P01.
POTENTIAL NEUROPROTECTIVE EFFECTS OF DEHYDROEPIANDROSTERONE SULFATE (DHEAS) AND BRAIN NEUROTROPHIC FACTOR (BDNF) *IN VITRO*

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Introduction: Neurosteroid dehydroepiandrosterone sulphate (DHEAS) and neurotrophin brain derived neurotrophic factor (BDNF) are involved in various brain functions, including neural plasticity, learning, memory and behavior. The aim of the project was to elucidate the potential effects of DHEAS and BDNF in preventing neuronal death, induced by ischemic injury, and promoting the survival of neurons by using the *in vitro* approach.
Materials and methods: Potential neuroprotective effects of DHEAS and BDNF have been evaluated using the human neuroblastoma cell line (SH-SY5Y) and oxygen-glucose deprivation/reperfusion (OGD/R) as an *in vitro* model of ischemic brain injury. For inducing OGD/R, SH-SY5Y cells in glucose- and serum- free medium were placed in a specially designed airproof modular incubation chamber for 24 h. Cell cultures were treated with increasing concentrations of DHEAS (10, 100 and 1000 nM) or BDNF (0.1, 1 and 10 ng/ml) for 24 h, immediately before (pre-treatment) or after (post-treatment) OGD. The potential protective effects of DHEAS and BDNF were determined by monitoring cell viability using trypan blue staining.
Results: One-way ANOVA, followed by Tukey's multiple comparisons test, demonstrated that OGD/R significantly decreased the viability of SH-SY5Y cells. Pre-treatment, as well as post-treatment with BDNF, was not able to counteract the effects of OGD/R. However, there was an insignificant trend suggesting potential dose-dependent protective action of BDNF pre-treatment. Pre-treatment with DHEAS showed no significant protective effects on SH-SY5Y cells exposed to OGD. On the other hand, post-treatment with 10 nM DHEAS significantly increased cell survival rate after OGD/R.
Conclusion: These preliminary data suggest potential protective effects of DHEAS and BDNF on SH-SY5Y cells exposed to OGD/R. However, further studies using longer treatment duration, as well as other *in vitro* and *in vivo* models, are needed in order to confirm neuroprotective role of DHEAS and BDNF and their potential use for treatment and prevention of ischemic brain injury.

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Project: Neurosteroids as therapeutic opportunities in ischemic brain injury; WWN/SFN collaborative research network program (CRNP), IBRO; PI: Svob Strac Dubravka, Zlatkovic Jelena, Krsnik Zeljka

P02.
THE INFLUENCE OF OXIDATION ON ANTIMICROBIAL EFFECT OF WINE

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Introduction: It often happens that the wine stays in the open bottle and is susceptible to oxidation that significantly changes its chemical composition[1]. However, there are no reports of biological effects of oxidized wine. The aims of the study were to determine antimicrobial effect of macerated white wine *Graševina* after spontaneous oxidation; to examine whether wine oxidation affects its biochemical composition and antioxidant effect *in vitro*, and to compare the changes in antimicrobial and antioxidant effects induced by wine oxidation.
Materials and methods: Polyphenols-rich, macerated white wine *Graševina*, Krauthaker winery, 2015., was produced by prolonged maceration in which white grape juice is fermented and left in extended contact (120 days) with hard parts of grape[2]. Oxidized wine was obtained after opening the bottle by exposing the wine to the air for 48 hours. The content of total phenolics and certain phenolic groups was determined spectrophotometrically. Antioxidant effects were measured by FRAP (Ferric reducing antioxidant power) method, and enological physical-chemical parameters by standard enological methods. The antimicrobial effect was investigated by inoculation of bacterial suspensions (*E. coli* and *S. enterica, serovar Enteritidis*) with wine on blood agar plates at pre-determined time intervals, in duplicate. After 24-hour incubation, visualization of bacterial colonies was performed until the plate with no apparent bacterial growth was found[3].
Results: Spontaneous oxidation significantly changed the content of total phenols and certain phenolic groups. The contents of total phenols, flavonoids and total flavanols were higher after oxidation. In contrast, oxidation reduced the content of monomeric flavanols and non-flavonoids. Both of the tested wine samples showed antibacterial effect against *E. coli* and *S. enterica, serovar Enteritidis*. The intact wine showed superior antibacterial activity relative to oxidized one against both bacterial species with incubation time that was different for each combination of wine and bacterial species.
Conclusion: Spontaneous oxidation of wine caused opposite changes in various biological effects; the antimicrobial effect decreased and antioxidant increased. These changes are somewhat related to changes in the biochemical composition of oxidized wine.

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Project: This work was supported by Croatian Science Foundation (HRZZ), project 8652 "Biological effects of wine: the influence of vinification technology, dealcoholization and aging of wine", project leader prof. Mladen Boban.

P03.
THE INFLUENCE OF PROLONGED MACERATION AS TECHNOLOGICAL PROCESS ON THE ANTIMICROBIAL EFFECT OF WHITE WINE

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Introduction: Antimicrobial effect is important biological property of wine. Wine phenolics have been anticipated to play a major role in this biological effect. In order to estimate the role of wine phenolics content on its antimicrobial effect, we compared standard and polyphenols-rich white wine obtained by prolonged maceration in which white grape juice is fermented and left in extended contact with hard parts of grape[1].
Materials and methods: The antimicrobial effect of wine was tested against two food-born bacteria, *E. coli* and *S. enterica*, serovar *Enteritidis*. Aliquots of 200 μL of the bacterial suspension were added to 3.8 mL of standard or macerated white wine (Graševina, Krauthaker winery, 2015) yielding an initial concentration of 10^5 to 10^6 colony forming units/mL. 0.01 mL of those suspensions were then inoculated on blood agar plates at pre-determined time intervals. After 24-hour incubation, visualization of bacterial colonies was performed until the plate with no apparent bacterial growth was found[2]. The content of total phenolics and certain phenolic groups were determined spectrophotometrically. Antioxidant effect was measured by FRAP (Ferric reducing antioxidant power) method[3], and enological physical- chemical parameters by standard enological methods.
Results: Concentration of total phenolics was almost ten times, and flavonoids over several hundred times less in the standard wine sample in comparison to the macerated one. Accordingly, macerated wine had stronger antioxidant effect. On the other hand, the macerated wine had lower antibacterial effect against both bacterial species with incubation time that was different for each combination of wine and bacterial species.
Conclusion: The technological process of extended maceration resulted in a wine of richer total phenolic, flavonoid and non-flavonoid content and stronger antioxidant activity. The macerated white wine showed weaker antibacterial effect against *E. coli* and *S. enterica*, serovar *Enteritidis*. Increase in total phenolic content did not improve antibacterial activity of white wine.

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Project: This work was supported by Croatian Science Foundation (HRZZ), project 8652 "Biological effects of wine: the influence of vinification technology, dealcoholization and aging of wine", project leader prof. Mladen Boban.

P04.
TRACING THE INSULIN SIGNALLING CHANGES IN OLFACTORY BULB OF THE RAT MODEL OF ALZHEIMER'S DISEASE

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Introduction: Olfactory dysfunction is one of the early symptoms in sporadic Alzheimer disease (sAD). The exact pathophysiological mechanism of olfactory dysfunction in sAD is not fully understood but literature data suggests that it might be associated with hyperphosphorilation of tau protein. Streptozotocin-intracerebroventriculary (STZ-icv) treated rats is recognized as a non-transgenic sAD model. This study aimed to investigate possible relationshio between the changes of insulin receptor (IR), insulin degrading enzyme (IDE), total tau(t-tau) and (p-tau) in the olfactory bulb (OFB) at the different time points and doses after STZ-icv treatment.
Material and methods: Adult male Wistar rats were injected icv with STZ (0.3, 1, 3 mg/kg) or vehicle (controls) and sacrificed one and three months after the treatment. Cognitive functions were tested by Morris Water Maze Test (MWM) and Passive Avoidance Test (PA). Protein expression of IR, IDE, tau and p-tau in OFB was measured by Western blot. Data were analyzed by Kruskal-Wallis and Mann-Whitney U test ($p<0.05$).
Results: Learning and memory deficit was found as early as two weeks following the STZ-icv treatment, and persisted up to three months after STZ-icv. Dose of 0.3mg/kg had no effect on the expression of investigated proteins. Dose of 1mg/kg decreased IR (-41,5%) only 1 month after STZ-icv, while leaving IDE and p/t tau ratio unchanged at both time points. Dose of 3mg/kg increased IR (+52,8%) after 1 month but decreased its expression after 3 months (-33,9 %) when also increased ratio of p/t tau Ser 404 (+183,7%) and p/tau Ser396 (+105,9%) was found.
Conclusion: Changes observed in STZ-icv model seem to be dose- and time-dependent suggesting that decrement of IR could lead to increased tau phosphorylation in OFB which should be further explored as a potential drug target.

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P05.
CENTRAL EFFECTS OF BOTULINUM TOXIN TYPE A ON SPINAL CORD NEUROTRANSMITTERS AND INHIBITORY NEURON MARKERS IN RATS WITH LOCAL MUSCULAR SPASTICITY

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Introduction: Intramuscular botulinum toxin type A (BoNT/A) is one of the most widely used treatments for focal muscle hyperactivity disorders. Recent preclinical and clinical observations suggested a possible contribution of BoNT/A actions at the level of central motor nuclei in its therapeutic effect (Mazocchio and Caleo, 2015; Matak et al., 2016.) . Herein, we examine this hypothesis by investigating the effects of BoNT/A on central neurotransmitters and neuronal protein markers in tetanus toxin (TeNT)-induced local spasticity.
Materials and methods: Focal unilateral muscle spasm was induced by unilateral low-dose TeNT injection into rat gastrocnemius muscle (1.5 ng). At day 7, spastic rats were injected with BoNT/A (5 U/kg) into the ipsilateral muscle or sciatic nerve. Rat spinal cord tissue was analyzed for glutamate content by ELISA, and the expression of vesicle-associated membrane protein-2 (VAMP-2) and synaptic vesicle 2C (SV2C) by immunohistochemistry.
Results: A relative increase in glutamate concentration in the ipsilateral vs. contralateral spinal cord ventral horns was observed in rats treated with TeNT, while BoNT/A treatment did not alter the glutamate increase. TeNT did not change the overal concentration of SV2C and VAMP-2 in the ventral horn. However, local spherical areas of VAMP-2 reduced immunoreactivity surrounding the alfa motoneuron bodies were observed in ipsilateral ventral horn of animals treated with TeNT + saline, seemingly more pronounced compared to other treatment groups and contralateral side. Surprisingly, an increased VAMP-2 immunoreactivity was observed in bilateral ventral horns of TeNT + BoNT/A-treated rats. TeNT-induced local reduction of VAMP-2 immunoreactivity, and increased glutamate content, suggest its enzymatic activity and increased excitatory transmission in ipsilateral ventral horns. BoNT/A treatment does not counteract the increased glutamate content, but it might induce a more widespread effect on the expression of VAMP-2, the target of TeNT enzymatic action.
Conclusion: BoNT/A action in the ventral horn of spastic animals does not involve reduction of excitatory transmission, however, it might involve indirect effect on other neuronal markers, which will be further examined.

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Project: The research was funded by European Social Fund, Croatian Ministry of Science, Education and Sport (project no. HR3.2.01-0178), and Croatian Science Foundation (project no. IP-2014-09-4503)

P06.

EFFECT OF ACUTE ORAL OR INTRAPERITONEAL GALACTOSE ADMINISTRATION ON OXIDATIVE STRESS AND METABOLISM IN BRAIN STEM OF STREPTOZOTOCIN INDUCED RAT MODEL OF SPORADIC ALZHEIMER'S DISEASE

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Introduction: Chronic oral galactose treatment improves learning and memory in streptozotocin (STZ) induced rat model of sporadic Alzheimer's disease, contrary to its parenteral administration used for induction of accelerated aging animal model. Mechanisms responsible for both beneficial and detrimental effects in both models are not yet sufficiently explored. The aim of the study was to determine the effect of acute peroral or intraperitoneal galactose administration on oxidative stress and metabolic changes in brain stem of rats previously treated intracerebroventricularly by STZ (STZ-icv).
Materials and methods: Male Wistar rats (3 months old) were given STZ-icv (3 mg/kg) while controls received vehicle only. One month after icv injections both groups were divided in 3 subgroups (no galactose treatment, peroral or intraperitoneal treatment (200mg/kg)) and sacrificed 15 minutes after. Oxidative stress was measured by colorimetric assays for malondialdehyde, catalase activity and reduced glutathion. Expression of GLP-1R, ERK, p70s6k, ULK1 was measured by Western blotting. Expression of GLP-1R, GLUT3, GLUT4 and pULK1 was visualised by immunofluorescence. Data were analysed by Kruskal-Wallis followed by Man-Whitney test.
Results: In comparison with control rats, STZ treated animals have higher levels of lipid peroxidation, increased catalase activity and decreased levels of reduced glutathion. Galactose administration increases lipid peroxidation but differently regulates CAT and reduced GSH in STZ treated animals when compared to controls. Western blot revealed no significant changes in GLP-1R, ERK, pERK, p70s6k, Pp70s6k, ULK1, pULK1 in whole brain stem lysate. Changes seen with immunofluorescence were dependent on the area analysed.
Conclusion: Acute administration of both peroral and intraperitoneal galactose induced changes in oxidative and metabolic parameters with a different trend observed in STZ treated animals in comparison to controls.

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Project: Supported by HRZZ IP-09-2014-4639

P07.
GALACTOSE EFFECT ON AUTOPHAGY IN THE BRAIN OF RATS AFTER ORAL/PARENTERAL ADMINISTRATION

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Introduction: Literature shows that parenteral administration of galactose can induce changes similar to those found in neurodegenerative disorders. Contrary to that chronic oral administration of galactose have beneficial effects on cognitive deficits observed in a rat model of sporadic Alzheimer's disease (AD). Aim of this study is to investigate the acute and chronic effects of galactose in the brain at the level of activation of astrocytes, neurons and autophagy.
Materials and methods: Adult male Wistar rats were given galactose chronically or in a single-dose. Oral galactose treatment (200 mg/kg/day in drinking water) was initiated 1 month after icv injections (citrate buffer) and continued for 2 months on daily basis until sacrifice (long-term). In a single-dose experiment animals were sacrificed 15 min after treatment (oral/ intraperitoneal 200 mg/kg galactose in a single bolus). The expression of extracellular signal–regulated kinase (ERK) and unc-51-like kinase 1 (ULK1) was measured by Western blot and immunofluorescence. Data were analyzed by Kruskal-Wallis and Mann-Whitney U-test ($p<0.05$).
Results: In comparison to the controls, chronic oral galactose treated rats showed decreased phosphorylation of ULK (-61% vs CTR and -42% vs CTRicv), while there was no change in ERK expression and activation in frontal and prefrontal cortex. Decreased phosphorylation of ULK at the Ser 757 is important for activation of autophagy. Contrary to that immunofluorescence results at the level of prefrontal cortex showed increased pULK expression after chronic galactose treatment in neurons and surrounding tissue and no expression in astrocytes. Astrogliosis was not present.
Conclusion: Autophagy deficits are likely major contributors to the etiology of AD and chronic-galactose treatment, which has beneficial effect in rat model of sAD (previously published), influences autophagy activation. Further research is needed to elucidate the effect of galactose on autophagy.

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Project: Supported by HRZZ IP-09-2014-4639

P08.
EFFECT OF CYCLAMATE ON ETHANOL ABSORPTION IN RATS

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Introduction: Ethanol absorption is determined by the rate of gastric emptying and previous researches showed that gastric emptying was affected by different sweeteners. Cyclamate is popular non-caloric artificial sweetener. The rats were intragastrically administered cyclamate-sweetened and non-sweetened ethanol solution. The aim of the study was to assess whether blood ethanol concentration differs when administered in cyclamate-sweetened ethanol solution comparing to non-sweetened ethanol solution.
Materials and Methods: 72 male Witsar rats were divided into 2 groups, each being intragastrically administered with 1500 mg/kg of ethanol in the form of differently sweetened v/v=16% alcoholic solutions: non-sweetened (NS) and cyclamate sweetened (CS). Blood samples were obtained via intravenous cannula from the tail vain at time t=10, 20, 40, 60, 90, 120 min after administration. Blood ethanol concentration was measured using enzymatic assay of alcohol dehydrogenase with spectrophotometric analysis of final product. Blood ethanol concentration at 10 min (C_{10}), maximal blood alcohol concentration (C_{MAX}) and area under concertation-time curve (AUC) were determined as pharmacokinetic parameters of ethanol absorption.
Results: Median value of C_{10} was significantly lower in CS group compared to NS group (0,51 vs. 0,81 , g/L, p=0,02). Median value of C_{MAX} was significantly lower in CS group compared to NS group (0,97 vs. 1,65 , g/L , p=0,03). Median value of AUC was significantly lower in CS group compared to NS group (195 vs. 286 , g*min/L , p=0,01). The exact mechanism, through which cyclamate affects gastric emptying, and consequently ethanol absorption, is not known but can possibly involve sweet taste receptors expressed on the gut epithelium.
Conclusion: Our data clearly show that alcoholic solution sweetened with cyclamate is absorbed more slowly than non-sweetened alcoholic solution.

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Project: All experiments were approved by animal ethics committee of Medical School University of Zagreb. This study was supported by project of prof. Predrag Sikirić (BM009 - grant from the University of Zagreb).

P09.
THE EFFECT OF SALIVA AND FLUORIDE TOOTHPASTE ON THE FORMATION OF KOH-SOLUBLE FLUORIDES: IN VITRO STUDY

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Mentors: Ivana Šutej, Assist. Prof.; K Bašić, PhD; Kristina Peroš, Assist. Prof.; Department of Pharmacology, University of Zagreb School of Dental Medicine, Zageb

Introduction: When fluoride is applied topically on the surface of hard dental tissue, it forms fluorite, CaF_2, which leads to higher fluoride level during acid activity, thus preventing tooth decay. The aim of this study was to assess the effect of smokers' and nonsmoker's saliva in combination with fluoridated toothpaste on the enamel uptake of alkali-soluble fluoride.
Materials and methods: Four enamel slabs were cut from each of 10 teeth and randomly assigned into 4 groups. Unstimulated saliva samples were collected from 12 male volunteers. Groups A and B were shaken in saliva (A in smoker's saliva, B in nonsmoker's saliva) for 5 min and then shaken for 3 min in toothpaste/deionized water slurry. Group C was treated only with toothpaste slurry. The procedure was repeated after 6-hour period. Group without treatment served as control group. The amount of KOH-soluble fluoride was determined by the method of Caslavska et al.
Results and discussion: The amount of KOH-soluble fluoride on the enamel in the group A was significantly higher than those of groups B, C and D. The amount of alkali soluble fluoride in the group B was significantly higher than those of control group, but it wasn't significantly different when compared to group C. Salivary flow was significantly higher in non-smoker's saliva. Salivary parameters were not significantly different. The calcium and magnesium ratio was nearly twice as large in non-smokers saliva.
Conclusion: The enamel uptake of alkali soluble fluoride depends whether saliva was included in treatment. The highest concentration of alkali soluble fluoride was found in samples treated with smokers' saliva.

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Approvals: The Ethics Committee of the School of Dental Medicine University of Zagreb, Croatia, approved the study protocol.
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P10.
THE EFFECT OF SMOKING ON SERUM LEVEL OF OLANZAPINE IN PATIENTS WITH SCHIZOPHRENIA

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Introduction: Olanzapine is a widely used second generation antipsychotic. Its extensive metabolism with smoking-inducible cytochrome P450 (CYP) 1A2 reduces serum levels of olanzapine in smokers. The aim of this study was to examine how smoking and the number of cigarettes per day affect olanzapine serum level in schizophrenic patients.
Material and Methods: We tested 45 blood samples of patients with schizophrenia; 28 treated with olanzapine intramuscularly and 17 treated with olanzapine per os. 57.8% (26/45) of them are smokers classified according to the number of cigarettes per day and nicotine dependence test (Fagerstrom). Olanzapine was analyzed with HPLC DAD method. The protocol of the project was approved by the Research Ethics Committee at the University Hospital Centre Zagreb, and all the participants gave written informed consent.
Results and Discussion: Median olanzapine trough concentration in nonsmoker patients in different dose groups was 104.9, 92.2, 308.0, and 183 nmol/L, and in smokers was 42.7, 78.5, 131, and 66.7 nmol/L, respectively. Our results suggest that serum levels of olanzapine decrease with increasing number of cigarettes per day and higher Fragerstrom test scores (best seen in i.m. 405 mg per month). Also, we can read lower average olanzapine serum levels in smokers' columns compared to nonsmokers' in four dosage regimens.
Conclusion: Based on results, smoking may have a significant effect on metabolism of olanzapine inducing CYP1A2. Reduced serum levels can decrease efficacy of the therapy and be below minimum effective concentration (64-256 nmol/L-therapeutic interval).

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P11.
THE ASSOCIATION OF CATECHOL-O-METHYLTRANSFERASE GENETIC VARIANTS WITH THERAPEUTIC RESPONSE TO ANTIPSYCHOTICS IN SCHIZOPHRENIA PATIENTS

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Introduction: Catechol-O-methyltransferase (COMT) is responsible for degrading dopamine, especially in the prefrontal cortex. *COMT* polymorphisms rs4680 and rs4818 significantly affect the enzyme activity and therefore modulate prefrontal dopamine levels and function. Study aimed to evaluate the association of genetic *COMT* variants and treatment response to atypical antipsychotics in schizophrenia.
Materials and methods: Study included 521 patients with schizophrenia, treated with olanzapine, risperidone, clozapine as monotherapy, or other antipsychotics as a comparator group. After 8 weeks of treatment, patients were subdivided into responders and non-responders according to a 50% reduction of the baseline Positive and Negative Syndrome Scale (PANSS) total and subscale scores. *COMT* rs4680 and rs4818 were genotyped using TaqMan® Drug Metabolism Genotyping Assays from Applied Biosystems, and following manufacture's procedures. LD pairwise values and haplotype frequencies were determined using the Haploview 4.2. The PLINK 1.07 was used to assign best-estimate haplotype pairs to each individual. This study was conducted with the approval of the Ethics Committee of the Psychiatric Hospital Vrapce, Zagreb, Croatia, and in accordance with the ethical standards established by the 1975 Declaration of Helsinki. All participants agreed to sign written informed consent.
Results: Treatment response to olanzapine was significantly associated with *COMT* rs4680. A significantly higher frequency of rs4680 A allele carriers vs. GG homozygotes was found in patients who responded well to olanzapine therapy. Also, the greater PANSS total and factor score reduction in rs4680 A allele carriers vs. GG homozygotes confirmed that A allele carriers achieved significantly greater improvement of overall schizophrenia symptoms. Haplotype analysis also suggested a higher frequency of C-A haplotype carriers in patients who responded well to olanzapine therapy than carriers of other haplotype combinations.
Conclusion: The data suggest that COMT genetic variants might predict good response to olanzapine in schizophrenia.

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Project: The impact of religiosity on the outcome of treatment of depression: clinical and biological indicators; University of Zagreb; PI: Marina Šagud
The association between stress, genetic variants of the catechol-O-methyltransferase and mu opioid receptor gene polymorphisms and tobacco smoking in patients with schizophrenia; collaborative project between University of Michigan (USA), Ruđer Bošković Institute (Croatia) and University Psychiatric Hospital Vrapce (Croatia); PI: Nela Pivac (CRO) and Eduard Domino (USA)

P12.
THE EFFECT OF SALIVA AND CALCIUM PHOSPHATE WITH FLUORIDE TOOTHPASTE ON THE FORMATION OF KOH-SOLUBLE FLUORIDES

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Introduction: The aim of this in vitro study was to assess the effect of smoker's saliva in combination with tooth crème containing calcium, phosphate and flouride on the enammel uptake of alkali-soluble flouride (KOH-soluble).
Materials and methods: Four enamel slabs were cut from each of 10 healthy, non carious permanent molars and arranged into 4 groups (A,B,C,D) . Unstimulated saliva samples were collected from twelve male volunteers, students of Universty of Zgreb average age of 21.75+-0.86. Six of the samples was taken from a smokers who smokes more than 20 cigaretts a day for the past 5 years and the other six samples was from a non-smokers. Two groups (A and B) were shaken in saliva (A in smoker's saliva, B in nonsmoker's saliva) for 5 min and then shaken for 3 min in a tooth crème /deionized water slurry (1:3 w/w). One of the groups (group C) received no saliva treatment and was only shaken in tooth crème slurry for 3 min. The treatment was repeated after a 6-hour period. One of the groups (D) served as a control group with no treatment. The amount of KOH-soluble fluoride was determined by the method of Caslavska et al.
Results and discussion: The enamel uptake of KOH-soluble fluoride among groups didn't show any significant differences. Salivary flow was significantly higher in non-smoker's saliva. Salivary parameters were not significantly different. The calcium and magnesium ratio was nearly twice as large in non-smokers saliva.
Conclusion: The enamel uptake of alkali soluble fluoride from tooth crème containing calcium, phosphate and flouride does not depend on whether saliva was included in treatment. These results demonstrate that calcium and flouride create very stong bonds, wich unables the enamel uptake of KOH-soluble fluoride.

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Approvals: The Ethics Committee of the School of Dental Medicine University of Zagreb, Croatia, approved the study protocol.
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P13.
TOLERABILITY OF OPIOID ANALGESICS - IMPORTANCE OF DRUG INTERACTIONS AND PHARMACOGENETICS

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Introduction: Opioids are commonly used for treatment of acute and chronic pain. They are often prescribed in polytherapy. The final analgesic effects highly depend on pharmacokinetic and pharmacodynamic properties of applied drugs, which are closely related to the capacity of patient's metabolic enzymes, drug transporters and opioid-binding receptors. Understanding of biotransformation processes of opioids and concomitantly prescribed drugs is crucial in predicting potential adverse drug-drug-gene interactions. Aim of this paper is presentation of clinical cases to discus drug interactions and pharmacogenetic variability of relevance for individual differences in effect and tolerability of opioid analgesics, which physicians and other healthcare professionals should be aware.
Patients and methods: Patients who experienced adverse drug reactions (ADRs) like tardive dystonia (TD) caused by oxycodone or tramadol in concomitant use with antidepressants will be described. Genetic analysis of variants relevant for opioids and antidepressants metabolism and transport, CYP2D6*3*4*5*6*41, CYP3A4*22, CYP2C19*2*17, UGT1A1*28, UGT2B7-161C>T, ABCB1 3435C>T was performed by Real-time PCR method. Clinical data on ADRs were recorded. Case series will be presented.
Results: Patients who developed TD were carriers of some inactivating alleles for CYP2D6, CYP2C19, CYP3A4, UGT2B7 and/or ABCB1, which resulted in prolonged bioavailability of opioids and/or antidepressants, elevating their potential for interactions. In presented cases predispositions for TD existed: Opioid system has been postulated to play an important role in the pathogenesis of dystonia. Studies suggest that opioids regulate some of dopaminergic neuronal activities. Due to inactivating gene-variants patients have prolonged bioavailability of oxycodone/ tramadol and escitalopram. Prolonged bioavailability of these drugs and its metabolites enlarge their interacting potential resulting in higher impact on opioid receptors (especially relevant for TD is kappa) and elevated interplay with dopaminergic pathway which in turn may cause TD.
Conclusion: Pharmacogenetics may encourage personalized therapy by identifying patients at increased risk of developing side effects or therapeutic failure due to drug-drug-gene interactions of some opioid analgesics and antidepressants, which can add to the severity of TD.

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Project - Support of the University of Zagreb: "The role of pharmacogenetics in drug interactions and side effects of opioid analgesics "

P14.
IS PLACEBO EFFECTIVE?

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Introduction: Until 2006. number of citations on PubMed for the term the „placebo effect“ was 1675. Eleven years later that number almost exponentially rose to 3325. citations. The goal of this paper is to show improvements in the conceptualization of placebo effect through the years.
Materials and methods: Narrative review paper. PubMed database was searched for review articles and studies (until 24.7.2017) which deal with „placebo effect“ in medicine. Studies included deal with topics from different fields, including: placebo and nocebo, clinical perspective, psychiatry, surgery, clinical research and ethics.
Results: Perception of placebo as an inert substance given to the patients changed to perception of stimulation of patient wellbeing as whole. Research shows that placebo effect isn't mediated by one single mechanism, but it is influenced by a spectrum of psychological and neurobiological mechanisms depending on the special medical context of illness. Psychosocial factors promoting beneficial effect of placebo can also produce harmful effect (so called nocebo). Placebo therapy in treatment of depression shows areas of possible improvement without changing the pharmacological active substance. Placebo in surgery subjectively may seem least feasible one. For better conceptualization of effect in surgery one needs to include perception of „ medical rituals“ in treatment. Even though placebo is probably most commonly prescribed medication in history, today it represents hot topic in ethical discussions. Generally, there are two perspectives in discussions. One deals with clinical research and second deals with use of placebo in everyday clinical practice. Predominantly scientific data about placebo comes from potentially unwillingly influenced experiments or it included only healthy participants. There are many researches which evaluate usefulness of placebo in clinical surroundings, but because of methodological mistakes there is room for different interpretations.
Conclusion: Even though there is scientific evidence that placebo in certain conditions provides relief of symptoms, it stills stays controversial topic in our society.

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P15.
SALIVA AS A DIAGNOSTIC FLUID

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Introduction: Saliva is a biofllluid which according to its various bio-molecules may be an indicator for oral health as well as numerous systematic diseases. Also, the non-invasive nature of collecting saliva is another advantage of running laboratory testing using saliva ahead of blood or other biological fluids.
Materials and methods: PubMed database was searched with keyword "saliva" and limits on human, and published from January 2016 to November 2017. Results were filtered for diagnosis.
Results: A total of 950 published studies were found using PubMed database. Most of this studies elaborate usage of saliva for virus detection, hormones testing, measurements of saliva flow, detecting DNA, biomarkers and salivary enzymes, valuation of pH and buffer capacity and microbiological testing.
Conclusion: Taking into account various advantages of using saliva as a diagnostic specimen it is obvious that this type of diagnostics may be very advantageous for clinical work. Nevertheless, there is still room for improvement in salivary analysis, methods and usage.

Literatura:
www.ncbi.nlm.nih.gov/pubmed

P16.

POTAGRUMAB (KLIO) AS A NEW THERAPY FOR GLIOBLASTOMA – STUDENTS' APPROACH

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Introduction: Glioblastoma (GBM) is a high-grade astrocytic brain tumour often refractory to the standard therapy with temozolomide and bevacizumab. The aim of this study was to create a new drug for treatment of GBM which would be more efficient than existing therapy.
Materials and methods: We synthesized PAP-1 derivate, AS-78, an inhibitor of Kv1.3 channels which we bound to an antibody against EGFRvIII, designed by the BiorByt, via protease labile linker, MC-VC-PABC, to create an antibody-drug conjugate, potagrumab. In *in vitro* binding studies of the IgG2/4 antibody to the EGFRvIII was proved using the BiorByt kit and effect of Kv1.3 inhibitors on cell death was tested on GL261, LN308 and A172 cell lines. Toxicokinetic studies were performed on mice and rabbits. Genetically modified mouse model and syngeneic mouse glioma model was used to test pharmacodynamics of the i.v. applied conjugate. After insuring the approval of the regulatory agency clinical studies were conducted and included patients with GBM, older than 18, with adequate haematologic, renal and hepatic function. FUS was used to increase the blood-brain barrier and MRI was performed to show tumour resolution.
Results: Results showed that antibody against EGFRvIII bound to glioma cell lines and up to 90% of the cells tested underwent apoptosis when they were treated with Kv1.3 inhibitor. Both antibody and Kv1.3 inhibitor showed no acute, subchronic or chronic systemic toxicity apart from the hearing problems for the channel inhibitor and mild hypotension with the antibody. Furthermore, the channel inhibitor proved to be teratogenic and both the inhibitor and the antibody proved not to be carcinogenic. T1/2 was 26 days and the distribution volume was 2.9 L/ 70 kg. The potassium channel inhibitor is eliminated unmetabolised by the kidneys. Clinical studies showed mean 33.87% tumour resolution and PFS was 8.9 months.
Conclusion: Potagrumab has almost three times greater effect on glioblastoma cells than other known therapies for GBM.

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