the CRKL protein in developing human kidneys.

Materials and Methods: Fetal kidney tissue embedded in paraffin between the 13/14th and 30th developmental weeks (dw) was stained with CRKL antibodies and distal tubule markers by double immunofluorescence. The collected data was analyzed by the Kruskal Wallis test.

Results: Between the various developmental periods analysed, there was a statistically significant difference in the temporal expression patterns of glomeruli ($P<0.01$). As the weeks of development progress, the extent and intensity of fluorescence increase. Additionally, there was a notable difference in expression in all weeks analysed between convoluted tubules (proximal and distal) and glomeruli ($P<0.001$). The expression in the glomeruli was less intense in comparison to their accompanying convoluted tubules. The expression of CRKL was mild in all kidney structures.

Conclusion: CRKL expression is an important factor in providing normal human kidney development. Further studys are nescesary to establish the role of CRKL in the Reelin/Dab1 pathway.

PII-O2-1

WEIGHT MANAGEMENT PROGRAM - IMPORTANCE OF PHARMACY CONSULTATION, PHYSICAL ACTIVITY AND REGULAR NUTRITION IN THE PREVENTION OF OBESITY

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Introduction: Croatia is ranked eighth among the member states of the European Union by the number of obese people, 18.7% of people in Croatia are obese. Of particular concern is the high prevalence of obesity in children (11.2% - fifth rank). The aim of study was to examine the importance of pharmacy consultation and the correlation between physical activity and nutrition in the improvement of health and to confirm that people with the right nutritional habits are more likely to avoid sedentary lifestyle.

Materials and Methods: Subjects were at the age 18-45 years, regular users of physiotherapy services to improve the overall health status. The sample was random and included 320 subjects, of which 110 men and 210 women. An anonymous questionnaire of 25 questions was used and contained questions on age, gender, body weight and height, nutritional, sports and other lifestyle habits.

Results: Subjects with regular physical activity had lower BMI. The majority of respondents regularly consumed vegetables and grains, and rarely fast food and sweets. Respondents are mainly engaged in physical activity several times a week, usually in order to preserve and improve health.

Conclusion: Respondents with regular physical activit rarely choose fast and calorie foods and prefer proper and balanced diet, which results in normal BMI. This study represents a guide map for the development of a comprehensive program adapted to the everyday use in community pharmacy practice and family medicine, which can be used by physicians, pharmacists, nutritionists and physiotherapists to prevent obesity and cardiovascular risk factors.

PII-O2-2

IMPACT OF THE COMPREHENSIVE MEDICATION MANAGEMENT SERVICES ON THE CLINICAL STATUS IN PATIENTS WITH HYPERTENSION IN THE HEALTH CENTRE ZAGREB – CENTRE

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Introduction: In 2016 cardiovascular diseases were the main cause of death globally, with hypertension as the leading risk factor. This study aims to evaluate the clinical impact of Comprehensive Medication Management (CMM) services provided to patients with chronic diseases on blood pressure as the main outcome measure.

Materials and Methods: A prospective, observational study was conducted with patients followed-up for 14 months at the primary care clinic, Health Centre Zagreb – Centre (HCZC). Patients with chronic conditions taking multiple medications were being referred to the pharmacist by their general practitioner (GP). Identified drug therapy problems (DTPs) were classified according to the Pharmacotherapy Workup method proposed by Cipolle et al (2012).

Results: Overall, 81 patients were included in the study, of which 51 (63%) were female, with a median age (range) of 71 (32 - 87) years. On average, patients used 8 (2 - 19) medications and had 5 (1 - 11) comorbidities. Hypertension was the most frequent medical condition (56.8%). The total number of 229 DTPs were identified with an average of 2.8 DTPs (± 1.7) per patient, of which 21.4% pertained to hypertension. Patients with hypertension and at least two consultations (N=46) were taken into consideration. Out of them, only 28.3% reached positive clinical outcomes at the first consultation. At the last consultation, the percentage of hypertensive patients with improved clinical status was significantly higher (63.0%) ($p<0.001$).

Conclusion: This study showed positive impact of CMM services on patients' blood pressure, thus implying an important role pharmacists have in the care of polymedicated patients with hypertension.

PII-O2-3

PATIENT'S READING PATTERN AND LEVEL OF SATISFACTION WITH PACKAGE LEAFLETS IN CROATIA

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Introduction: In EU Package Leaflet (PL) is the pivotal source of regulated and scientifically validated information intended for patients, written in a user friendly way. PL should be easily read with clear wording to convey the right information to the patients. PLs follow so called "QRD format", with specially defined chapters and wording and should also undergo "readability user testing" with specified target group. In order to ensure patients receive the right information, test of satisfaction with current PL format has, as well as general habits of reading PLs been performed on 53 volunteers.

Materials and Methods: Standardized questionnaire with 11 pre-defined questions (YES/NO and pick lists) and one with free text entry field (for comments) has been developed and uploaded as on-line survey to employees and students working for Bayer d.o.o., Croatia during March 2018. Survey was anonymous.

Results: In this poster we discuss findings obtained from on-line survey, carried out on 53 subjects (70% female and 30% male subjects, aged 18+, with different educational level). Majority of subjects don't read the whole PLs, but only those chapters they are particularly interested in (posology, adverse events, special warnings/ contraindications/ interactions). Also, majority of subjects find PLs in general too long. It has been found, that key information should be additionally summarized and emphasized and majority of subjects see e-format as advance. Furthermore, special attention should be brought for long-term therapy since revised information could not be easily identified.

Conclusions: Subjects were generally satisfied with current PL format and receive the right message, although there is a room for improvement (length, key information). With development of digital PLs majority of these observations will be overcome.

PII-O2-4

USE OF ADDICTIVE SUPSTANCES IN HIGH SCHOOL AND STUDENT POPULATION IN THE SIBENIK KNIN COUNTY

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Introduction: Substance abuse is a serious health and social problem in Croatia. An additional problem is easier access to addictive substances and the emergence of new drugs. We studied the type and frequency of consumption of addictive substances, as well as psychological and social factors related to substance abuse. Our objective was to determine the prevalence of risk behaviours, including smoking, alcohol and psychoactive drug consumption, gambling and Internet addiction among students, as well as the characteristics of the respondents.

Materials and Methods: The survey was conducted among 773 high school students and students in Sibenik-Knin County using an anonymous questionnaire consisting of 46 questions.

Results: 36.5% of the students smoke cigarettes. Marijuana is consumed by 16.5% of the students. 86% of the students sometimes consume alcohol or drink at least once a week. Girls smoke more cigarettes, whereas boys consume more alcohol. They spend 4 or more hours a day on the Internet; girls more than boys. Gambling was more prevalent among boys than girls. They are satisfied and happy about their lives, but worried about their future.

Conclusion: It is necessary to continue tracking data and developing a comprehensive program of health promotion and drug prevention as well as an effective strategy to prevent risk behaviours. The data obtained are useful for the purpose of further planning and development of systems for prevention and protection of young people against addiction. What we should definitely pay attention to is the emergence and spread of new types of addiction.

PII-O2-5
REPORTING OF OVER-THE-COUNTER DRUGS ADVERSE DRUG REACTIONS IN 2018

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Introduction: A growing popularity of self-med bication process has been acknowledged. This trend could be explained due to the fact that it has several advantages for both consumer and health care professionals. However, over-the-counter (OTC) drug use could also result with adverse drug reactions and these reactions should be reported to national authority.

Materials and Methods: This was a cross sectional study containing data on reported adverse drug reactions from Agency for Medicinal Products and Medical devices of Croatia (HALMED). The Croatian drug database (available online) was searched before requesting HALMED for the data reports. Inclusion criteria for database search was OTC drug, and it consisted of both analgesic and non-analgesic drugs marketed in Croatia. In 2019 overall 466 drugs were registered as OTC drugs, and were included in this study. Adverse drug reaction reports of OTC drugs from 1 January to 31 December 2018 were obtained. The following reports data were analysed: reporter qualification, patient gender and age, seriousness and active substance name.

Results: Number of adverse drug reaction reports during the examined period was 165. The adverse drug reactions were most frequently reported by pharmacists, followed by consumers. Fifty-three percent of all reports were classified as serious. Most commonly reported drugs were ibuprofen, paracetamol and Hedera helix. Accidental exposures to product by child was reported in 26 cases of adverse drug reaction.

Conclusion: The major risk associated with OTC drug consumption in 2018 was accidental exposures to products by child, and awareness of childproofing mechanisms should be raised.

POSTER SEKCIJA III - STUDENSTKA POSTER SEKCIJA S ORGANIZIRANOM DISKUSIJOM /
POSTER SESSION III - STUDENT POSTER SESSION WITH ORGANIZED DISCUSSION:

EKSPERIMENTALNA FARMAKOLOGIJA 1 / EXPERIMENTAL PHARMACOLOGY 1 (PIII-SE: 1-10)
OSTALE TEME U FARMAKOLOGIJI / OTHER TOPICS IN PHARMACOLOGY (PIII-SO: 1-10)

SUBOTA, 28. RUJNA 2019. / SATURDAY, 28 SEPTEMBER 2019

10:15 – 11:15

PIII-SE-01
ACUTE EFFECTS OF INTRANASAL INSULIN IN THE BRAIN OF RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Introduction: Sporadic Alzheimer's disease (sAD) is a common neurodegenerative disease characterized by central insulin resistance, glucose hypometabolism, amyloid plaques and abnormal neurofibrillary tangles. Central administration of streptozotocin intracerebroventricularly (STZ-icv) has been shown to induce Alzheimer-like changes and STZ-icv rat model has been proposed as a non-transgenic sAD animal model. Intranasal insulin administration (INS) is being investigated as a possible pharmacological treatment to improve brain insulin dysfunction, but exact mechanism is not yet clear.

Materials and Methods: Activation of c-fos was evaluated 2 hours after INS in the brain of STZ-icv rat model of sAD. Immunofluorescent analysis of c-fos activity was colocalized with neuronal, astrocytic and microglial markers and western blot analyses of synaptic and metabolic markers were performed in hypothalamus (HPT) and temporal cortex (TC) 2h after INS administration in STZ-icv rat model. Spectrophotometry was used to measure glucose in plasma and CSF and β-amyloid concentration in plasma samples (ELISA).

Results: Intranasal insulin significantly increases c-fos expression in TC only. Importantly, most of c-fos in TC was located in neurons whereas microglia were the main source for c-fos expression in HPT. When metabolic and synaptic changes in HPT and TC were analyzed it was found that STZ and INS treatments have different and opposite effects in these two brain regions. Plasma and CSF glucose levels were unaltered after INS.

Conclusion: In this research, we have shown that STZ and insulin have different and opposite effects on metabolic and synaptic changes in HPT compared to TC in rat model of sAD.

This work has been supported in part by Croatian Science Foundation under the project (IP-2018-01-8938 and IP-09-2014-4639). Co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007 funded by the European Union through the European Regional Development Fund).

PIII-SE-02
ORAL CONTRACEPTIVES THERAPY IN POLYCYSTIC OVARY SYNDROME: THE EFFECTS ON HORMONAL STATUS AND OVARIAN RESERVE

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Introduction: Our aim was to show whether three months of therapy with oral contraceptives changed the hormonal status of patients with polycystic ovary syndrome (PCOS). The concentration of Anti-Müllerian hormone (AMH), as one of the parameters of ovarian reserve, was also measured.

Materials and Methods: The study included 19 patients with PCOS and clinical/laboratory signs of hyperandrogenism without additional comorbidity and therapy. All participants were treated with the same therapy (a fixed combination of ethinyl estradiol (0.035 mg) and cyproterone acetate (2 mg)). The main outcome measures were concentrations of reproductive hormones measured before the therapy and during the first menstrual cycle after the end of the therapy, as well as age, body height, weight and body mass index. Hormone concentrations were analyzed by the immunochemical electrochemiluminiscent method (ECLIA).

Results: Mean age of the participants was 29 years, body weight 69 kg, and body mass index 24.2 kg / m^2. Initial concentrations of total and free testosterone, and AMH were elevated while those of other reproductive hormones were within reference range. Due to the treatment, AMH, luteinizing hormone and estradiol concentration decreased by 20% and free testosterone by more than 85%. Expectedly, the concentration of sex-hormone binding globulin increased by 40%.

Conclusions: Based on our results, three months of oral contraceptives therapy induced changes in the concentration of reproductive hormones and apparently slightly reduced the ovarian reserve. However, for better assessment of ovarian reserve, further research based on a longer-term monitoring of the patients' hormonal status with PCOS is needed.

PIII-SE-03
ANTIMICROBIAL ACTIVITY OF SELECTED RED AND WHITE WINES AGAINST *ESCHERICHIA COLI* IN MARINATED FISH FILLETS

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Five different wines (standard Grasevina, macerated Grasevina with and without sulphites, rosé and standard Plavac Mali), all typical Croatian wines, were tested to determine the antimicrobial activity against *Escherichia coli* (ATCC® 25922) using sea bass (*Dicentrarchuslabrax*) fillets as food matrix. Fish fillets were marinated for two hours in a 1:1 wine:water mixture. Sterile water was used for the control. The filets were minced and the *E. coli* inoculum (7 log CFU/25g) was added before the samples were vacuum packed and refrigerated. Enumeration of the bacteria was done on days three and seven, after 24 hrs incubation on Tryptone Bile X-GLUC (TBX) agar. The minimal inhibitory concentration (MIC) and total phenolic (TP) content of the wine samples were determined. The highest number of E. coli was detected in the control sample (6.81 and 7.22 log CFU/25g), while the lowest counts of 4.95 and 6.04 log CFU/25g were detected in fillets marinated in macerated Grasevina (MIC 1284mg GAE/L, TP = 2699 mg GAE/L) after three and seven days, respectively. Addition of sulphites in macerated white wine did not enhance antimicrobial effect. Samples marinated in rose showed bacterial growth from 5.46 to 6.73 log CFU/25g. Standard Grasevina and standard Plavac Mali had a similar effect on the E. coli after three days (5.32 and 5.42 log CFU/25g), with Plavac Mali showing reduced growth after seven days. The most prominent inhibitory effect was detected for macerated Grasevina and standard Plavac mali, as these wines resulted in the lowest MIC and TP values.

This study was supported by the Croatian Science Foundation (Project No. 8652).

PIII-SE-04
THE EFFECT OF SMOKING ON SERUM LEVELS OF OLANZAPINE IN PATIENTS WITH SCHIZOPHRENIA

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Introduction: Olanzapine is atypical antipsychotic effective in treatment of symptoms of schizophrenia. Except of oral administration, olanzapine is often administered parenterally as a long-acting injectable formulation in order to improve patient adherence and faster action. Its extensive metabolism with smoking-inducible cytochrome P450 (CYP) 1A2 reduces serum levels of olanzapine in smokers. Prevalence of smoking in psychiatric patients is more than 60%. It is also known that men have higher clearance of olanzapine. The aim of this study was to examine effect of smoking, gender and administration route on serum levels of olanzapine in schizophrenic patients.

Materials and methods: This study included 58 patients with schizophrenia; 65,5% are smokers and 34,5% are nonsmokers; 70,7% are men and 29,3% are women; 70,7% receive i.m. olanzapine and 29,3% receive p.o. olanzapine. HPLC-DAD method was used to determine serum levels of olanzapine. For statistical analysis, Microsoft Excel 2013 and MedCalc were used.

Results: There were no statistically significant differences found between men and women for olanzapine serum concentration (P=0,682). No statistically significant difference was also found between p.o and i.m. group for olanzapine serum concentration (P=0,066) and between smokers and nonsmokers for olanzapine serum concentration (P=0,071).

Conclusions: Although there were no statistically significant differences between studied groups, it is recommended to expand the study with more patients and more equally distributed groups. P values for smoking status and route of drug administration were close to significant (P=0,050) meaning that this could affect the therapeutic response in schizophrenics.

PIII-SE-05
DOSE-DEPENDENT EFFECTS OF GLUCAGON-LIKE PEPTIDE-1 RECEPTOR INHIBITOR ON THE SIGNALING PATHWAYS IN THE RAT HIPPOCAMPUS

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Introduction: Previous research in a rat model of sporadic Alzheimer's disease (sAD) suggested that oral galactose-induced normalization of cognitive deficits is mediated by a glucagon-like peptide-1 (GLP-1)1,2. The activation of the central GLP-1 receptors (GLP-1R) plays a role in neuroprotection3 but the exact signalization pathways of GLP-1 in the brain and the consequences of its inhibition are still not fully understood. We aimed to determine changes in the hippocampal (HPC) expression of c-fos, insulin receptor (IR), extracellular signal-regulated kinase (ERK) and its phosphorylated form (p-ERK) after the intracerebroventricular (icv) administration of different doses of GLP-1R inhibitor exendin 9 (Ex9) in healthy rats.

Materials and methods: Male Wistar rats were icv injected with Ex9 (60, 85, and 125 μg/kg) or vehicle (controls) and sacrificed 20 minutes after the treatment. Protein expression of c-fos, IR, ERK and pERK in HPC was measured by Western blot. Data were analysed by Kruskal-Wallis and Mann-Whitney U test ($p<0.05$).

Results: The dose of 60 μg/kg Ex 9 had no effects on the expression of investigated proteins. The dose of 85μg/kg decreased the expression of c-fos (-58%) and IR (-56%) while the dose of 125μg/kg Ex9 increased the ratio of pERK/tERK (+85%).

Conclusion: The icv administration of GLP-1R inhibitor Ex9 induces dose-dependent changes in neuronal signaling pathways in rat HPC. Medium dose –induced effects indicates a reduction of both energy metabolism and neuronal activation (decreased IR and c-fos respectively), while even higher inhibition of GLP-1R (125μg/kg of Ex9) seems to point to the increased apoptosis (increased pERK/tERK ratio). Further research is needed to elucidate the dose-dependent metabolic and apoptotic consequences of GLP-1R inhibition in the rat brain.

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PIII-SE-06
INFLUENCE OF PIOGLITAZONE ON THE MICROGLIAL REACTION AND SOME MARKERS OF INFLAMMATION AND CELL STRESS IN THE RAT HIPPOCAMPUS FOLLOWING LATERAL FLUID PERCUSSION INJURY

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Introduction: One of the leading causes of death and disability throughout the world, traumatic brain injury (TBI), still lacks clinically effective neuroprotective therapy. Previously it was found that in the TBI in rats, application of a single dose of pioglitazone, a peroxisome proliferator-activated receptor-γ (PPARγ) agonist, had some limited beneficial effects on trauma-induced oxidative hippocampal damage and neurodegeneration. The aim of this study was to examine the effects of pioglitazone, applied twice daily, on the microglial activation and the expressions of inflammation (COX-2) and cell stress (HSP70) markers in the rat hippocampus following lateral fluid percussion injury (LFPI).

Materials and methods: Moderate brain trauma was performed over the left parietal cortex by the LFPI method in adult male Wistar rats. Pioglitazone or vehicle were administered i.p. 10 min and 12 h after the injury. Sham-operated, vehicle-treated animals were used as the control group. Rats were sacrificed 24 h after TBI and their hippocampi were prepared for the histological and western blotting analyses.

Results: Pioglitazone significantly decreased TBI-induced microglial activation in the dentate gyrus of the hippocampus. TBI caused an increase in the expressions of COX-2 and HSP70, but the applied PPARγ agonist did not affect the levels of these proteins.

Conclusions: Our study showed that pioglitazone, applied twice daily, reduced microglial reaction in the hippocampus after TBI in the rat, but did not influence the levels of measured inflammatory and cell stress markers.

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PIII-SE-07
INFLUENCE OF SALIVA ON THE RESULTS OF LABORATORY COAGULATION TESTS

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Introduction: Oral wounds inevitably come into contact with saliva which can affect time needed for bleeding to stop. Its effect can be nonspecific, related to dilution of blood, or, potentially, mediated by salivary factors that affect haemostasis directly. The aim of this study was to examine if mixing blood with individual's saliva would influence the rate of its coagulation measured by global haemostatic tests, prothrombin time (PT) and activated partial thromboplastin time (aPTT).

Materials and Methods: The study included 30 healthy nonsmoking volunteers who maintain good oral hygiene and health. Paired blood and unstimulated saliva samples were obtained from each study participant and PT and aPTT were determined in blood, blood+saliva and blood+water mixtures. Coagulation tests were performed using the mechanical clot detection method.

Results: PT was significantly longer and PT% significantly lower in both blood+saliva and blood+water mixtures compared to blood alone. However, aPTT was significantly longer only in blood+water mixture compared to blood.

Conclusions: Equally prolonged PT in both mixtures could suggest that both saliva and water prolong coagulation evenly due to their nonspecific effect of blood dilution (i.e. dilution of blood procoagulants). However, the finding that aPTT was significantly prolonged only when blood was mixed with water could indicate presence of tissue factor (TF) in saliva. Its concentration is likely too low to influence the results of PT. However, as reagents for performing aPTT do not contain recombinant TF, salivary TF could be an additional factor in stimulating coagulation thereby counteracting the anticoagulant effect of dilution.

PIII-SE-08
EFFECT OF RENAL FUNCTION ON SERUM CONCENTRATIONS OF LEVETIRACETAM IN TREATED PATIENTS

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Introduction: Therapeutic drug monitoring of levetiracetam, the adjunctive drug indicated for partial epileptic seizures, might seem unnecessary due to its linear pharmacokinetics, low variability and probability of interaction with other drugs. However, previous studies have demonstrated some sources of pharmacological variability: age, induction effects of other anticonvulsants or comedications and renal dysfunction. Therefore, relative influence of renal function on patients' serum drug concentration has been checked experimentally in order to adjust the dosage since both too low and too high concentrations lead to the same consequence (uncontrolled seizures).

Materials and methods: The routine analytical method of high performance liquid chromatography (HPLC) and the photodiode detector system was used for quantification of serum levetiracetam concentrations in randomly selected patients (N=78) with levetiracetam in their therapy. The dosing regimen was monitored for each patient, as well as their biochemical parameters: creatinine, urea as renal function indicators; aspartate and alanine aminotransferase (AST, ALT) catalytic concentrations as liver function indicators.

Results: Based on the evaluated data and calculated linear regression model, statistically significant correlation was observed between some biochemical parameters and concentration/dose ratio of levetiracetam. The reported correlation with the renal parameters is statistically significant for creatinine and urea ($r=0.37, P=0.001$ and $r=0.36, P=0.002$ respectively, significance value $\alpha=0.05$), but statistically insignificant with the liver enzyme AST and ALT ($P=0.198$, $P=0.163$ respectively).

Conclusion: Insignificant correlation between levetiracetam serum levels and liver enzyme, but significant correlation with creatinine and urea is the evidence of levetiracetam pharmacokinetic dependence on kidney function. Patients with reduced renal function should be placed under special surveillance and dosage adjustment.

PIII-SE-09
COMPARISON OF FLUORIDE ION RELEASE FROM FLUORIDE GEL IN VARIOUS SOLVENTS

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Introduction: High concentration fluoride gels are used for caries prevention and therapy. The aim of this study was to determine the concentration of fluoride ions in various solutions of fluoride gels pertaining to the label amount of fluoride concentration.

Materials and methods: Two fluoride gels containing different active ingredients were used in this study. Mirafluor gel with sodium fluoride and Elmex gel with amine fluorides and sodium fluoride. Solvents used in this study were distilled water, redistilled water, tap water, 0.9% sodium chloride solution and the artificial saliva product Axerosta. Pure solvents samples were prepared, as well as one gel solution per each particular solvent. Next, the amount of fluoride ion (F^-) was determined by use of ion-selective electrode (ISO 19448:2018 standard).

Results: F^- ion concentrations in pure solvent samples were negligibly low and could not increase F^- ion concentrations in fluoride gels solutions. The differences between F^- ion release in various solvents were statistically significant (ANOVA, $p < 0.05$). The F^- ion concentrations measured in both gels' redistilled water solutions were higher than the label concentration. There was a significant difference in fluoride ion release in every Mirafluor gel solution except between distilled and redistilled water solutions. Fluoride ion release was significantly different in every Elmex gel solution except between distilled and redistilled water solutions and between redistilled water and artificial saliva Axerosta solutions.

Conclusions: Amine fluorides do not interact with other ions present in the solution, whereas F^- ion release from sodium fluoride alters when other ions are dissolved in the same solution.

PIII-SE-10
TRANS-SYNAPTIC ACTIONS OF BOTULINUM TOXIN AFTER INTRAMUSCULAR APPLICATION

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Introduction: Botulinum neurotoxin type A (BoNT/A) is used for treatment of spasticity and hyperkinetic movement disorders. It was recently discovered that BoNT/A may induce central antispastic effects in rats due to its retrograde axonal transport and transcytosis. In present study we examined the BoNT/A peripheral muscular vs central spinal effect on abnormal muscle tone during extended observation period.

Materials and methods: Tetanus toxin (TeNT) (2 ng) injection into rat gastrocnemius induced a reversible spastic paralysis lasting for 3 weeks. After induction of muscle spasm, BoNT/A (5 U / kg) was applied i.m. into the spastic muscle. Its central effects were evaluated by lumbar intrathecal (i.t.) application of BoNT/A-neutralising antitoxin. Behavioral motor effects were studied by Basso Beattie Bresnahan (BBB) gait scoring, digit abduction score (DAS) and resistance to passive ankle dorsiflexion. Long-term BoNT/A effects were studied by repeated TeNT-evoked spasticity (7 and 13 weeks after BoNT/A).

Results: The local spasticity of the muscle, characterized by sustained rigid hind-paw extension and resistance to passive ankle flexion, was reduced by i.m. BoNT/A which lasted throughout the duration of first TeNT-evoked spasm. During the period of BoNT/A-evoked flaccid paralysis, central effects did not influence the antispastic action of i.m. BoNT/A. However, upon second induction of spasticity by TeNT (35d post BoNT/A), the rats treated i.t. with antitoxin that prevented central toxin effects displayed a smaller reduction of spasticity. Upon third TeNT injection, the animals injected with BoNT/A still exhibited lower magnitude of spastic paralysis, apparently due to a lasting muscle atrophy.

Conclusion: BoNT/A effects on abnormal muscle tone were influenced by a.) peripheral flaccid paralysis; b.) central effects which became apparent after the completion of peripheral flaccid paralysis; and c.) by muscle atrophy which outlasted both peripheral and central toxin effects.

PIII-SO-01
ABILIFY MYCITE-FIRST HIGH-TECH DRUG

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Introduction: Abilify Mycite system is a drug-device combination product comprised of oral aripiprazole tablets (*Otsuka Pharmaceutical)* embedded with an ingestible sensor (*Proteus Digital Health)*, which allows tracking if medicine was taken. This digital medicine system (DMS) has been approved by FDA in 2017 for treatment of schizophrenia and bipolar I disorder and as adjunctive therapy for major depressive disorder.

Materials and Methods: PubMed and Google databases were searched in order to assess Abilify Mycite's safety, effectiveness, detection accuracy, latency period between ingestion and detection, and usability rate. Alongside with scientific literature and reports, clinical trial data used to support the FDA's approval has been also reviewed.

Results: Studies demonstrated 96,6% ingestion detection rate and 1.1-1.3 min latency period between ingestion and detection. 81,2% of patients successfully replaced wearable sensor and 78% patients were satisfied with DMS. 783 out of 805 performance tasks attempts were successful, there was one close call, 15 failures and 6 difficulties occurred. Difference between digital and non-digital formulation of medicine is yet to be determined. No study provided data on remission, quality of life or any efficacy outcome.

Conclusions: DMS has the capability to detect and report tablet ingestion with high accuracy and acceptable latency time. High proportion of patients was able to use DMS and were satisfied. However, regulatory approval was permitted without evidence of better adherence of digital version of aripiprazole in comparison to non-digital. Furthermore, scientific literature and news reports conveyed an unsupported impression of clinical benefit.

PIII-SO-02
MUSIC AS MEDICINE

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Introduction: Music is a very complex stimulus, with many different psychological and physiological effects. Although the ways it works on us remain largely elusive, music has been recommended as therapeutic tool for a variety of physical, psychological, cognitive, as well as social problems. In order to get a better understanding of music as medicine, we have reviewed a number of articles and collected information about music medical usage in the treatment of various disorders and conditions.

Materials and Methods: PubMed base has been searched for English-language articles of interest. Selected topics associated with music included stress, anxiety and depression, sleep disorders, as well as neurorehabilitation with focus on specific medical conditions.

Results: Various studies demonstrated that listening to music can improve sleep, by promoting relaxation or serving as a distraction, and it has been recommended for treatment of both acute and chronic sleep disorders. Music can be used to alleviate anxiety, depression and pain, or for motivation and energizing. Positive influence of music on concentration, mood, stress reduction, as well as heart rate and blood pressure, have been also reported. In addition, various subtypes of music therapy can be a useful tool in neurorehabilitation of disorders such as stroke, Alzheimer's and Parkinson's disease, due to beneficial effects on patient's motor, cognitive and psychological disturbances.

Conclusion: Unlike drugs or medical procedures, music needs no government approval or clinical trials— it is inexpensive and simple way to improve the quality of people's lives and cannot hurt even if it fails to help.

PIII-SO-03
ARE DIGITAL DRUGS ACTUALLY DRUGS?

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Introduction: Digital drugs or binaural beats represent auditory phenomenon perceived by the listener when two tones with slightly different frequencies are provided separately to each ear. It has been suggested that they positively affect anxiety reduction, memory, attention, pain sensation and overall quality of life. However, although digital drugs are gaining popularity, especially due to their Internet availability, there are some controversial claims about their potential abuse for euphoric and high feelings, and possible negative effects.

Materials and methods: PubMed database was searched for English-language articles by using "binaural beats" as keyword. No time or article type constraints were applied.

Results: Literature search yielded 89 results, including a review of 30 selected articles and recent meta-analysis of 22 studies. Search revealed contradictory outcomes reported in the literature and suggested that many factors (frequency, exposure duration and timing, age, gender, additional stimuli) influence binaural beats efficacy. However, there is growing evidence that binaural beats affect cognition over and above reducing anxiety and pain perception. Four individual articles investigating the effects of binaural beats on memory, attention, anxiety and pain perception were selected as examples for presentation.

Conclusions: Exposure to digital drugs can be an effective way to positively affect memory, attention, reduction in anxiety and pain perception, which correlate with increased quality of life. However, further research, including larger number of participants, more accurate reporting of stimulation parameters and experimental protocols, especially in clinical studies, is needed in order to clarify the most promising effects, and to elucidate underlying neural mechanisms.

PIII-SO-04
DENTISTRY STUDENTS' KNOWLEDGE AND AWARENESS ABOUT THE USE OF ANTIBIOTICS IN DENTAL MEDICINE

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Introduction: Microorganisms' antibiotics resistance is significantly increasing and the use of antibiotics in dental medicine has, in smaller proportions, contributed to this. The purpose of this research was to establish knowledge about antibiotics among 3rd and 6th year students of School of Dental Medicine University in Zagreb.

Materials and methods: The research included 106 participants. 48% were third-year and 52% were sixth-year students. The research was conducted based on a 25 questions questionnaire designed specifically for the purpose of this research. The questions were divided based on their difficulty and theoretical or practical knowledge. Questions on self-assessment were separated part of the questionnaire.

Results: The analysis of the results showed that sixth-year students were more successful in completing the questionnaire (67%) than third-year (59%). The most significant difference occured in the area of practical knowledge, with sixth-year students answering better (67%). Theoretical knowledge was similar between sixth and third year students. The participants assessed their knowledge as good and very good. Some of the areas that students think they are not informed enough, include the guidelines for antibiotics prescribing, right dosage of antibiotics depending on patient's symptoms, the mechanism of drug action, as well as written prescribing.

Conclusion: By analysing the results of the research, it can be concluded that the students' knowledge and awareness should be on a higher level. These findings show that there is a need for additional education, as a part of School of Dental Medicine's courses, and through life with the focus on practical usage.

PIII-SO-05
DO GRADUATING DENTAL STUDENTS FEAR THEIR FUTURE PATIENTS ON ANTITHROMBOTIC THERAPY?

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Introduction: Patients undergoing oral antithrombotic therapy have a reduced ability to form clots. Consequently, invasive dental procedures in these patients might lead to heavier bleeding. The aim of this study was to investigate whether Croatian final-year dental students experience discomfort when treating patients undergoing antithrombotic therapy and if the discomfort would influence their decision to provide care to these patients independently after graduation.

Participants and Methods: Final-year dental students from Zagreb, Split and Rijeka were invited to participate in the study by filling in our online questionnaire regarding, among other, self-assessed knowledge of these drugs, experience in managing patients on antithrombotics, and readiness to provide care to them independently.

Results: We received 77 filled questionnaires. All students have been in contact with patients undergoing antithrombotic therapy. Despite a poor self-assessed knowledge of the latest drugs among antiplatelet agents and novel oral anticoagulants, students expressed a high degree of willingness to provide care to patients who use antithrombotics without fear or in spite of it. Thereby, all students consider that it is important to consult with patients' physician in all or in some cases. Lower levels of stress were associated with better knowledge of medical indications for antithrombotic therapy, greater self-confidence in performing specific dental procedures, and better knowledge of other factors that can be related to the risk of prolonged bleeding after dental procedures.

Conclusions: Croatian final-year dental students mostly feel prepared to manage patients undergoing antithrombotic therapy on their own after graduation, whether it provokes anxiety or not.

PIII-SO-06
ADVERSE DRUG REACTIONS OF TYROSINE KINASE INHIBITORS APPROVED FOR TREATMENT OF NON-SMALL CELL LUNG CANCER

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Introduction: The identification of oncogenic activation of particular tyrosine kinases in some advanced non-small cell lung cancer (NSCLC) tumors, especially mutations in the epidermal growth factor receptor (EGFR) or rearrangements of the anaplastic lymphoma kinase (ALK) gene has led to a paradigm shift and the development of specific molecular treatments for patients. The aim of our study is to describe adverse drug reactions of tyrosine kinase inhibitors (TKI) approved only for the treatment of NSCLC reported to the Agency for Medicinal Products and Medical Devices of Croatia (HALMED).

Materials and Methods: All cases were reported to HALMED until June 23, 2019. Data was analyzed in respect to the total number of reports, demographic characteristics of the patients, System Organ Class MedRA Preferred Terms and suspected/interacting active compounds.

Results: HALMED received 188 reports related to NSCLC TKIs. Most patients belong to 45 – 64 age group (41.48 % of cases). The highest number of cases were reported for erlotinib and afatinib. Most ADRs belonged to the following System Organ Classes: Skin and subcutaneous tissue disorders (58.51%) and Gastrointestinal disorders (43.61%). Most frequently reported MedRA Preferred Terms were rash (39.89%) and diarrhea (33.51%). In the highest number of cases pantoprazole was reported as the suspected/interacting drug.

Conclusion: Our study described the reported ADRs of NSCLC TKIs in Croatia. The knowledge of ADRs enables prevention and adequate management of the ADRs of these important class of drugs.

PIII-SO-07
STUDENTS' PROJECT: OLIVESPIB (CROHUNTIN) – SELECTIVE CYTOSOLIC HSP90 ISOFORM INHIBITOR IN THERAPY OF HUNTINGTON'S DISEASE

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Introduction: Huntington's disease (HD) is a neurodegenerative, autosomal dominant disorder caused by trinucleotide CAG repeats on chromosome 4p16.3. The mutated form of protein huntingtin (Htt) accumulates, causing reactive gliosis and loss of neurons in striatum resulting in motor symptoms followed by cognitive deterioration. Key role in Htt pathway is attributed to HSP90, which prevents Htt intracellular degradation. This project aimed to develop and characterize selective inhibitors of a cytosolic isoform of HSP90.

Materials and methods: The selectivity of molecules was examined by measuring Ki values using the FPBA method. Potency (IC50) was examined on HEK cell line which expresses mHtt Q73 gene using immunochemical techniques. Pharmacokinetics and toxicokinetics were tested on Sprague-Dawley rats and C56BL/6 mice. To test effectiveness, R6/2 transgenic mice were subjected to RotaRod and Two-choice Swim Test and concentration of Htt was measured in the brain tissue and plasma (Western blot). Clinical studies followed after obtaining regulatory approval.

Results: Molecule 3 (olivespib) showed the best selectivity and potency (Ki = 9pM; IC= 22pM). Except for teratogenicity, it was negative in other toxicological screening tests. It showed an acceptable pharmacokinetic profile after oral application. In behavioral studies on transgenic mice, olivespib improved all tested parameters. Moreover, Htt brain concentration was reduced while number of astrocytes didn't change. Phase 1 clinical trials showed good tolerability after p.o. 10 mg dose. In Phase 2 olivespib lowered plasma Htt concentration in patients with HD. Additionally, improvements in cognitive and functional capacity were demonstrated using Mini-mental state examination and UHDRS scoring.

Conclusion: Olivespib is the first oral selective cytosolic HSP90 isoform inhibitor showing significant efficacy and good tolerability in the treatment of HD.

PIII-SO-08
INTRAORAL FLUORIDE DELIVERY SYSTEMS DURING ORTHODONTIC TREATMENT

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Introduction: There are different fluoride regimens aimed to reduce caries during orthodontic treatment with fixed appliances. Due to unpredictable compliance, there is a need for a long-term low-dose fluoride release delivery system. The aim of this study was to evaluate reported fluoride regimes in current use.

Materials and Methods: The literature search with keywords „fluoride release orthodontic"was reached through PubMed website (US National Library of Medicine, National Institute of Health). The literature search with keywords „polycaprolactone fluoride" was done through the same website, as well.

Results: A total of 132 published studies reported fluoride use during orthodontic treatment with fixed appliances. Twenty five studies were dealing with wire corrosion and ion release, reviews and other topics, and were excluded from further analysis. One hundred and seven studies used 6 different sources of fluoride for orthodontic treatment with fixed appliances. The most used were orthodontic adhesives (70%) followed by topical fluorides (10%), elastomeric ligatures (7%), fluoride sustained release devices (6%), removable appliances and retainers (5%) and coated wires or brackets (2%). The literature search on polycaprolactone fluoride revealed 18 relevant studies. Among those, there were stuides about developing new clinical applications of polycprolactone flouride, with existing laboratory methods for incorporation and release of fluorides in/from polycaprolactone flouride.

Conclusions: Intraoral fluoride release delivery systems take a small part in caries prevention. There is a need for improvement of existing and development of new fluoride release delivery systems. Polycaprolactone is promising material for sustained fluoride release.

PIII-SO-09
MEDICAL PRESCRIPTION PRACTICE OF DOCTORS OF DENTAL MEDICINE IN THE REPUBLIC OF CROATIA

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Introduction: The use of medicaments in dental clinical practice is common, due to frequent odontogenic infections, toothache (dental pain) and many other conditions (illnesses) that require medical treatment. Uncritical and unnecessary use of any medicament may result in multiple complications at a local and/or systematic level, such as antimicrobial resistance or drug interactions. The aim of this study was to show medical prescription practice in the Republic of Croatia in the period 2013 to 2017 by doctors of dental medicine.

Materials and methods: Data related to the use of drug prescription practice used in this research were delivered by the Croatian Health Insurance Institute, a national insurance company.

Results: The results demonstrated that antibiotics were the most frequently prescribed medicaments by dentists. Penicillin group were the most commonly prescribed class of antibiotics, especially amoxicillin with clavulanic acid. Lincosamides were also broadly used, especially clindamycin, followed by cephalosporines and metronidazole. Broad-spectrum antibiotics have been more used than narrow-spectrum ones, which ultimately leads to the increasing bacterial resistance to antibiotics. Other most often prescribed medicaments were the anti-inflammatory drugs, with ibuprofen as most frequent. The results also showed a small increase in prescribing corticosteroids, both for local and systematic use, as well as the increase in antifungal and antiviral medical treatments.

Conclusions: The Republic of Croatia is among the countries with a high rate of medicaments' consumption. In the period 2013 to 2017, trend of increase in medicaments prescription by doctors of dental medicine is observed.

PIII-SO-10
METABOLOMICS IN PHARMACOLOGY

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Introduction: Metabolomics is the emerging scientific discipline dealing with small (up to 1.5 kDa) molecules called metabolites. Metabolites, as the final products of gene regulation and protein expression, highly reflect environmental influence as well as different pathologies or internal changes in biochemical pathways of studied system. Development of a human disease is a complex process associated with the malfunctioning of cellular system ranging from genome to metabolism. Personal disease pathogenesis and therapy response are highly influenced by genetic background, but it is obvious that effects of environment and gut microorganisms should also be taken into account.

Materials and Methods: There are two approaches that can be used in metabolomics. Targeted, hypothesis driven, approaches have a low detection limit, but enable the absolute quantification of the sample. Untargeted, hypothesis generating, approaches enable the discovery of unknown compounds and provide a global view of a sample.

Results: Pharmacometabolomics could be, or already is, implemented in: a) individual drug-response prediction indicating the overall biochemical changes induced by a drug; b) evaluation of drug-response variability identifying metabolic pathways affected by a specific drug treatment; c) identification of biomarkers by profiling of drug-induced changes in metabolites or creating metabolite signatures of different disease subtypes; d) drug discovery and development by characterizing drug metabolism and pharmacology as well as their side-effects.

Conclusions: Pharmacometabolomics captures environmental and microbiome-level influences on response to drug therapy transforming pharmacology and clinical pharmacology and contributing to the development of personalized therapy.

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