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# PERIODICUM BIOLOGORUM

## FINAL PROGRAMME AND ABSTRACT BOOK

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# PERIODICUM BIOLOGORUM

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PERIODICUM BIOLOGORUM

# 5th Croatian Congress of Pharmacology
# and
# 2nd Congress of Croatian Physiological Society

**with international participation**

**Supplement editors:**

Banfić H.
Boban M.
Francetić I.
Klarica M.
Mück-Šeler D.
Pivac N.
Sabolić I.
Tvrdeić A.
Župan G.

Osijek, Croatia
September 19–22, 2007

Croatian Pharmacological Society

Croatian Physiological Society

HDF CPS

**5th Croatian Congress of Pharmacology and**
**2nd Congress of Croatian Physiological Cociety**
**with international participation**

# FINAL PROGRAMME AND ABSTRACT BOOK

# GENERAL INFORMATIONS

## Organizing Committee

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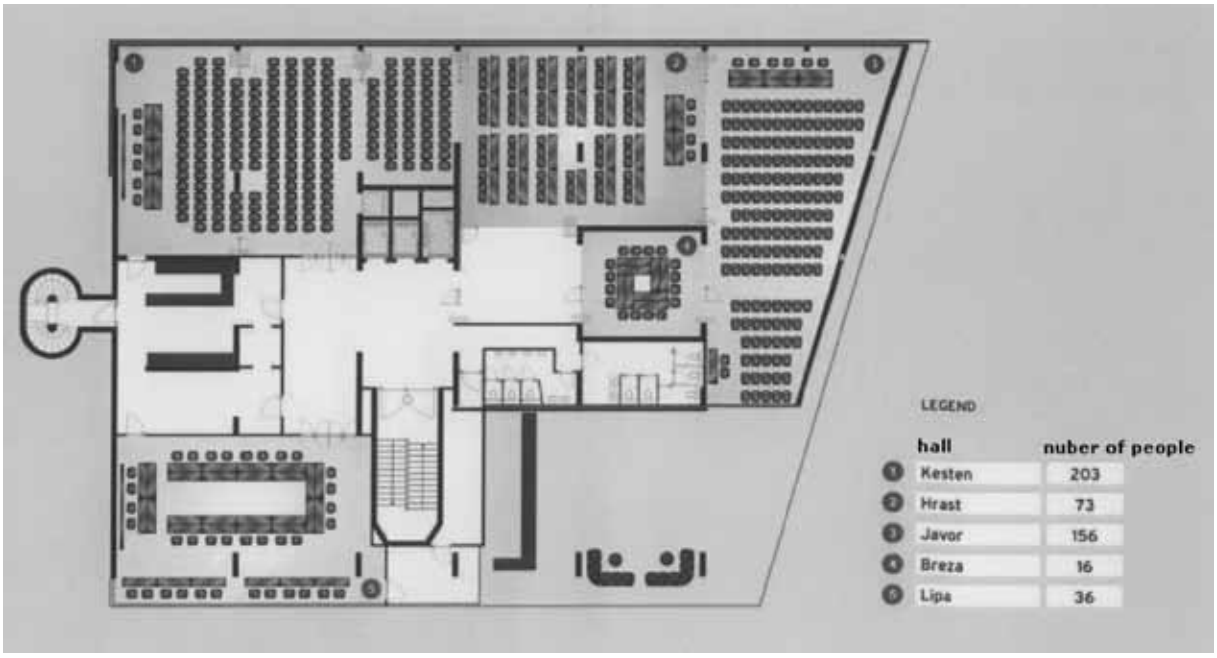
## Congress Venues

### 1) Hotel Osijek, Šamačka 4, Osijek

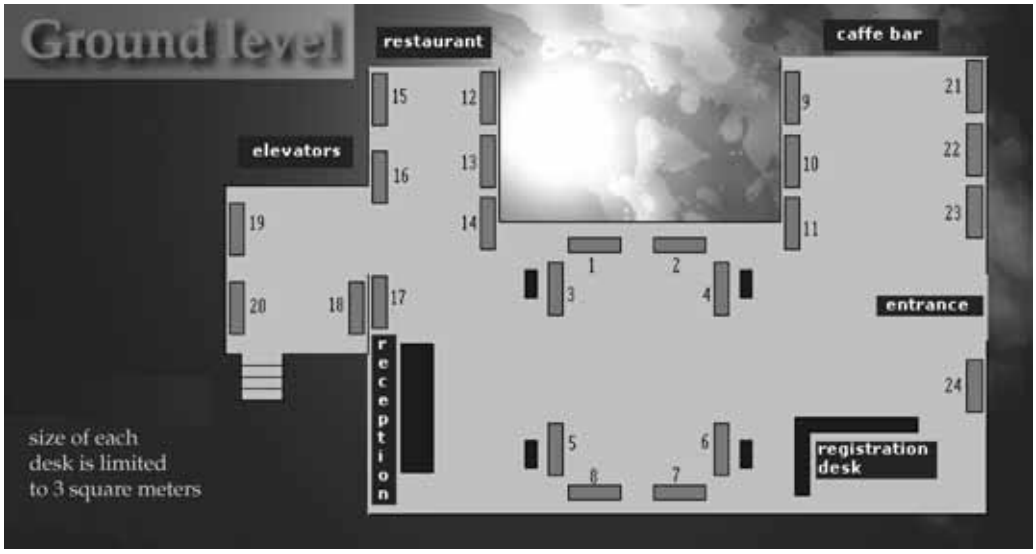
Hotel Osijek is located in the center of Osijek, five minutes a walk from main City square (Trg Ante Starčevića).

Congress halls: 1st floor: Hrast, Javor, Lipa (Lecture halls), Kesten (Poster session display), Breza (Coffee breaks)




Registration desk: Hotel Osijek, ground floor. Opening hours on September 19th: 16.00–19.00; on September 20th–22nd: 8.30–15.00

Exhibitions: Hotel Osijek, ground floor, stands 1–24




**2) University Josip Juraj Strossmayer of Osijek, Faculty of Medicine, Josipa Huttlera 4, Osijek**

Poster session 1, on 20th September will be displayed in Biomedical Science Building, Faculty of Medicine Osijek.

Faculty of Medicine is located on the field of Clinical Hospital Osijek. Clinical Hospital Osijek is positioned five streetcar stations a way from main City square.

## Official Languages

Croatian and English

## Registration Fees

Only registered participants may take part in scientific sessions and attend to the social events. Registered participants are required to wear identification badge.

| | Before June 15th, 2007. | After June 15th, 2007. |
|---|---|---|
| **The members of both societies** | 850,00 kn | 900,00 kn |
| **Non members** | 1000,00 kn | 1100,00 kn |
| **One day participation** | 400,00 kn | 450,00 kn |
| **Students** | 300,00 kn | 350,00 kn |
| **Accompanying persons** | 400,00 kn | 450,00 kn |

**The registration fee for society members, non-members and students includes:**

a. Participation in scientific/promotional events and certificate of attendance (upon request)
b. Congress bag, identification badge, Program and Book of abstracts
c. Welcome party
d. Coffee breaks
e. Launch
f. Visit to Zoological Reserve and Nature Park Kopački rit with Gala dinner
g. Visit to Vukovar and Ilok (option)

The registration fee for one day participants includes: a + b + d on the selected day. The registration fee for accompanying persons includes: c + f + g.

## Instructions for Lecturers

Presentation should be prepared in English. Technical assistant will be at your disposal during specified period of symposium session. Presentation should be handed to the technical assistant 30 minutes before the start of symposium. Immediately, after finishing the symposium, please ask the technical assistant to return your presentation.

## Poster Instructions

Poster number corresponding to the author abstract will be posted on the top of the board. Poster should be mounted 15 minutes before the start of Poster session. Presenting authors are required to attend his/her position during specified period of the poster session. Immediately, after finishing the poster session, please, remove your poster from the board.

## Instructions for Submission of Full Paper

Selected full papers will be published in the special issue of Periodicum biologorum. The specific instructions to authors are published by the journal Periodicum biologorum. Authors should follow those instructions http://mcc.irb.hr/instruct.html. The papers should be sent before September 22nd to:

**Prof. dr. Branko Vitale**
Editor-in-Chief
Periodicum biologorum
Hrvatsko prirodoslovno društvo
Frankopanska 1, 10 000 Zagreb, Croatia

## Certificate of Attendance and Credits for Participants

Certificate of attendance and/or credits of professional chambers (Croatian participants only), will be issued at the Registration desk only to registered participants, upon request.

## Available services

Please, contact the Registration desk stuff for:

Registration and on site payment of registration fee
Congress materials (Congress bag, identification badge, Program and abstract book)
Technical assistance (oral and poster presentations)
Contacts and information about Congress organization and events
Certificates for attendance and credits

# PROGRAMME OVERVIEW

## SEPTEMBER 19 (WEDNESDAY)

**16:00 – 19:00** **REGISTRATION (Hotel Osijek, Šamačka 4)**

**19:00 – 24:00** **OPENING CEREMONY, WELCOME PARTY (Hotel Osijek, Šamačka 4, Hrast and Javor halls)**

## SEPTEMBER 20 (THURSDAY)

**8:30 – 15.00** **REGISTRATION (Hotel Osijek, Šamačka 4)**

**9:00 – 11:00** **Symposium 1 (S1), Hotel Osijek, Hrast hall**
**Symposium 2(S2), Hotel Osijek, Javor hall**
**Symposium 3(S3), Hotel Osijek, Lipa hall**

**S1** *Cell signaling* **(***chairs: Banfić H. and Đikić I.***)**
S1.1. **Volarević S.**, Panić L.: Response of mammalian cells to a defect in ribosome biogenesis
S1.2. **Đikić I.**: Ubiquitin signaling networks
S1.3. Jan Keune W., Bultsma Y., Elouarrat D., Jones D., **Divecha N.**: Phosphoinositide regulation of nuclear function
S1.4. **Banfić H.**: Cellular functions of phosphoinositide 3-kinase C2ß

**S2** *Neurobiological basis and treatment of posttraumatic stress disorder* **(***chairs: Pivac N. and Manev H.***)**
S2.1. **Manev H.**, Manev R., Uz T., Zhang Z., Dimitrijević N., Imbesi M., Dzitoyeva S: Stress and neuronal epigenetic mechanisms
S2.2. **Pivac N.**, Kozarić-Kovačić D., Jovanović T.: Neurobiological and genetic markers in posttraumatic stress disorder
S2.3. **Mück-Šeler D.**, Pivac N., Mustapić M., Deželjin M.: Dopamine betahydoxylase in posttraumatic stress disorder
S2.4. **Kozarić-Kovačić D.**: Novel approaches to the treatment of posttraumatic stress disorder
S2.5. **Vukšić-Mihaljević Ž.:** Sleep disturbances in posttraumatic stress disorder
S2.6. **Francisković T.**, Grković J.: Dissociations: rediscovered concept in psycho trauma

**S3** *Diagnosis and treatment of patients with chronic Hepatitis C* **(***chairs:* Včev *A. and Mihaljević S.***)**
S3.1 **Včev A.**: Hepatitis C: Epidemiology and natural course of disease
S3.2 **Božić D.**: Prevention of Hepatitis C
S3.3 **Balen S.**: Laboratory diagnosis of viral Hepatitis C
S3.4 **Miše S.**: Hepatitis C: Who should be tretaed?
S3.5 **Ostojić R.**: Treatment of Hepatitis C
S3.6 **Dželalija B.**: Antiviral treatment for patients with HCV and HIV co-infection
S3.7 **Zildžić M.**: Treatment the side effects of antiviral therapy
S3.8 **Mihaljević S.**: Future of HCV therapy
S3.9 **Sikirić P.**: Experimental models of hepatitis

**11:00 – 11:30** **COFFEE BREAK (Breza hall)**

**11:30 – 13:30** **Symposium 4 (S4), Hotel Osijek, Hrast hall**
**Symposium 5 (S5), Hotel Osijek, Javor hall**

**S4** *Organic anion and cation transporters in mammalian organs* **(***chairs: Sabolić I. and Buckhardt G.***)**
S4.1 **Buckhardt G.**, Bahn A., Burckhardt B.C., Hagos Y.: Physiology and pathophysiology of organic anion transporters (OATs)
S4.2 **Koepsell H.**: Structure and function of transporters that mediate hepatic and renal excretion of cationic drugs
S4.3 **Sabolić I.**, Ljubojević M., Breljak D., Balen D., Brzica H., Zlender V.: Gender and species differences in renal organic anion and cation transporters
S4.4 **Zlender V.**, Breljak D., Ljubojević M., Balen D., Brzica H., Anzai N., Fuchs R., Sabolić I.: Organic anion and cation transporters in experimental ochratoxin A nephrotoxicity

**S5** *Pathophysiology and treatment of Alzheimer's disease* **(***chairs: Šalković M. and Mimica N.***)**

S5.1 **Mimica N.**: Contemporary treatment and care for people with Alzheimer's disease and Croatian reality

S5.2 **Šimić G.**: CSF phosphorylated tau proteins as predictors of Alzheimer's disease in subjects with mild cognitive impairment

S5.3 **Šalković Petrišić M.**, Grünblatt E., Osmanović J., Hoyer S., Riederer P.: »Amyloid cascade« hypothesis: is it true for sporadic Alzheimer's disease?

S5.4 **Babić T.**: Clinical trials with new drugs for the treatment of neurodegenerative disorders

**13:30 – 15:00 LUNCH (Hotel Osijek, restaurant Zimska luka)**

**15:00 – 16:00 Arrival at Faculty of Medicine. Poster mounting.**

**16:00 – 17:00 Welcome address and visit of Building of Biomedical Sciences**

**17:00 – 19:00 POSTER SESSION P1 (Faculty of Medicine Osijek, Josipa Huttlera 4)**

**P1** – *Clinical Pharmacology I (chairs: J. Čulig and D. Vitezić)*

P1.1 **Štimac D.**, Čulig J., Šostar Z., Bucalić M.: Comparison of outpatient utilization of psychopharmaceuticals between Zagreb and Scandinavian countries (2001.–2006.)

P1.2 **Leppee M.**, Štimac D., Klepac-Pulanić T., Blajić I., Čulig J.: Cross-sectional analysis of drug use in pregnancy in Zagreb's hospitals

P1.3 **Erić M.**, Sabo A., Mihić N.: How safe are antibiotics during pregnancy?

P1.4 **Antolović-Amidžić A.**, Virovkić-Žunec B., Stiblik-Stipešević S., Šamšalović G.: Consumption of the drugs ATC A in Clinical hospital Osijek during the period of 2004.–2006.

P1.5 **Prološčić D.**, Kvolik S., Ivić D., Haršanji Drenjančević I., Vešara-Hazurović V.: Reporting adverse drug reactions occurring during anaesthesia

P1.6 **Stipčević T.**, Kusačić-Kuna S., Deželjin M., Ciglar M., Koršić M., Pivac N., Mück-Šeler D.: Peripheral serotonergic markers after total thyroidectomy: the effect of antihypertensive medications

P1.7 **Pavličević I.**, Rumboldt M., Kuzmanić M., Rumboldt Z.: Clinical significance of the interaction between antihypertensive and antirheumatic drugs

P1.8 **Nenadić-Šviglin K.**, Nedić G., Kozarić-Kovačić D., Deželjin M., Stipčević T., Mustapić M., Mück-Šeler D., Pivac N.: Suicidity and platelet serotonin concentration in alcoholism

P1.9 **Dodig-Ćurković K.**, Ćurković M., Degmečić D., Dovhanj J., Požgain I., Filaković P.: Drug–induced disturbances of serum glucose and lipid profile during treatment with antipsychotic and antidepressive drugs

P1.10 **Nedić G.**, Knežević J., Dezeljin M., Balija M., Kozarić-Kovačić D., Pavelić J., Mück-Šeler D., Pivac N.: Monoamine oxidase type B polymorphism in combat related posttraumatic stress disorder

P1.11 **Varda R.**, Mimica N.: Color reproductions of psychopharmacs registered in Croatia

**P1** – *Clinical Pharmacology II (chairs: N. Božina and I. Francetić)*

P1.12 **Cuković-Čavka S.**, Brinar M., Božina N., Grubelić-Ravić K., Krznarić Ž., Rojnić Kuzman M., Sertić J., Vucelić B.: Allelic variants of the multidrug resistance gene (MDR1/ABCB1) and response to corticosteroid therapy in patients with inflammatory bowel disease

P1.13 **Cuković-Čavka S.**, Božina N., Krznarić Ž., Grubelić-Ravić K., Brinar M., Vucelić B.: Azathioprine-induced allergic hepatitis associated with thiopurine methyltransferase (TPMT) genotype; case report

P1.14 **Mandac I.**, Planinc-Peraica A., Jakšić B.: Thalidomide for the treatment of multiple myeloma

P1.15 **Badel T.**, Pandurić J., Marotti M., Keros J., Kern J., Kocijan Lovko S., Ročin Grget K.: Therapy of temporomandibular joint displaced disc according to anxiety

P1.16 **Šutej I.**, Ročin-Grget K., Plančak D., Linčir I.: Nicotine and periodontal health of adolescents

P1.17 Ivić J., **Haršanji Drenjančević I.**, Ivić D., Pelc B., Krešić M.: Case report: could the oral contraceptive pills be the cause of the internal jugular vein thrombosis during the perioperative period?

P1.18 **Puretić Z.**, Granić P., Bubić-Filipi Lj., Lalić Z., Lovrić M., Humar I., Mustapić Z., Sertić J., Kes P.: Therapeutic drug monitoring of mycophenolic acid in patients with kidney transplant

P.19 **Bubić-Filipi Lj.**, Bašić-Jukić N., Puretić Z., Kes P.: Oral valganciclovir is as effective and safe as iv ganciclovir for the treatment of cytomegalovirus infection in renal transplantation

P1.20 **Majnarić Lj.**: Soluble P-selectin in hypertensive patients might be a marker of disturbed hemorheology

P1.21 **Ivić D.**, Marijanović K., Ivić J., Mimica-Matanović S., Dobrić I., Došen G.: Toxic epidermal necrolysis after linezolid administration

P1.22 **Čandrlić J.**, Takač I., Ivić D., Čandrlić K.: Oxygen as a drug – a presentation of method

P1.23 **Selthofer-Relatić K.**, Radić R., Vizjak V., Kosović P.: Effect of leptin levels and selective leptin resistence on hypertension and consequence myocardial hypertrophy in obese individuals

P1.24 **Zibar L.**, Barbić J., Sabolović D., Wagner J., Pavlinić D., Pasini J.: Association between interferon gamma gene polymorphism and cyclosporine a dose requirement in kidney transplant patients

**P1** – *Preclinical Pharmacology I (chairs: V. Bradamante and I. Samaržija)*

P1.25 **Boban Blagaić A.**, Štambuk N.: The influence of alpha-melanotropin and tetracosactid on ethanol-induced gastritis in rats

P1.26 **Samaržija I.**: Prostaglandins are involved in the regulation of renal tubular glucose cotransport

P1.27 **Osmanović J.**, Šalković-Petrišić M., Riederer P.: Altered expression of insulin receptor in hippocampus of streptozotocin intracerebroventricullary treated rats

P1.28 **Brizić I.**, Lukšić B., Modun D., Vuković J., Mudnić I., Boban M.: Cardioprotective effects of 2,3-butanendione monoxamine against the vipera Ammodytes ammpodytes venom in the isolated rat heart

P1.29 **Lovrić J.**, Macan M., Koprivanec M., Kelava M., Bradamante V.: Effect of simvastatin treatment on MDA level in rat plasma measured by spectrophotometric and HPLC-MS analysis

P1.30 **Čičin-Šain L.**, Bordukalo-Nikšić T., Štefulj J., Mokrović G., Hranilović D., Jernej B.: Wistar: Zagreb-5HT rat: further progress in development of the model

P1.31 **Krnić Ž.**, Bradamante V., Kujundžić Tiljak M., Zrinski Topić R.: Correlation between serum butyryl-cholinesterase activity and serum lipids concentration in rats treated with different antagonists of the adrenergic system

P1.32 **Bradamante V.**, Macan M., Vrkić N., Konjevoda P.: The effects of statins on rats butyrycholinesterase

P1.33 **Macan M.**, Bradamante V., Vrkić N., Radić B., Lucić Vrdoljak A., Konjevoda P.: The opposite effects of simvastatin and atorvastatin on serum and liver paraoxonase activity in normolipidemic rats

**P1** – *Neuropharmacology (chairs: M. Klarica and A. Tvrdeić)*

P1.34 **Bach – Rojecky L.**, Šalković Petrišić M., Lacković Z.: Long-lasting antinociceptive effect of botulinum toxin type a in experimental diabetic neuropathy

P1.35 Tvrdeić A., **Kocevski D.**: Effects of exploration behaviour, locomotor activity and emotionallity on paw withdrawal latencies in Hargreaves test

P1.36 **Puljak L.**, Lovrić Kojundžić S., Sapunar D.: Behavioral and morphological changes in rat DRG following lidocaine injection

P1.37 **Hrabrić K.**, Buljan R.: Therapeutic effect of ropinirol on polysomnographic characteristics of sleep in patients with PLMD/RLS

P1.38 **Božina N.**, Medved V., Rojnić-Kuzman M., Sertić J.: Association study of therapeutic response with SERT, MDR1 and 5-HT2c gene polymorphisms in female schizophrenic patients

P1.39 **Peternel S.**, Blagaić A., Pilipović K., Mršić-Pelčić J., Župan G.: Effects of pioglitazone in the lithium-pilocarpine model of status epilepticus in rats

P1.40 **Pilipović K.**, Frković V., Dangubić B., Župan Ž., Peternel S., Župan G.: Pioglitazone limits the cortical oxidative damage following traumatic brain injury in the rat

P1.41 **Klarica M.**, Vukić M., Radoš M., Orešković D., Bulat M.: Development of transmantle pressure gradient in cats with acute aqueductal blockage

P1.42 **Bulat M.**, Lupret V., Orešković D., Klarica M.: Mechanism of absorbtion of the cerebrospinal fluid

P1.43 **Orešković D.**, Maraković J., Vukić M., Radoš M., Klarica M.: Evaluation of perfusion method for measuring of cerebrospinal fluid secretion

P1.44 **Klarica M.**, Kuzman T., Mandac I., Radoš M., Orešković D., Bulat M.: The effect of body position on intracranial and intraocular pressure in cats

P1.45 **Ćurić G.**, Pavlinić D., Wagner J., Đurković M., Braš M., Lauc G.: Absence of association between genetic polymorpisms in CRFR1 and PTSD

P1.46 **Erdeljić V.**, Francetić I., Makar-Aušperger K., Merćep I., Šimić P., Likić R.: Does positive history of allergic drug reactions or atopy justify does positive history of allergy to local anesthetics?

P1.47 **Lalić Z.**, Lovrić M., Božina N., Granić P., Sertić J.: Quantitative determination of gabapentin and vigabatrin by HPLC in the serum of patients with epilepsy

P1.48 **Lovrić M.**, Lalić Z., Božina N., Granić P., Sertić J.: Therapeutic drug monitoring: quantitation of oxcarbazepine and its active metabolite in human serum

## SEPTEMBER 21 (FRIDAY)

**8:30 – 15.00** **REGISTRATION (Hotel Osijek, Šamačka 4)**

**9:00 – 11:00** **Round table (RT), Hotel Osijek, Hrast hall**
**Symposium 6 (S6), Hotel Osijek, Javor hall**

**RT:** *Regulation of medicine products in Croatia (chairs: Tomić S. and Martinac Ilić A.)*

RT1 **Tomić S.**: Medicinal products regulation in Croatia – The new Pharmaceutical legislation

RT2 **Ilić Martinac A.**: Counterfeit medicines – impact on national healthcare system

RT3 **Trkulja V.**: Emerging issues in regulatory views on bioequivalence: Highly variable drugs & drug products – how should they be treated?

RT4 **Vitezić D.**: Clinical trials in Croatia

RT5 **Draganić P.**, Macolić-Šarinić V., Tomić S: Drugs consumption in Croatia in 2005

**S6** *Diabetes and stress* **(***chairs: Marotti T. and Hadžija M.***)**

S6.1 **Hadžija Popović M.**, Korolija M., Pavlić Renar I., Kapitanović S.: Polymorphism in type I diabetes mellitus

S6.2 **Korolija M.**, Popović Hadžija M., Hadžija M.: ß-cells development *in vitro*

S6.3 **Četković Cvrlje M.**: Signal transducer and activator of transcription (Stat) 4 and Stat 6 genes in autoimmune diabetes

S6.4 **Balog T.**: Opioids and oxidation stress

S6.5 **Sobočanec S.,** Šarić A., Korolija M., Marjanović M., Kralj M., Knežević J., Čretnik M., Marotti T.: Dual effect of native propolis *in vivo* as a consequence of selective gene expression

S6.6 **Šarić A.**, Balog T., Marotti T.: Endomorphyns modulate nitric oxide release

**11:00 – 11:30** **COFEE BREAK (Breza hall)**

**11:30 – 13:30** **Symposium 7 (S7), Hotel Osijek, Hrast hall**
**Symposium 8 (S8), Hotel Osijek, Lipa hall**
**Symposium 9 (S9), Hotel Osijek, Javor hall**

**S7** *Natural sources of pharmacology active compounds (chairs: Boban M. and Medić Šarić M.)*

S7.1 **Medić Šarić M.**: Investigation of polyphenols from Croatian natural sources

S7.2 **Katalinić V.**: Natural sources and chemistry of resveratrol

S7.3 **Miloš M.**: Pharmacologically active volatile compounds from some aromatic plants

S7.4 **Boban M.**: Mechanisms of antioxidatory and vasodilatatory effects of red wine

S7.5 **Cvek J.**, Milčić N.: Regulative aspects of herbal medicines

**S8** *Pharmacogenomics (chairs: Henigsberg N. and Božina N.)*

S8.1 **Henigsberg N.**: Pharmacogenetics in Psychiatry

S8.2 **Božina N.**, Makar-Aušperger K., Lovrić M.: Pharmacogenomics in clinical practice – warfarine therapy individualization

S8.3 **Mihaljević Peleš A.**, Božina N., Šagud M.: Serotonine transporter polymorphism and response to paroxetine therapy

S8.4 **Sporiš D.** Božina N., Hajnšek S., Bašić S., Lovrić M., Kovačević I.: The role of polymorphic P-glycoprotein in the therapy of epilepsy

**S9** *Clinical pharmacology (chairs: I. Francetić and J. Bagatin)*

S9.1 **Francetić I.**: Therapeutic guidelines

S9.2 **Bagatin J.**: The role of beta adrenergic blockers in therapy of hypertension

S9.3 **Vitezić D.**: Pharmacotherapy of hypertension and blood pressure control in Croatia

S9.4 **Vlahović Palčevski V.**: Drug prescribing – can it be better? Contributions of pharmacoepidemiology to clinical pharmacology.

S9.5 **Makar Aušperger K.**: Individualization of therapy in clinical practice – importance of pharmacogenomics

S9.6 **Mimica Matanović S.**: Clinical pharmacologist in therapeutic problem solving

**14:00 – 15:30 General Assembly of Croatian Pharmacological Society, Hotel Osijek, Javor hall**

**14:00 – 15:30 POSTER SESSION P2 (Hotel Osijek, Kesten hall)**

**P2** – *Physiology I (D. Višnjić and S. Kukolja Taradi)*

P2.1 **Rastija V.**, Medić-Šarić M.: Analysis of biologically active polyphenols in wines from Croatia

P2.2 **Jasprica I.**, Mornar A., Medić-Šarić M.: Effects of flavonoids on production of reactive oxygen species in differentiated THP-1 cell line

P2.3 **Mudnić I.**, Modun D., Vuković J., Brizić I., Višnja Katalinić, Bernard Kozina, Mladen Boban.: Antioxidative and vasodilatatory effects of phenolic acids from wine

P2.4 **Generalić I.**, Katalinić V., Ljubenkov I., Pezo I., Stričević O., Miloš M., Modun D., Boban M.: Free resveratrol monomers in varietal red and white wines from Dalmatia (Croatia)

P2.5 **Modun D.**, Mudnić I., Vuković J., Brizić I., Katalinić V., Bilušić T., Boban M.: Antioxidative capacity and vasodilatatory activity of strawberry leaves extract

P2.6 **Vuković J.**, Mudnić I., Brizić I., Modun D., Katalinić V., Kozina B., Karoglan M., Maslov L., Mladen Boban M.: Modification of red wine vinification process: addition of white grape seeds, skins and ethanol causes changes in biochemical properties and vasodilatatory activity *in vitro*

P2.7 **Politeo O.**, Jukić M., Miloš M.: Chemical composition and antioxidant capacity of selected spice volatile aglycones in two lipid model systems

P2.8 **Jukić M.**, Miloš M., Politeo O., Ivanković S., Stojković R., Jurin M.: *In vitro* and *in vivo* antitumor activity of thymoquinone and thymohydroquinone

P2.9 **Martin-Kleiner I.**, Bombek S., Košmrlj J., Čupić B., Čimbora-Zovko T., Jakopec S., Polanc S., Osmak M., Gabrilovac J.: Selective cytotoxicity of diazenecarboxamides towards human leukemic cell lines

P2.10 **Čimbora Zovko T.**, Ambrilović Ristov A., Lnčarek J., Osmak M.: Altered cell-cell adhesion in cisplatin-resistant human carcinoma cells: a link between beta-catenin/plakoglobin ratio and cisplatin resistance

P2.11 **Radačić M.**, Pavlak M., Zinić B., Jerčić J., Vlahović K., Radačić Aumiler M., Kašnar-Šamprec J., Stojković R., Ivanković S.: The influence of hyperthermia and chemotherapy on the tumor growth in mice

P2.12 **Kukolja Taradi S.**, Taradi M., Urano M: Effect and mechanism of thermoimmunotherapy with OK-432 on the tumour growth and lung metastases in mice

P2.13 **Mišković K.**, Suver M., Stolić I., Bajić M., Baus Lončar M., Glavaš Obrovac Lj.: Cytotoxic effects of new synthesized bisbenzimidazole derivatives on different tumour cell lines

P2.14 **Matković K.**, Banfić H., Višnjić D.: The role of the nuclear protein kinase b/Akt activation in HL-60 leukemia cells

P2.15 Matković K., Banfić H., **Višnjić D.**: The effects of phosphoinositide 3-kinase/Akt inhibitors on two retinoid--responsive leukemia cell lines

P2.16 **Višnjić D.**, Lukinović-Škudar V., Matković K., Banfić H.: Two distinct peaks of nuclear PI-PLCbeta1b activity occur in serum-stimulated HL-60 cells

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P2.21 **Micek V.**, Brzica H., Balen D., Ljubojević M., Breljak D., Anzai N., Koepsell H., Sabolić I.: G-protein-coupled -ketoglutarate (GPR99) are $\alpha$-receptors for succinate (GPR91) and differently localized in the human and mouse nephron

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P2.33 **Šimpraga M.**, Matanović K., Vojta A., Filipović I., Radin L.: Hematological and biochemical values of ecologically bred Cres sheep

P2.34 **Šimpraga M.**, Lukač Novak I., Mazija H., Štoković I., Vojta A.: Hematologic and biochemical parameters of ostriches after vaccination against Newcastle disease

P2.35 **Krčmar S.**, Marić S.: The role of blood meal in the life of hematophagous horse flies (diptera: *Tabanidae*)

**16:00 – 23:00** **Visit to Baranja and Kopački rit with Gala Dinner in Restaurant »Kormoran«**

## SEPTEMBER 22 (SATURDAY)

**8:30 – 15.00** **REGISTRATION (Hotel Osijek, Šamačka 4)**

**9:00 – 11:00** **Symposium 10 (S10), Hotel Osijek, Javor hall**
**Workshop, Part 1, Hotel Osijek, Hrast hall**

**S10** *Regulation of blood pressure and microcirculation (chairs: Drenjačević Perić I. and Salihagić Kadić A.)*

S10.1 **Drenjančević-Perić I.**: Role of the renin-angiotensin system in regulation of vascular reactivity

S10.2 **Salihagić-Kadić A.**, Arbeille P.: Fetal cerebrovascular response to chronic hypoxia-implications for the prevention of brain damage

S10.3 **Valić Z.**: Regulation of skeletal muscle blood flow

S10.4 **Phillips S.A.**: Exercise, reactive oxygen species and human endothelial function

*Workshop: The role of the health care professionals in adverse reaction reporting and pharmacovigilance system in Croatia. Part 1 (lectures).*

**11:00 – 11:30** **COFFEE BREAK (Breza hall)**

**11:30 – 13:30 Symposium 11 (S11), Hotel Osijek, Javor hall**
**Workshop, Part 2, Hotel Osijek, Hrast hall**

**S11** *Diving physiology (chair: Dujić Ž.)*
S11.1 **Dujić Ž.**: Introduction to diving physiology
S11.2 **Obad A.**: Antioxidants and SCUBA diving
S11.3 **Baković D.**: Spleen as a part of diving response
S11.4 **Palada I.**: Muscle and cerebral oxygenation during breath-hold

*Workshop: The role of the health care professionals in adverse reaction reporting and pharmacovigilance system in Croatia. Part 2 (practical and test).*

**13:30 – 14:00 CLOSING CEREMONY (Hotel Osijek, Hrast hall)**

**15:00 – 22:00 Visit to Vukovar and Ilok**

# ABSTRACTS
## Symposia, round table and workshop

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Abstract

# S1.1 Ribosomal protein S6 deficiency activities a p53 dependent checkpoint

**S. VOLAREVIĆ, L. PANIĆ**

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The capacity to detect and appropriately respond to many different stresses that interfere with functional homeostasis is essential for survival. Recent evidence suggests that the nucleolus, the site of ribosome biogenesis, plays a critical role in sensing and responding to many external and internal stresses. To understand these processes, we have recently used a genetically defined *in vivo* mouse model in which ribosome biogenesis could be manipulated during oogenesis and embryo development. In these mice ribosomal biogenesis is impaired by a conditional deletion of one allele of the gene encoding 40S ribosomal protein S6. Embryos from these animals fail during gastrulation, apparently due to a p53-dependent checkpoint activation, rather than a deficit in translational capacity. These findings imply that molecular mechanisms have evolved during mammalian evolution to strongly guard against potential heterozygosity for ribosomal protein genes.

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Abstract

# S1.2 Targeting ubiquitin signaling networks

**I. ĐIKIĆ**

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Originally described as a destruction tag for misfolded or disused proteins ubiquitin (Ub) has recently entered centre stage in many fundamental processes like cell cycle, apoptosis, DNA repair or endocytosis. In these processes Ub acts as a signalling component able to trigger molecular events in cells. Ub does so by operating as a reversible and highly versatile regulatory signal for an expanding number of Ub-binding domains (UBD) present in cellular proteins that convey Ub signals into appropriate cellular phenotypes. In addition, it is becoming apparent that deregulation of Ub pathways results in the development of human diseases including many types of tumours. The common principles and specific features of Ub and other Ub-like modifiers and their binding domains in the regulation of signaling pathways will be discussed. The concept of inhibition by monoubiquitination of UBD-proteins will be described in more details. In adittion, Ub-like domains in TBK1 and IKKi kinases are involved in intramolecular binding, regulation of their kinase activities and presentation of substrates, such as IRF3 and IRF7, thus linking signalling from Toll-like receptors with interferon-inducible genes.

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Abstract

# S1.3 Regulation of the levels of nuclear PtdIns5P: a nuclear lipid involved in the regulation of the tumor suppressor protein p53

**W. J. KEUNE, Y. BULTSMA, D. ELOUARRAT, D. JONES, N. DIVECHA**

Netherlands Cancer Instiutute, Division of Cellular Biochemistry, Amsterdam, Netherland

Phosphoinositides regulate many different cellular functions in part by the regulation of their synthesis and degradation in various subcellular compartments. For example the protein kinase PKB is activated as a consequence of receptor-mediated increases in PtdIns(3,4,5)$P_3$ at the plasma membrane, while EEA-1, a protein which regulates membrane trafficking is modulated by changes in the concentration of PtdIns3P in early endosomal vesicles. We and many others have demonstrated the presence of a pool of phosphoinositides within the nucleus that is regulated in a distinct manner from phosphoinositides in other sub-cellular compartments. In order to comprise a functional signalling pathway we expect to find that in response to activation of specific upstream pathways that nuclear proteins can impinge on nuclear phosphoinositide synthesis or degradation to control their intranuclear levels. Furthermore in response to changes in nuclear phosphoinositides levels we expect to find specific nuclear sensors whose activities/subnuclear localisation is changed in order to modulate specific nuclear functions. As an example of a nuclear specific phosphoinositide pathway we will describe how the levels of PtdIns5P are regulated in response to DNA damage and oxidative stress and how changes in PtdIns5P impinges on downstream sensors to regulate the activity of the tumour suppressor protein p53.

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# S1.4 Cellular functions of phosphoinositide 3-kinase C2β

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The large family of phosphoinositide 3-kinase (PI3K) enzymes can be divided into three distinct classes (I, II and III) based on the sequence similarity and lipid products they generate *in vitro*. In mammalian cells there are three class II PI3K isoforms, and PI3K-C2β act as downstream targets of growth factor, chemokine and integrin receptors. The proline-rich motifs within the N-terminal region of PI3K-C2β mediate its association with activated receptor, and the enzyme regulates cell migration, suggesting its potential role in tumour metasthasis. In *Drosophila melanogaster,* ectopic expression of the enzyme in the larval marginal disks affected the development of the wings, and the number of external sense organs, suggesting a role of the enzyme in the signalling pathways affecting pattering. Furthermore, the enzyme is localized in the nucleus and is activated during $G_2$/M phase of the cell cycle *via* calpain-mediated proteolysis? Enzyme contains nuclear localization sequence within C2 domain, which is crucial for its nuclear localization and translocation.

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Abstract

# S2.1 Stress and neuronal epigenetic mechanisms

**H. MANEV, R. MANEV, T. UZ, Z. ZHANG, N. DIMITRIJEVIĆ, M. IMBESI, S. DZITOYEVA**

The Psychiatric Institute, Department of Psychiatry, University of Illinois at Chicago, Chicago, Illinois, USA

Recent research suggests that mechanisms for the physical marking of DNA and DNA-associated proteins, called epigenetic mechanisms or epigenetic code, are operative in the nervous system both during development and adulthood. An epigenetic code regulates the activity of genes and in contrast to genetic code mutations, epigenetic changes, which are influenced by the environment and occur throughout life, are reversible. In this presentation, we will review the current concepts regarding epigenetic mechanisms that involve DNA methylation and histone acetylation and their relevance for neuronal functioning. Stress and the glucocorticoid system have been linked to depression and post traumatic stress disorder (PTSD). Preclinical studies demonstrated the importance of the epigenetic code in regulating the glucocorticoid receptor promoter and its association with maternal programming of stress responses. Clinically, drugs such as valproate, which is clinically effective for the treatment of PTSD, are known epigenetic modifiers, e.g., histone deacetylase (HDAC) inhibitors. Our research concerns epigenetic regulation of the 5-lipoxygenase (5-LOX) gene. 5-LOX, an enzyme that metabolizes arachidonic acid, is typically considered for its role in the vascular endothelium and inflammation. In addition, 5-LOX is also expressed in neurons. Neuronal 5-LOX expression increases during aging and upon stimulation of the glucocorticoid receptors. Alterations of 5-LOX have been postulated as a contributing factor in depression and may be important in regulating glutamate GluR1 receptors. Using *in vivo* mouse models, primary cultures of cerebellar granule neurons, and neural cell lines, we demonstrated that both histone acetylation and DNA methylation are involved in the regulation of neural 5-LOX expression. A better understanding of the epigenetic regulation of neuronal gene expression is needed to therapeutically target the epigenetic code with brain-specific pharmacological tools.

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Abstract

# S2.2 Neurobiology of posttraumatic stress disorder

**N. PIVAC**[1], **D. KOZARIĆ-KOVAČIĆ, T. JOVANOVIĆ**

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Department of Psychiatry and Referral Centre of the Ministry of Health and Social Welfare for the Stress related Disorders, Dubrava University Hospital, Zagreb, Croatia

Posttraumatic stress disorder (PTSD) is a debilitating and severe, common psychiatric disorder, affecting 10% of all men and 18% of women. PTSD is a person's reaction to a life-threatening event that elicited fear, helplessness, or horror. PTSD is the only psychiatric disorder that requires an external event for diagnosis, however, the majority of individuals who experience severe trauma will not develop the disorder. Due to the increase in global conflicts and subsequent trauma exposure, PTSD will continue to increase in frequency. PTSD is, according to the DSM criteria, characterized by three major clusters, contributing to the complexity of its clinical presentation: a) symptoms of re-experiencing the event (intrusive thoughts, nightmares, flashbacks, and hyperarousal induced by reminders of the event); b) avoidance of stimuli associated with the trauma (avoiding activities, places, thoughts or feelings related to the trauma, as well as emotional numbing); and c) increased arousal (insomnia, irritability, impaired concentration, hypervigilance, and an exaggerated startle response). PTSD is a serious illness which includes functional impairment, occupational instability, marital problems, discord with family and friends, and difficulties in parenting. In addition, PTSD is often accompanied by substance abuse, and by different comorbidities, such as major depression, other anxiety disorders, somatization, personality disorders, and dissociative disorders. One of the major risks frequently associated with a PTSD diagnosis is suicidal behavior. Combat-related PTSD is an especially difficult and severe form of PTSD. According to USA data, around 830,000 Vietnam war veterans still fulfill the diagnostic criteria for current PTSD, with 31% of male and 27% of female veterans suffering from PTSD at some point during their lives after combat. The situation with current conflict in Iraq is even more serious, resulting in 13.18% of PTSD among active soldiers. During the 1991–1995 Homeland war in Croatia an estimated 1,000,000 people (i.e. war veterans with combat-related PTSD and prisoners of war, but also traumatized civilians in the combat zones, displaced persons and refugees, victims of terrorist attacks, civilian relatives of traumatized war veterans and terrorist attacks victims, and traumatized children and adolescents) were exposed to tremendous suffering, and about 10,000 of the Homeland War veterans (15% prevalence) developed PTSD. Approximately 60% of the 680 combat soldiers with PTSD had comorbid diagnoses, such as alcohol abuse, major depressive disorder, anxiety disorders, panic disorder and phobia, psychosomatic disorder, psychotic disorders, drug abuse, dementia, and suicidal behaviour. A significant proportion of combat veterans with PTSD developed psychotic features. The neurobiological basis of PTSD is far from clear, although a significant progress in the understanding of particular neurobiological alterations has been made in recent years. PTSD is associated with persistent morphologic changes in the brain, and alterations in neurotransmitter and neuroendrocrine function. Neuroanatomical changes include reduced hippocampal volume in PTSD patients, and these morphological changes might explain divergent cognitive disturbances (i.e. of sensory and memory processing) found in PTSD. Neuroendocrine changes include dysregulated activity of the hypothalamic-pituitary-adrenal axis, with reductions in cortisol and elevations in corticotrophin-releasing factor secretion, and a supersuppression of cortisol after a low dose of dexamethasone. Alterations in different neurotransmitter systems (noradrenaline, serotonin, glutamate, γ-aminobutyric acid, and endogenous opioids) are associated with PTSD. Both neuroendocrine and neurotransmitter systems are regulated by multiple genes *via* proteins involved in the synthesis, degradation, transport, reuptake, and receptors of these neurochemicals. These genetic, environmental, early traumatic and neurobiological factors are both inter-correlated and interdependent. To elucidate the neurobiological basis of PTSD these risk factors should be linked to smaller psychological sub-symptoms that can serve as endophenotypes of vulnerability and resilience to develop PTSD. Such heritable biomarkers will improve risk assessment, diagnosis, and treatment strategies for PTSD.

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Abstract

# S2.3 Genotype controlled analysis of plasma dopamin-beta-hydroxilase activity in posttraumatic stress disorder

**D. MÜCK-ŠELER**, N. PIVAC, M. MUSTAPIĆ, M. DEŽELJIN

Ruđer Bošković Institute, Division of Molecular Medicine, Molecular Neuropharmacology, Zagreb, Croatia

**Introduction**: Dopamine beta-hydroxylase (DBH) is the enzyme that converts dopamine to norepinephrine. Altered plasma DBH activity has been reported in various psychiatric disorders. Polymorphisms within the dopamine beta-hydroxylase (*DBH*) gene could be related to etiology of posttraumatic stress disorder (PTSD), given the well-documented changes in the catecholamine-mediated neurotransmission that occurs in this disorder. The aim of the present study was to investigate whether the functional polymorphism *DBH*-1021C/T (rs1611115) and plasma DBH activity were associated with PTSD, or with psychotic and non-psychotic subtypes of PTSD. **Methods**: Plasma DBH activity and *DBH*-1021C/T polymorphisms were determined in 329 male subjects: 162 healthy controls, 133 combat veterans with current and chronic PTSD (subdivided further into two subgroups according to the presence or absence of psychotic features) and in 34 veterans without chronic PTSD. PTSD diagnosis was done using the Structured Clinical Interview (SCID) for DSM-IV. Plasma DBH activity was determined by a photometric method and genotyping by standard RFLP technique. **Results**: There was a significant decrease in plasma DBH activity among war veterans with PTSD, regardless of the presence of psychotic symptoms, as compared to enzyme activity in war veterans without PTSD and healthy controls. No significant differences were detected in genotype or allele frequencies between healthy controls, war veterans with and without PTSD. War veterans with PTSD carrying CC genotype had significantly lower plasma DBH activity compared to all other subjects with the CC genotype. There was no relationship between smoking status, plasma DBH activity and polymorphisms within *DBH* gene. **Conclusion**: Since both groups of war veterans were exposed to the same trauma, it is possible that a pre-existing trait difference in regulation of noradrenergic function contributed to a differential vulnerability to develop PTSD, or that the regulation of DBH expression was different in response to trauma. The results suggest that genotype-controlled measurement of plasma DBH activity might be used as a potential biological marker of the response to trauma, and that further studies of *DBH* and other loci related to dopamine and noradreneline in PTSD are warranted.

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Abstract

# S2.4 Novel approaches to the treatment of posttraumatic stress disorder

**D. KOZARIĆ-KOVAČIĆ**

Department of Psychiatry and Referral Centre of the Ministry of Health and Social Welfare for the Stress related Disorders, Dubrava University Hospital, Zagreb, Croatia

Posttraumatic stress disorder (PTSD) can be treated with educational and psychosocial support, as well as psychotherapy. Another significant aspect of symptom management involves psychopharmacological treatment. The choice of drug, dosage, and duration of treatment depends upon the most prominent symptoms of PTSD, their chronicity, comorbidity of other psychiatric and physical disorders, side effects of the drugs, contraindication for other medication, and the patient's compliance. Pharmacotherapy can be indicated for the specific symptoms of anxiety, depression, impulse control, aggression, etc., or for one of the clusters of PTSD symptoms: core symptoms, intrusive re-experiencing of the traumatic events, avoidance of stimuli associated with the traumatic experience, numbing and anhedonia, hyperarousal, and secondary symptoms (comorbid diagnoses, poor functioning and resilience to stress). The goals of pharmacological treatment for PTSD include: reduction of distress symptoms, bolstering resilience, and restoration of functioning. Several neurobiological systems are involved in the etiology of PTSD. Because of the limited knowledge of the pathophysiology of PTSD and a lack of placebo-controlled clinical pharmacological studies, current clinical findings lead to the conclusion that serotonergic drugs are more effective than dopaminergic treatments, although both neurotransmitter systems are involved in the pathophysiology of PTSD. Benzodiazepines are not effective in the treatment for PTSD because of potential addiction and a lack of efficacy. Mood stabilizers can be effective because PTSD patients have affective instability and the mechanism of kindling can contribute to increased reactions to stressors. In chronic and resistant patients, and patients with psychotic features of PTSD, atypical antipsychotics are beneficial.

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Abstract

# S2.5 Posttraumatic stress disorder and insomnia

**Ž. VUKŠIĆ-MIHALJEVIĆ**

Clinical Hospital Osijek, Psychiatry Clinic, Regional Centre for Psychotrauma, Osijek, Croatia

Insomnia is an experience of inadequate or poor-quality sleep characterized by one or more of the following problems: difficulty in falling asleep, difficulty in maintaining sleep, waking up too early in the morning and sleep that is not refreshing. Insomnia also involves daytime consequences such as fatigue, lack of energy, difficulty in concentrating and irritability. Chronic insomnia is an important symptom of chronic posttraumatic stress disorder (PTSD). In recent years studies have suggested the emergence of changing perspective of etiology and pathophysiology and management of chronic insomnia in PTSD patients.This article reviews the findings about the disturbed sleeping in PTSD patients. Special emphasis is given to advantages and disadvantages of medications employed to promote improved sleep.

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Abstract

# S2.6 Dissociations: rediscovered concept in psychotrauma

**T. FRANCISKOVIĆ, J. GRKOVIĆ**

Clinical Hospital Center Rijeka, Psychiatric Clinic, Center for Psychotraumatology, Rijeka, Croatia

Dissociations take place when emotions, thoughts or sensations are separated from the rest of the mind. Although dissociation theory has been established almost a hundred years ago, especially in Pierre Janet's work, today's psychiatry is paying far more attention to this dissociative structuring of the mind, rediscovering the concept and thus finding new understanding of a number of diagnostic entities within psychiatry. The current formulation of PTSD is based on the notion that dissociated memories of trauma could be expressed in intrusive thoughts, affect states, sensory perceptions etc. Contemporary researchers and practitioners in the field of psychotraumatology conceptualized new theories and insights regarding structural dissociation of the personality, multiple self states model and somatoform dissociations.

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Abstract

# S3.1 Hepatitis C: Epidemiology and natural course of disease

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Hepatitis C is a blood-borne, infectious, disease that is caused by a hepatotropic virus called Hepatitis C virus (HCV). The infection can cause liver inflammation that is often asymptomatic, but ensuing chronic hepatitis can result later in cirrhosis (fibrotic scarring of the liver) and liver cancer. The Hepatitis C virus (HCV) is spread by blood-to-blood contact with an infected person's blood. The symptoms can be medically managed, and a proportion of patients can be cleared of the virus by a long course of anti-viral medicines. Although early medical intervention is helpful, people with HCV infection often experience mild symptoms, and consequently do not seek treatment. An estimated 150–200 million people worldwide are infected with Hepatitis C. In Europe and the U.S., those with a history of intravenous drug use, inhaled drug usage, tattoos, or who have been exposed to blood *via* unsafe sex or social practices are increased risk for this disease. Hepatitis C is the leading cause of liver transplant in the United States.

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# S3.2 Prevention of Hepatitis C

**B.BOŽIĆ**

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The following guidelines will prevent infection with the Hepatitis C virus, which is spread by blood:

avoid sharing drug needles or any other drug paraphernalia including works for injection or bills or straws;

avoid unsanitary tattoo methods;

avoid unsanitary body piercing methods and acupuncture;

avoid needle stick injury;

avoid sharing grooming utensils;

avoid sharing personal items such as toothbrushes, razors, and nail clippers.

Proponents of believe that strategies such are the provision of new needles and syringes or education about procedures, greatly decreases the risk of Hepatitis C spreading between injecting drug users. There is no vaccination that protects against contracting Hepatitis C.

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# S3.3 Laboratory diagnosis of viral Hepatitis C

**S. BALEN**

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Serological and molecular testing of the presence of Hepatitis C virus (HCV) plays a key role in the diagnosis of the infection and helps the clinician in making the appropriate therapeutic decision necessary for confirmation of the virological response to treatment and the long-term follow up of the patients. Hepatitis C virus infection is usually diagnosed by means of detection of antibody against HCV (anti-HCV) using enzyme immunoassays (EIA). These serologic assays detect HCV antibodies that indicate present and previous infection but they cannot discriminate acute from chronic or resolved infection. The average window period between infection and seroconversion is approximately 70 days. Screening-test-positive samples require serological or nucleic acid supplemental testing (NAT). Confirmation by recombinant immunoblot assay (RIBA) is needed only for low-risk patients. Individuals with indeterminate RIBA results should be evaluated by a sensitive HCV RNA detection test. The presence of HCV RNA in plasma defines active infection and can be detected 1 to 3 weeks post exposure. Patients with acute hepatitis of uncertain origin and negative hepatitis serology panels should undergo qualitative HCV RNA testing. Qualitative assays should be used to confirm viremia and asses the therapeutic response until quantitative assays with comparable sensitivities are available. An alternative approach to molecular techniques in reducing the window period is the detection of HCV antigen and anti-HCV in a single test that would speed up the diagnosis of infection and simplify the flowcharts in virology and transfusion units.

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# S3.4 Hepatitis C: Who should be tretaed?

**S. MIŠE**

Abstract not received.

# S3.5 Treatment of Hepatitis C

**R. OSTOJIĆ**

Abstract not received.

# S3.6 Antiviral treatment for patients with HCV and HIV coinfection

**B. DŽELALIJA**

Abstract not received.

# S3.7 Treatment the side effects of antiviral therapy

**M. ZILDŽIĆ**

Abstract not received.

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# S3.8 Future of HCV therapy

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Infection with hepatitis C virus (HCV) is a major worldwide cause of chronic liver disease, cirrhosis and liver cancer. Therapy has improved substantially since the introduction of interferon-α monotherapy in the late 1980 s. Responce rate was less then 10%. Now, combination therapy with peginterferon and ribavirin has a succes rate of about more then 50%. Important factors associated with treatment response include the race, age, sex, weight, biocemical and histologic characteristics of patients and aspecialy viral genotype. The current HCV therapy has limited efficasy and there is pressing need fornew and more effective therapies. A crucial challenge now is to manipulate such cellular targets pharmacologically for chronic HCV treatment, without being limited side effects. New therapeutic approaches to HCV include antisense therapy, hammerhead ribosymes, oral IFNs and specific enzyme inhibitors, such as protease, helicase and polymerase. We need oral drugs that are easy to take, non-toxic, inexpensive and efficacious.

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# S3.9 Experimental models of hepatitis

**P. SIKIRIĆ**

Abstract not received.

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Abstract

# S4.1 Physiology and pathophysiology of organic anion transporters (OATs)

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The efficient renal excretion of various endogenous and exogenous organic anions involves active secretion in proximal tubules. To accomplish this task, proximal tubule cells are equipped with a battery of organic anion transporters belonging to the SLC22 gene family. The organic anion transporters 1 and 3 (OAT1, OAT3) located in the basolateral membrane of proximal tubule cells release $\alpha$-ketoglutarate from the cells and »by exchange« take up organic anions from the blood. In the apical membrane, OAT4 mediates the release of organic anions into the urine in exchange for chloride ions. The physiological role of OAT2 that is located in the apical membrane in rodents and in the basolateral membrane in humans is not yet clear. OATs display a broad substrate specificity and handle anionic drugs such as analgesics, antibiotics, antihypertensives, antivirals, diuretics, uricosurics, and some cytostatics. Interaction of OATs with more than one compound can cause drug-drug interactions. OAT3, for instance, is the site of potentially life-threatening interaction between analgesics and methotrexate. OATs also interact with anionic toxins such as Ochratoxin A that is translocated by all OATs. Organomercurials are taken up from the blood by OAT1 causing a selective heavy metal damage of proximal tubule cells. OAT1 is also involved in the uptake of nephrotoxic antiviral drugs as well as of uremic toxins. Finally, OAT1 and OAT3 contribute to the uptake of the sulfooxymethylesters and could therefore have a role in the development of renal cancer. Organic anion transporters can also be used to prevent nephrotoxicity. OAT1 and OAT3 transport the chelator 2,3-dimercaptopropane-1-sulfonate (DMPS) that binds intracellular mercury and greatly facilitates the detoxification. Probenecid can be used to inhibit OATs and thereby to decrease the nephrotoxicity of antivirals during the therapy of AIDS patients. Since OATs contribute significantly to renal drug excretion, drug accumulation will occur when the activity of these transporters is decreased. In this respect it is noteworthy that female rats and mice express considerably less OAT1 than male animals. A chronic elevation of prostaglandins, e.g. during prolonged fever or after renal ischemia, can down-regulate OAT1 and OAT3 expression with possible side effects on the excretion of drugs. Taken together, OATs play a significant role in the excretion of various, widely prescribed drugs, and can cause drug-drug interactions and nephrotoxicity. In case of decreased OAT activity, drug accumulation and unwanted side effects can occur.

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Abstract

# S4.2 Structure and function of transporters that mediate hepatic and renal excretion of cationic drugs

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Organic cation transporters (OCTs) of the *SLC22* transporter family contain three subtypes. In renal proximal tubules and liver OCTs were localized to basolateral membranes and mediate the first step in renal and hepatic excretion of cationic drugs. Functional characterization showed that the three subtypes OCT1, OCT2 and OCT3 translocate organic cations with diverse structures in both directions across the plasma membrane. Their substrates include endogenous cations such as choline and monoamine neurotransmitters and a large variety of cationic drugs. New insights into the transport mechanism of OCTs were obtained by (*1*) parallel tracer-flux and electrical measurements, (*2*) mutagenesis experiments, (*3*) fluorometric measurements after labeling of cysteine residues at defined positions, (*4*) and modelling of tertiary structures of OCTs. OCTs mediate electrogenic transport of organic cations in both directions across the membrane. The charge-to-substrate ratio varies with membrane potential and with different substrates. Non-transported competitive inhibitors inhibit OCTs after application from both sides of the plasma membrane. This indicates that the substrate binding region can be oriented to the extracellular or intracellular side. Mutagenesis experiments and modelling of OCTs in analogy to the resolved crystal structure of LacY permease suggest that OCTs contain a deep cleft with an extended substrate binding region. When we replaced an amino acid in the 11th transmembrane a-helix (TMH) by cysteine and labeled this cysteine with a fluorescent dye, fluoresence changes were induced by substrate and inhibitors. This indicates movement of the 11th TMH during transport. Using cation-induced fluorescent changes as readout, low and high affinity interaction sites for cationic substrates and a non-transported cationic inhibitor were distinguished. When amino acids in a modelled contact region between the 2nd and 11th TMH were exchanged, high affinity binding of tetrabutylammonium that does not influence cation transport in wildtype rOCT1, leads to inhibition of transport activity in the mutants. The data indicate that rOCT1 contains a substrate binding region within a large cleft. During transport, the cleft opens successively to the extracellular and intracellular side of the plasma membrane. Two or more organic cations and some small ions may bind within the cleft and may be translocated together.

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Abstract

# S4.3 Gender and species differences in renal organic anion and cation transporters

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The kidneys maintain body fluid and electrolyte homeostasis using polarized localization of various transporters/carriers in the apical (luminal) and basolateral membrane domains of the cells along nephron. These transporters use ATP or transmembrane ion gradients to drive vectorial transport of their substrates in direction of secretion or reabsorption. The ATP-dependent carriers, termed ATP-binding casette transporters (Abc/ABC for animal/human transporters; primary active transporters) and ATP-independent solute carriers (Slc/SLC for animal/human carriers; secondary and terciary active transporters) are important for: a) handling of endogenous organic compounds that are produced during normal metabolism, such as organic anions (OA) and cations (OC), peptides, nucleosides, and their products, b) handling of exogenous (xenobiotic) organic compounds, such as food constituents (flavonoids, mycotoxins, pesticides, other alimentary organic substances), and drugs (various antibiotics and chemotherapeutics), c) drug-drug interactions, and d) development of nephrotoxicity and specific transporter-related diseases. Because of their major importance in drug transport and drug-mediated nephrotoxicity relevant to humans, a bulk of current research in the field of renal transporters has been dedicated to the roles of OA and OC transporters (Oats for animal/OATs for human, and Octs for animal/OCTs for human transporters, respectively). However, most of these data were obtained from the experiments in animals, largely rats and mice; only a limited amount of data is from other animal species and humans. Recent observations in experimental animals and humans have indicated that renal secretory and reabsorptive functions under various physiological, pharmacological, and toxicological conditions may be different in males (M) and females (F), and that these differences may be related to sex hormone-regulated expression and action of specific transporters in one of the membrane domains of (mainly) proximal tubules (PT). Accordingly, a) M-dominant gender differences (GD) in renal excretion/clearance in rats and mice were observed for some OA, such as *p*-aminohippurate (PAH) and furosemide, due to androgen-stimulated expression of their carriers Oat1 and Oat3 in the PT basolateral membrane (BLM), b) renal accumulation and toxicity of mercury in rodents is M-dominant, because this toxic metal can enter the PT cells by molecular mimicry, e.g., bound to SH groups in some OA and thus transported *via* basolateral Oat1 and/or Oat3, c) M-dominant GD in renal handling of some OC in rats, such as tetraethylammonium (TEA), amantadine, and cisplatin, are determined by androgen-stimulated expression of Oct2 in the PT BLM, d) F-dominant renal clearance/excretion of various metabolic products and xenobiotics in rats (cyclacillin, carnitine, egualen sodium, perfluorooctanoic acid, estradiol-17β-glucuronide, taurocholate, dibromosulfophtalein, zenarestat) may be related to the lower expression of the respective transporters in the PT brush-border membrane (BBM) (Pept1, Octn2, Oatp1, Oat2), resulting in lower reabsorption of the filtered compounds in F. Where studied, the expression of several transporter (Oat1, Oat2, Oat3, Oat5, Oct2) were low and similar in M and F before the puberty; GD occurred after the puberty. Contrary to the numerous cases of gender-dependent, carrier-mediated transport of specific organic compounds in rats and mice, a very few reports have been related to other species, including humans. In rabbits the expression of Oat1, Oat2, Oat3, and Oct2, in the PT significantly increased after the puberty, however without exhibiting sex differences in adult animals. Accordingly, rabbits exhibit no GD in urinary excretion of OA PAH and OC TEA. Some comparable data were also found in dogs. Furthermore, studies in humans showed that: a) the plasma level of acetylsalicylate, which is a substrate of several OATs (OAT1-4), was higher in F, b) the renal clearance of urate in F is higher than in M, possibly due to lower reabsorption of urate *via* weakly expressed URAT1 in the PT brush-border membrane, c) the renal clearances of the OA ciprofloxacin (a substrate of OATs), and of the OC amantadine (a substrate of OCT2), were found to be higher in M, but gender-related expression of the respective carriers in the human nephron has not been

reported. Some other therapeutic drugs in humans (verapamil, metronidazole, vecuronium) also exhibit GD in their blood clearance, but a possible contribution of renal excretion/relevant transporters are not known. Humans and animals exhibit a similar set of renal transporters, but the presence, localization along the nephron, and intracellular localization of some carriers seem to be different from those in experimental animals. For example: a) Oat2 in rats and mice was localized to the PT BBM, whereas in humans, OAT2 was localized to the PT BLM, b) Oct1 was localized in the rat kidney largely to the PT BLM, whereas in the human kidney, OCT1 was not detected, c) Oct2 in the rat kidney was localized predominantly to the BLM of PT S3 segment, whereas OCT2 in the human kidney was localized to the BLM along the entire PT, and d) OAT4 in the human kidney was localized to the PT BBM, whereas a similar protein was absent from the rat nephron. These species differences in the expression and/or cellular localization of some transporters may influence the secretory and/or reabsorptive direction of renal transport of their substrates and thus may affect their urinary excretion. This further indicates that the findings regarding GD and effects of sex (and, possibly, other) hormones upon renal transporters in one species can not simply be regarded as relevant for other species.

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Abstract

# S4.4 Organic anion and cation transporters in experimental ochratoxin A nephrotoxicity

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Ochratoxin A (OTA) is a ubiquitous fungal metabolite with nephrotoxic and carcinogenic potential. Various animal models have demonstrated that OTA mainly impairs proximal tubule (PT) function and causes glucosuria, enzymuria, and diminished secretion of organic anion *p*-aminohippurate (PAH). The last indicates that OTA toxicity may target renal organic anion transporters (Oats; subfamily of Slc22a drug transporters). Oat1 (Slc22a6) and Oat3 (Slc22a8) proteins reside in the basolateral membrane (BLM) of PT epithelial cells and mediate both OTA and PAH transport in a noncompetitive mode. Previous studies have shown that subchronic intoxication of rats with OTA leads to a dramatic reduction of PAH clearance/excretion, indicating that Oat1 and Oat3 may be involved in OTA nephrotoxicity, possibly via inhibiting their transport activity and/or expression in the cell membrane. However, these possibilities have not been clarified. In order to study effects of OTA on cell/tissue morphology and on cellular expression of renal Oat1 and Oat3, especially in view of male-dominant expression of these transporters in renal proximal tubules in rats and mice, we used an established subchronic *in vivo* model of OTA intoxication in adult male (M) and female (F) Wistar rats. The animals of both sexes were treated with various doses of OTA (0, 50, 125, 250 & 500 μg/kg b.w., p.o., every 2nd day for 10 days). Using peptide-specific polyclonal antibodies, Oats were studied by Western blotting (WB) in total cell membranes (TCM) isolated from the cortex (C) and outer stripe (OS) tissues, and by immunofluorescence cytochemistry (IC) in tissue cryosections, whereas the expression of their mRNA was analyzed by RT-PCR using RNA from the renal C and OS. The study of tissue morphology revealed that OTA treatment resulted in damaged PT S3 segments in medullary rays, manifested by the loss of regular tubular structure and cell membranes, cell desquamation and other necrotic events. These phenomena were more severe at higher OTA doses, and were much more pronounced in M. As found in WB experiments, in spite of different levels of the Oat1 and Oat3 protein expression in isolated membranes (M>F), their expression in OTA-treated rats in both sexes followed a similar and dual, dose-dependent pattern. At low OTA doses (50–125 μg/kg b.w.) the expression of Oat1 and Oat3 protein increased ~50% and ~300%, respectively, whereas high OTA doses (250–500 μg/kg b.w.) decreased the expression of Oat1 (~70%) but not of Oat3 in respect to controls (vehicle-treated rats). By IC, the staining intensity of both transporters in proximal tubules completely matched the WB data. However, the RT-PCR data showed that the Oat1 mRNA expression was unaffected by low OTA doses, whereas high OTA doses downregulated it, and the expression of Oat3 mRNA was unaffected by any OTA dose. Also the expression of housekeeping gene GAPDH mRNA remained unchanged in all OTA-treated animals. Our data indicate that in rats: 1) the OTA treatment causes dose-related and M-dominant damage to cell morphology in proximal tubule S3 segments, 2) Oat1 and Oat3 have a similar pattern of protein expression for both sexes, with upregulation of both transporters at low but downregulation of only Oat1 at high OTA doses, 3) the upregulation of Oat1 and Oat3 proteins seems to be mRNA-unrelated and post-transcriptional, 4) previously observed inhibition of the renal PAH transport/secretion in rodents treated with high OTA doses may be explained by the loss of cell plasma membrane (transporting surface) and Oat1 protein in this membrane, and 5) upregulated Oat1 and Oat3 proteins at low OTA doses may possibly enhance OTA uptake and accelerate the development of nephrotoxicity.

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Abstract

# S5.1 Contemporary treatment and care for people with Alzheimer's disease and Croatian reality

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For the treatment of dementia in persons with Alzheimer's disease (AD), at the moment in the World, five drugs (tacrine; donepezil; rivastigmine; galantamine and memantine) are approved. Among them in Croatia, till now, only two e.g. donepezil (Aricept; Yasnal) and memantine (Ebixa) are registered, but not single one is on reimbursement list of Croatian Institute for Health Insurance. Thus, these drugs have relatively high cost which makes them almost unavailable to patients. Even though Croatian algorithm for psychopharmacological treatment of AD, done by Croatian association for clinical psychiatry, exists and suggests which anti-dementia drug should be given in particular stage of AD, these patients are more often prescribed alternative or adjuvant treatment (ginkgo biloba; nootropics; statins; nonsteroidal anti-inflammatory drugs; omega-3 fatty acids; vitamins; NADH; trace elements; etc.). Among people with dementia (PWD) along with cognitive deficits often there might be present behavioural disturbance, sleep disturbance, psychiatric symptoms (depression, anxiety, psychosis with or without hallucinations, mania, obsessive-compulsive disorder, alcohol dependence), involuntary emotional expression disorder and other. For treatment of these conditions, clinicians use antipsychotics, antidepressants, anxiolytics, hypnotics and other medication. Since current therapy of AD is not etiological, and AD is chronic and progressive disease, over period of time the care for patient becomes essential and the role of career becomes crucial. Therefore, education for caregivers and concern for there own health represent significant part in complete care and management of AD. Establishing daily-care centers for PWD will help their families a lot and lengthen patients period spent at home. In advanced phases of AD, in spite of contemporary psychopharmacotherapy and family care, these patients will demand accommodation in specialized institutions and therefore it is necessary to stimulate development of nursing homes for PWD which will provide adequate professional care and treatment 24-hours a day. Today we record tremendous increase in prevalence of AD everywhere in the World, and it is expected that the number of cases will quadruple by 2040 making AD leading health priority. Therefore, it is understandable that great effort and humongous amount of money is put in research, on a daily bases, of new treatment possibilities of this late-life disease. In the near future we expect greater improvements in practical psychopharmacological therapy of PWD, but also some positive movements in PWD care in Croatia.

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Abstract

# S5.2 CSF phosphorylated tau proteins as predictors of Alzheimer's disease in subjects with mild cognitive impairment

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Major efforts are under way to define reliable biomarkers of Alzheimer's disease (AD). With the advent of new drugs such as gamma-secretase inhibitors, early detection of elderly subjects with mild cognitive impairment (MCI) who are destined to develop AD is becoming increasingly important. Highly significant increases of hyperphosphorylated tau proteins in cerebrospinal fluid (CSF) have been recently reported in AD patients compared with controls by several independent groups, including ours. These findings support the notion that CSF phosphorylated tau proteins may be useful biomarkers in the early identification of AD in MCI subjects.

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Abstract

# S5.3 »Amyloid cascade« hypothesis: is it true for sporadic Alzheimer's disease?

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Huge progress in understanding and treating of Alzheimer's disease (AD) has started whith the era of AD modeling. However, experimental model that recapitulates all aspects of Alzheimer's disease (AD) has not yet been produced although various transgenic mouse lines have been designed that offer relatively faithful reproductions of some of the main neuropathologicall features; extracellular senile plaques composed of unsoluble amyloid beta (Aβ) fibrilles, and intraneuronal neurofibrillary tangles composed of hyperphosphorylated form of microtubule associated protein tau. Currently, the leading hypothesis assumes that pathological assemblies of Aβ are the cause of all forms of AD, whereas other neuropathological changes are downstream consequences of pathological Aβ accumulation. However, this hypothesis fits only to the familial, early onset type of AD caused by mutations in three particular Aβ-related genes, while the great majority of AD patients have sporadic late-onset type of disease (sAD) with age and several susceptibility genes as risk factors. In line with the brain insulin dysfunction found post-mortem in sAD patients, streptozotocin-intracerebroventricularly treated rats (STZ-icv) have been recently proposed as an experimental model for sAD because STZ is a drug selectively toxic for insulin producing/secreting cells. Central, icv application of STZ is associated with progressive deficits in learning and memory, decrement in cholinergic transmission and decreased glucose and energy metabolism in the brain. To further characterize this model we investigated the brain insulin system and influence of its dysfunction on tau protein and amyloid beta (Aβ) peptide following STZ-icv administration. Gene expression of insulin and insulin receptor (IR), alterations of IR-beta subunit, IR tyrosine kinase (TK) activity, expression of protein kinase B (Akt/PKB), glycogen synthase kinase 3 (GSK-3) and tau protein were measured by RT-PCR, ELISA, TK assay and Western blot in frontoparietal cortex (CTX) and hippocampus (HPC) of adult STZ-icv (1 mg/kg) treated rats. Aβ aggregates were visualised by Congo red staining. Cognitive deficits were measured in Morris Water Maze Test. Data were analysed by Cruscal-Walles ANOVA and Mann-Whitney U test ($P<0.05$). Expression of insulin-1 (HPC) and -2 (CTX) and IR mRNA (CTX, HPC) was decreased. Phosphorylated IR-beta subunit content was increased (CTX) and TK activity increased (HPC). Akt/PKB and phosphorylated/non-phosphorylated GSK-3α/β ratio were decreased (HPC) GSK-3-related hyperphosphorylation of tau protein (HPC) and Aβ aggregates in meningeal capillaries were found not earlier than 3 months after STZ-icv treatment. Cognitive deficits were found as early as 2 weeks after STZ-icv treatment. Results support STZ-icv rat as a representative experimental sAD model which suggests that instead of Aß, imbalance in IR signaling cascade phosphorylation/dephosphorylation and insulin resistant brain state could be the primary pathological event in sAD ethiopathogenesis.

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# S5.4 Clinical trials with new drugs for the treatment of neurodegenerative disorders

**T. BABIĆ**

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Abstract

# S6.1 Association of TNF $\alpha$ and PTPN22 gene polymorphisms with type I diabetes in Croatian patients

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**Introduction**: Type I diabetes is autoimmune disease characterized by activated T-cell-mediated destruction of the pancreatic β-cells. Polygenic component of the disease has been supported by identification of major susceptibility alleles in HLA class, insulin and CTLA-4 loci. However, single nucleotide polymorphism in *TNF*-α and *PTPN22* genes has been recently identified as susceptibility factor for inflammatory autoimmune disease. Variants of *TNF-alpha and PTPN22* genes have been investigated in diabetic patients of other ethnic groups, but there is no existing data for Croatian patients. We used association study to evaluate relationship between type I diabetes and single nucleotide polymorphisms in *TNF*-α and *PTPN22* genes. **Material and Methods:** *Subjects:* The study included 102 patients with type I diabetes. Patients were carefully assessed and categorized as per recent classification by the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2001. All patients were recruited from Vuk Vrhovac University Clinic Zagreb, after giving written informed consent for the participation. The control subjects included 154 unrelated, healthy volunteers, obtained from the Croatian Tumor and DNA bank. DNA extraction from venous blood was performed using sodium chloride method. *Single nucleotide polymorphisms* The genotyping of *TNF*-α G-238A, G-308A and *PTPN22* C1858T variants was determined by PCR-RFLP method. Amplified products were digested overnight with restriction enzymes. *Nco*I and *Msp*I were used for detection G to A transition in promoter of *TNF*-α gene (uncut *A* allele and cut G allele). *Rsa*I enzyme is used for detection C to T nucleotide variants in coding region of *PTPN22* gene (uncut T allele and cut C allele). Digested products were separated by electrophoresis on 10% polyacrilamide gel and stained by silver. *Statistical analysis:* The distribution of tested polymorphisms was compared between patients and healthy controls by the $X^2$ test. A p value $=0.05$ was considered significant. **Results**: The frequency of AG/AA genotype and A allele at *TNF*-α -308 site was significantly higher in patients with type I diabetes than in controls ($X^2=66.27607$, df=2, $p<0.001$; $X^2=37.08470$, df=1, $p<0.001$, respectively). The frequency of AG/GG genotype and A allele at *TNF*-α –238 site was not significantly different between these two groups. The frequency of CT/TT genotypes as well as T allele at 1858 position in *PTPN22* gene was significantly higher in patients in comparison to healthy volunteers ($X^2=33.70588$, df=2, $p<0.001$; $X^2=30.61254$, df=1, $p<0.001$, respectively). **Conclusion**: These results present evidence of association between *TNF*-α A-308G and *PTPN22* C1858T genetic polymorphisms and type I diabetes in Croatian population.

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Abstract

# S6.2 Influence of hyperglycemia on gene expression during embryonic development

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**Introduction**: Diabetic embryopathy is a complication of diabetes in which the embryo of a diabetic mother develops congenital malformations, mostly of the hart and the neural tube. These malformations arise at the beginning of organogenesis during the first 8 weeks of gestation in human embryos, and during the first 7–10 days in mouse and rat embryos. Our intention was to test whether and to what extent hyperglycemia influences the expression of several genes involved in neural embryonic development in NOD (non-obese diabetic) mice. In order to asses if altered gene expression detected *in vivo* is due to hyperglycemia, we tested *in vitro* system that mimics early embryonic development (differentiation of mouse embryonic stem cells as embryoid bodies in culture) at different glucose concentrations. **Materials and Methods**: *In vitro* R1 mouse embryonic stem cell line was cultured and propagated on mitotically inactive mouse primary fibroblasts in a culture medium supplemented with leukemia inhibitory factor (LIF). Differentiation was triggered by omitting LIF from culture medium and growing embryonic stem cells as aggregates (embryoid bodies) in non-adherent Petri dishes. Embryoid bodies were cultured under three glucose concentrations (5,5 mmol/L; 25 mmol/L; 36 mmol/L). At different time points embryoid bodies were harvested, homogenized and RNA was extracted. Semi quantitative RT-PCR was performed by three sets of primers representing following genes: pax3, neuroD1and ngn3. *In vivo* hyperglycemic and normoglycemic NOD mice were mated and embryos were recovered 9.5 days *post coitum*. Blood of the female mice was extracted just before mating and immediately after the sacrifice, and the glucose level was determined by glucose oxydase. Embryos from each mother were pooled and RNA was extracted. Semi quantitative RT-PCR was performed by nine sets of primers representing following genes: pax3, pax4, pax6, neuroD1, ngn3, sod1, sod2, gpx1, and cat. **Results**: Preliminary results of our study confirmed previous findings that the expression of transcription factor pax3 gene is significantly inhibited in embryos of diabetic NOD mice (in contrast to embryos of normoglycemic NOD mice). Gene for transcription factor neuroD1 displayed even more dramatic down regulation. In contrast, embryoid bodies grown in culture at various glucose concentrations (5.5 mM, 24.8 mM, 35.8 mM) and harvested for RNA isolation after 3, 12 and 14 days displayed strong and equal neuroD1 expression in all tested samples, while pax3 expression was completely undetectable in the same samples. Gene for transcription factor ngn3 could not be detected in any sample both *in vivo* and *in vitro*. Since all other tested genes (transcription factors pax4 and pax6 as well as genes coding antioxydative enzymes sod1, sod2, gpx1 and cat) were expressed equally in all embryos regardless of mother's glycemic status, they have not been tested *in vitro*. **Conclusion**: Our preliminary study indicates strong down regulation of neuroD1 expression in embryos of diabetic mothers. Since NeuroD1 transcription factor is required during development of the central as well as the peripheral nervous system, consequences of its loss should be investigated further. Since we did not observe the similar effect *in vitro,* higher glucose concentrations for culturing embryoid bodies should be applied. Morover, analysis of the protein expression is needed.

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# S6.3 Targeting JAK3 with WHI-P131 for the prevention of autoimmune type 1 diabetes in NOD mice

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Here we show that Janus kinase (JAK) 3 is an important molecular target for the treatment of autoimmune insulin-dependent (type 1) diabetes mellitus. The rationally designed JAK3 inhibitor WHI-P131 exhibited a potent immunomodulatory activity and delayed the onset of diabetes in the NOD mouse model of autoimmune type 1 diabetes. Whereas 60% of vehicle-treated control NOD mice became diabetic by 25 weeks, the incidence of diabetes at 25 weeks was only 9% for NOD females treated with daily injections of WHI-P131 (100 mg/kg/day) from week 10 through week 25 ($P = 0.007$). Furthermore, WHI-P131 prevented the development of insulitis and diabetes in NOD-scid/scid females after adoptive transfer of splenocytes from diabetic NOD females. The increased secretion of IL-10 was found to be the potential mechanism of the protective anti-diabetogenic action of WHI-P131 treatment. Chemical inhibitors of JAK3, such as WHI-P131, may provide the basis for effective treatment modalities against human type 1 diabetes. To our knowledge, this is the first report of the immunosuppressive activity of a JAK3 inhibitor in the context of an autoimmune disease.

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# S6.4 Opioids and oxidation stress

**T. BALOG**

Abstract not received.

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Abstract

# S6.5 Dual effect of native propolis *in vivo* as a consequence of selective gene expression

**S. SOBOČANEC, A. ŠARIĆ, M. KOROLIJA, M. MARJANOVIĆ, M. KRALJ, J. KNEŽEVIĆ, M. ČRETNIK, T. MAROTTI**

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Propolis is a resinous substance considered to have a broad spectrum of biological activities. Previously we showed that propolis had the opposite dose- and tissue- dependent effects on oxidative/antioxidative status in murine liver, lungs and brain: it decreased lipid peroxidation (LPO) in a lower dose by increasing copper-zinc superoxide dismutase (CuZnSOD, sod-1) activity in liver, mangan superoxide dismutase (MnSOD, sod-2) and catalase (cat) activity in lungs while brain remained unaffected while in higher dose it showed prooxidative effect by reverting LPO to control level and increasing catalase activity. In this paper we investigated the effect of native propolis on expression pattern of sod-1, sod-2, cat and glutathione peroxidase-1 (gpx-1) on RNA and protein level using semiquantitative RT-PCR and Western blott analysis. Since we observed no correlation among RNA and protein level of antioxidant enzymes with their corresponding activities, the gene expression profile of 96 genes indicative for stress and toxicity (SuperArray Inc.) and Real-time PCR was performed. These results showed that native propolis induced the upregulation of interleukin-1 beta (il-1β) and nitric oxide syntethase-2 (nos-2) in liver at higher dose and downregulation of interleukin-1 alpha (il-1α) at both doses in lungs. Brain remained intact upon treatment in any dose. Thus, its dual effect on oxidant/antioxidant parameters might involve some other regulation mechanisms beside antioxidant enzyme system.

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Abstract

# S6.6 Endomorphins modulate nitric oxide release

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**Introduction**: Endomorphin 1 and 2 are newly discovered opioid tetrapeptides which structure is more resistant to enzymatic degradation then other opioid peptides. Endomorphins 1 and 2 are considered as endogenous ligands with high affinity for $\mu$ receptors. Number of studies have shown that opioid peptides *per se* can induce nitric oxide release from rodent and human immune cells. Endomorphins seemed to be involved in the process of vasodilatation by stimulating nitric oxide release. **Materials and Methods**: In our study we investigated both *in vitro* and *in vivo* modulating effect of endomorphins on nitric oxide release from mice macrophages. For the *in vitro* experiments, J774 mice macrophages were stimulated with different concentration of endomorphins 1 or 2 for measuring nitric oxide release using Griess assay. For the determination of the *in vivo* effect of endomorphins on macrophage-derived NO, female CBA mice were injected i.p. with endomorphins 1 or 2 (either 1 or 2.5 mg/kg bw). In order to investigate whether the effect of endomorphins was $\mu$ opioid receptor mediated, mice were injected with β-funaltrexamine hydrochloride (β-FNA, a μ-selective antagonist) 30 min before endomorphins. The animals were sacrificed two hours after endomorphin treatment and isolated peritoneal macrophages were either left unstimulated or exposed to 10 μg/ml LPS for 48 h. Following stimulation the nitrite concentration in the culture medium was measured using Griess method. **Results and Conclusion:** Since a 48 h incubation of J774 macrophages did not change nitric oxide release when measured with Griess method, we further investigated *in vivo* effect of endomorphins on mice peritoneal macrophages. Results showed that endomorphins modulate NO release *in vivo* in a dose-dependent manner and also depending on LPS stimulation response. Since a μ-opioid receptor specific antagonist β-FNA inhibited nitric oxide release from endomorphin treated mice, the effect seems to be μ-opioid receptor mediated.

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Abstract

# S7.1 Investigation of polyphenols from Croatian natural sources

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Various properties of polyphenols isolated from propolis and wines from Croatia have been investigated. These dietary compounds have been reported to have multiple biological activities like vasodilatatory, anti-inflammatory, antiviral, antibacterial and antioxidant effects. Also, beneficial effect in the prevention of various degenerative diseases, types of cancer and cardiovascular diseases, have been observed. In addition of being one of the essential nutrients, plant phenolics exert a remarkable array of biochemical and pharmacological activities including modulation of immune functions. Propolis is a natural substance collected by honeybees from a variety of plant sources. Its composition and thus its content of pharmacologically active compounds (flavonoids and phenolic acids) depend on geographic origin. Wines contain a wide range of polyphenols that include phenolic acids, trihydroxystilbene- resveratrol, the flavonols (e.g. quercetin and myricetin), flavan-3-ols (e.g. catehin and epicatehin), as well as polymers of the latter, defined as procyanidins and anthocyanins. Different analitycal techniques have been used for determination of polyphenols in wine and propolis samples such as thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The best mobile phases for separation of polyphenols on silica gel TLC plates were selected using methods of numerical taxonomy. This method includes three mathematical techniques: calculation of the information content (*I*) derived from *Shannon's* equation; determination of discriminating power (*DP*) and formation of clusters and dendrograms. In QSAR study of antioxidant activity of polyphenols from Croatian wines we demonstrated that antioxidant activity of polyphenols, as hydrogen donating free radical scavengers, is closely related to their chemical structure, especially with number and arrangement of free hydroxyl groups on flavonoid skeleton, or on phenol ring of phenolic acids. Early screening of physicochemical and ADME (Absorption, Distribution, Metabolism and Elimination) properties has become the key interest in drug discovery. The aim of these investigations was to compare chromatographic parameters obtained by reversed-phase high performance liquid chromatography (RP-HPLC), immobilized artificial membrane high performance liquid chromatography (IAM-HPLC) and human serum albumin high performance liquid chromatography (HSA-HPLC) in order to predict pharmacokinetic behaviour of flavonoids and phenolic acids. Furthermore, we wanted to investigate correlations between experimentally obtained chromatographic parameters and ADME parameters predicted by different computer programs. In our study we also investigated immunomodulatory effects of 31 structurally diverse flavonoids, 9 phenolic acids and 12 propolis samples from different Croatian regions. To establish the influence of chosen standard compounds and propolis samples on production of reactive oxygen species and activity of antioxidative enzymes (superoxide dismutase and glutathione peroxidase), together with the influence on cytokine (TNFa, IL-1ß, IL-2) and chemokine (IL-8) production, we used appropriate cell culture models. Using mathematical modeling, we established the relationship between the structures of investigated flavonoids and their immunomodulatory activity. After performed chromatographic analysis, we compared the activity of complex mixtures (propolis samples) and standard compounds as well. The results of our investigations have shown that Croatian natural products contain curative substances which should be used in therapy.

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Abstract

# S7.2 Natural sources and chemistry of resveratrol

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The link between nutrition and a number of diseases is becoming clearer every day. While the consumer is advised to enrich the nutrition with antioxidative compounds, antioxidant mechanisms are being investigated, natural antioxidant sources identified and bioactive molecules isolated. Resveratrol (3,5,4'-trihydroxystilbene) has emerged as one of the key molecules among bioactive phenols, due to its antioxidative, cardioprotective, antimutagenic, antiinflammatory, and especially emphasized, antitumor effect. The number of plants able to synthesize stilbenes is limited. It is mostly present in small amounts, being one of the phytoalexins, synthesized by plants as a response to injury. Although resveratrol has been identified as active ingredient of some appreciated medicinal herbs, such as *Polygonum cuspidatum*, a plant with special position in traditional medicine of East, its content in our medicinal herbs has not been investigated. It can be supposed that some plants traditionally used in Croatian folk medicine are rich with resveratrol or its closely related conjugates and derivatives which also show biological activity and have potential therapeutic value. The results of screening of 70 medicinal plant extracts confirm that individual medicinal plants can be an important dietary source of phenolic compounds with high antioxidant capacity comparable with red wine or beverages like tea. The presence of free resveratrol monomers was confirmed in individual plant infusions prepared in common way in which teas are prepared for human consumption. During further investigations, extraction of phenolic compounds from plant material was conducted using alcoholic solvents. As the structure-activity relationship examination showed that glycosylation of the stilbenes could implicate with their biological properties, extraction was conducted under mild conditions to protect true structure of phenolic compounds. Polyphenolic composition and antioxidant properties of medicinal plant extracts were analyzed. Free radical scavenging ability and ferric reducing antioxidant power of plant extracts were compared at equal concentration value of total phenols. The separation and quantification of stilbenes by HPLC-RP-PAD, using internal standard method, indicated significant differences in the polyphenolic composition of selected plant extracts. Free resveratrol monomers, if found, were present in very small amounts, mostly as E-isomer. The results of investigations strongly indicate that resveratrol or its hydroxy or methoxy derivatives are present in row plant material as glucosides. Resveratrol derivatives piceid (3,4',5-trihydroxystilbene-3-β-D-glucoside), astringin (3,3',4',5-tetrahydroxystilbene-3-β-D-glucoside) and especially isorhapontin (3'-methoxy-3,4', 5-trihydroxystilbene-3-β-D-glucoside) were confirmed as dominant stilbenes in controlled medicinal plant extracts. Among the controlled plant material the *Teucrii montani herba, Salviae folium*, and *Fragariae folium* extracts can be considered the best sources of resveratrol isorhapontin and piceid. As these medicinal plants are well-known in phytomedicine their medicinal properties may be related to presence of these resveratrol derivatives. The results of plant potential research, regarding the content of biologically active stilbenes, could motivate the development of new ways of using resveratrol-rich plant material, and find its application in the food industry (biologically valuable food production), as well as pharmaceutical industry.

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Abstract

# S7.3 Pharmacologically active volatile compounds from some aromatic plants

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Reactive oxygen species, which are generated during metabolic process of aerobic cell life, cause the damage of various macromolecules like proteins, nucleic acids and lipids. That may contribute to numerous chronic diseases like atherosclerosis, rheumatic diseases, tumors and different neurodegenerative diseases like Alzheimer's and Parkinson's diseases. By ingestion with foods of diverse antioxidants it is possible to influence oxidative processes in living organisms. For this reason there is a growing interest for biologically active natural compounds from medicinal plants. The goals of these investigations are the isolation, fractionation, identification and examination of biological activities of volatile components of selected aromatic plants. Their antioxidant potential, antitumor activity inhibitory properties on LDL proteins and acetylcholinesterase will be presented. In process of isolation and identification of antioxidant compounds, the conventional extraction methods have been used. Separation of active components is performed using chromatographic techniques. Identification of chemical composition and amount of volatile compounds has been performed using chromatography and spectrometry (GC-MS, HPLC, UV/VIS). Antioxidant activity is examined using following standard methods: DPPH radical scavenging, FRAP the ferric reducing ability of plasma, TBARS method with thiobarbituic acid and RANCIMAT automatic method for identification of oxidative stability of fats and oils. Research on acetylcholinesterase activity, in absence and presence of inhibitory compounds, is performed with standard UV/VIS spectrophotometric methods. Antitumor activity is demonstrated *in vitro* and *in vivo* using one L929 mouse fibroblasts and two tumor cell lines: squamous cell carcinoma – SCC VII and fibrosarcoma – FsaR. It was showed that aromatic plants possess biologic active volatile compounds with antioxidant, inhibitor properties on LDL proteins, acetylcholinesterase and antitumor activity. They are present as free volatiles in essential oil or as hidden potential in form of volatile aglycones which are glycosidically bound on sugar unities. Their biologic activity is demonstrated after catalytic transformation or hydrolysis.

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Abstract

# S7.4 Mechanisms of antioxidatory and vasodilatatory effects of red wine

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Numerous studies have shown that moderate consumption of wine, especially red wine, has beneficial effects on cardiovascular system. These effects are mainly attributed to wine polyphenolics. Plasma antioxidant capacity elevation and change in human vascular reactivity after wine consumption are important biological effects which mechanisms are still undetermined in many aspects. Wine polyphenolics are insufficient to explain theacute increase in plasma antioxidant capacity after red wine consumption, as their bioavailability is rather low. It is now recognized that parallel increase in plasma urate concentration after red wine consumption significantly contributes to the observed increase in plasma antioxidant capacity. Vasodilatation induced by red wine is also mainly attributed to the polyphenolics, especially to flavonoids. The role of non-flavonoids and non-polyphenolics from wine is less studied, and their effects are mainly unknown. Ethanol induced effect is especially interesting, as ethanol induces vasodilatation in *in vivo* conditions, which makes interpretation of specific vasoactive properties of other wine constituents difficult. *In vitro* antioxidant activity of wine polyphenolics is clearly related to its chemical structure, and *in vitro* antioxidant activity of wine and its derivatives are mainly dependent on their polyphenolic content. In *in vivo* conditions this is not necessarily the case. In the series of studies, by using red wine (RW), dealcoholized red wine (DARW), polyphenols-stripped red wine (PSRW), ethanol-water solution (ET) and water (W), we investigated the effects of red wine and its derivatives on endothelial function, both in human subjects and isolated rat vessels, in order to distinguish the role of wine polyphenols from other wine constituents. The role of wine polyphenols and induction of plasma urate elevation on plasma antioxidant capacity in humans, as well as constituents in the red wine responsible for the plasma urate increase, were also examined.We have determined in what extent the effects observed in *in vitro* biochemical measurements and isolated organ studies can be linked with the effects in humans after intake of tested wine and beverages based on that wine. Particularly, we determined the correlation between antioxidant activity of wine constituents with endothelial function and vascular response *in vitro* and *in vivo*. Antioxidant activity was measured by »Ferric Reducing Antioxidant Power« and »Trolox Equivalent Antioxidant Capacity« methods. Chemical analysis of wine and plasma were performed by spectrophotometer and chromatographic methods. The endothelial function in humans following acute intake of red wine and its derivatives was estimated by »flow mediated dilatation« method that measures reactive hyperemia after occlusion of brachial artery.

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Abstract

# S7.5 Regulative aspects of herbal medicines

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Herbal medicine is any medicine, exclusively containing as active ingredients one or more herbal drugs, herbal preparations or their combination. The use of herbal medicines as the first choice in the treatment of minor diseases or disorders continues to expand rapidly across the world. Safety of herbal medicines is therefore an important public health issue. Among consumers, there is a widespread misconception that »natural« always means »safe«, and a common belief that remedies from natural origin are harmless and carry no risk. However, some medicinal plants are inherently toxic. Further, like with all medicines, there is a risk of side effects, mainly due to lack of regulation, weaker quality control systems and loose distribution channels (including mail order and Internet sales). Major causes of adverse events are adulteration of herbal products with undeclared other medicines and potent pharmaceutical substances, such as corticosteroids. Adverse reactions may also arise from the mistaken use of the wrong species of medicinal plants, incorrect dosing, errors in the use of herbal medicines (both by health-care providers and consumers), interactions with other medicines, and use of products contaminated with potentially hazardous substances, such as toxic metals, pathogenic microorganisms and agrochemical residues. As with other medicines for human use, herbal medicines should be covered by a drug regulatory framework to ensure that they conform to required standards of safety, quality and efficacy. According to current medicines act and rules in Croatia, herbal medicines can be authorized *via* well established use procedure which requires at least ten years of medicinal use. In this case safety and efficacy are recognized by means of a detailed scientific bibliography, and results of toxicological and pharmacological tests and clinical trials don't need to be provided. In European Union for herbal medicines that could not satisfy above described criteria, but have a long-standing traditional use, a simplified registration procedure is in force (Traditional herbal medicinal products Directive 2004/24/EC). In a close future we are expecting the integration of European special provisions for registration of traditional herbal medicines into national legislation. In both procedures, the documentation on quality of herbal medicine is essential part of registration application.

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Abstract

# S8.1 Pharmacogenetics in psychiatry

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Researches in psychiatric genetics consistently displayed the role of genes in determining majority of psychiatric disorders, particularly through twin and family studies. Although the input of genetic loading is substantial, no psychiatric disorder could be solely explained by genetic determinants. At present, there are no available means to accurately predict therapeutic response to psychotropic medications, both in relation to clinical response and to development of adverse drug reactions. It is recognized that therapeutic response is dependent on range of various factors, including organ function, age, psychological factors, drug interactions and genetic factors. Pharmacogenetics in psychiatry is an emerging field encompassing issues as prediction of clinical response to psychotropic medications and identification of genes associated with the development of adverse drug reactions. Recent pharmacogenetic research has identified several response-related variants in metabolic enzymes and drug-targeted receptors that could partially explain variation in response. While allelic variations related to metabolic enzymes were shown to be related with toxic effects due to drug accumulation, allelic variants of dopaminergic and serotonergic receptors, inter alia have been shown to be associated with clinical outcome and adverse events such as movement disorders. While early researches in that field were targeted to identification of individual genes modifying the clinical response, more recent approaches, after sequencing of the human genome, are oriented towards pharmagocenomics, i.e. to identification of more than one gene and to recognition of interplay of individual genes in modification of response. Contrary to pharmacogenetic research, no formal hypothesis in relation to pharmacodynamics or pharmacokinetics of medication under study is prerequisite of research, consequently resulting in considerably larger sample requirements to perform a study. Following genes expression and their related protein products through transcriptomic and proteomic researches could facilitate identification of genetic variations that are modifying clinical response. Pharmacogenomic research will not only aid to more accurate pharmacological treatment plan formulation, but will also help in identification of potential targets for new drug development, resulting in development of more effective medications.

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Abstract

# S8.2 Pharmacogenomics in clinical practice – warfarine therapy individualization

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**Introduction**: The use of anticoagulants of the coumarin type is accompanied by considerable problems as the consequences of high interindividual variability. This variability can partly be explained by polymorphisms of the CYP2C9 gene which encodes the main metabolizing enzyme of coumarins, and by polymorphisms of vitamin K epoxide reductase (VKOR). Coumarins act by inhibiting VKOR activity, their target having been identified as the protein vitamin K reductase complex subunit 1 (VKORC1) which is encoded by the homonymous gene VKORC1. Carriers of a combination of CYP2C9 polymorphism and VKORC1 polymorphism had an increased risk of severe overanticoagulation compared to subjects with no polymorphism or only one polymorphism. Patients with VKORC1 polymorphism required significantly lower doses than VKORC1 CC wild-type patients. **Methods**: The aim of this study was to assess the CYP2C9 genotype (CYP2C9*2 and CYP2C9*3 polymorphisms) and the VKORC1- C1173T genotype in Croatian population (n=82). The genotyping of CYP2C9 (alleles *2 and *3) was performed by Real time PCR method, using TIB MOLBIOL LightMix® in Roche LightCycler® instrument. The genotyping of VKORC1- C1173T was performed by Real time PCR method in LightCycler® Fast Start DNA Master plus HybProbe master mix. **Results**: Genotype was obtained for 82 subjects. 29 (35.4 %) had CYP2C9*2 or CYP2C9*3 allele and 50 (61%) had VKORC-C1173T polymorphism. The prevalence of subjects with CYP2C9 *2 or *3 allele and VKORC1-T variant was 28%. **Conclusion**: Polymorphisms of CYP2C9 and VKORC1 genes should be considered as important genetic markers for coumarin type drug response. Other alleles of CYP2C9 and VKOR could additionally contribute to therapeutic variability and should therefore be also included in pharmacogenetic analysis.

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# S8.3 Genetic polymorphism of the serotonin transporter (SERT) and therapeutic response to paroxetine

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**Introduction**: The selective serotonin reuptake inhibitors (SSRIs) continue to be the most widely prescribed class of antidepressants and their mechanism of action is believed to involve the blocking of the neuronal serotonin transporter (SERT) and thus modulating serotonergic activity. Genetic factors could play an important role in antidepressant response. The gene encoding for SERT maps to chromosome 17q11.1-q12. It is polymorphic and is considered to be a strong candidate for pharmacogenetic marker that could predict treatment response to antidepressants. Two polymorphic regions of SERT: a 44-base-pair (bp) insertion/deletion polymorphism in the promoter region (SERTPR) and variable number of tandem repeats in second intron (SERT-in2) seem to modulate gene's transcription in allele-dependent manner. Genetic polymorphisms of the SERT have been investigated in relationship to the antidepressant response to SSRIs. Aim of the study was to investigate the relationships between *LS* promoter (SERTPR) and *ls* intron2 (SERTin2) genetic variants of serotonin transporter polymorphisms with treatment response in 130 patients with major depressive disorder (MDD) treated with paroxetine (20mg/day) for 6 weeks. **Patients and Methods:** To assess therapeutic response to paroxetine, all patients were rated using the HAMD-17 scale. Responders were defined as those subjects with a decrease in HAMD scale by = 50% at week 6 of treatment. SERT genotyping was performed by the PCR methods. **Results**: Comparison of genotypes and allele frequency of the SERTPR between responders and non responders revealed significant differences among genotypes and overrepresentation of the *S* allele in the group of non responders (p= 0.0004). SERTin2-*ss* genotype bearing subjects showed better treatment response compared to *ls* and *ss* genotype from 4th week of treatment (p=0.035). Statistical differences were also found in distributions of the estimated haplotypes between responders and non responders, while subsequent analysis revealed overrepresentation of S/l haplotype (p=0.006) in the group of non responders. SERTPR and SERTin2 were found to be in linkage disequilibrium in studied population. **Conclusion**: These findings identify genetic factors associated with paroxetine treatment response in MDD patients.

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Abstract

# S8.4 The role of polymorphic P-glycoprotein in the therapy of epilepsy

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**Introduction**: The importance of drug efflux transporters in disease proceses and treatment has become increasingly evident in recent years. Often this efflux of therapeutic compounds is mediated by a large superfamily of proteins referred to as multidrug resistence proteins, most of which belong to the family of ATP-binding casette (ABC transporters). P-glycoprotein (Pgp), the encoded product of the human multidrug-resistance –1 (MDR1/ABCB1) gene, is of particular clinical relevance because several mayor antiepileptic drugs including phenytoin, phenobarbital, carbamazepine, lamotrigine, topiramate and gabapentin are substrates for Pgp. Pgp is expressed in the BBB or blood CSF barrier and combine to reduce the brain penetration of this drugs. Epilepsy is a common disorder affecting up to 1% of population. Twenty percent of all patients with epilepsy have uncontrolled seizures, refractory to anticonvulsant therapy. Functional polymorphism or overexpression of Pgp in the BBB of patients with epilepsy may play a role in pharmacoresistance. Mutations of MDR1 have been associated with alteration of Pgp expression and /or function. The aim of this research was to investigate the possible association of MDR1 gene polymorphisms (G2677T in exon 21 and C3435T in exon 26) with the development of resistance to antiepileptic therapy. **Patients and Methods:** In this prospective study fifty patients with cryptogenic partial complex epilepsy with or without secondary generalisation, who have been suffering for more than two years, were studied. They were devided into two groups. The first group comprised patients refractory to the current therapy, while the second group consisted of patients with well-controled seizures. Genotyping of G2677T and C3435T polymorphisms was performed by Real time PCR method in LightCycler® instrument and by polymerase chain reaction followed by restriction digestion (PCR-RFLP). **Result**: We found significant statistical difference in distribution of TT genotype in our patients. **Conclusion**: Our data support functional importance of the MDR1 mutations for the susceptibility and treatment response in patients with cryptogenic partial complex epilepsy.

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Abstract

# S9.1 Therapeutic guidelines

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Due to the plethora of drugs with same or similar efficacy and lack of money there is a pronounced need for rational drug prescribing. Among numerous other instruments, therapeutic guidelines are regarded and accepted as one of the key instruments providing rational drug prescribing. Therapeutic guidelines can be defined as systematically developed statement to assist both practitioner and patient's decision about appropriate health care for specific clinical circumstances. In essence, the purpose of therapeutic guidelines is to improve drug prescribing, improve the cost/benefit ratio and educate both prescriber and patient. Prerequisite for fulfilling these purposes is that therapeutic guidelines are based on randomized controlled trials. Numerous therapeutic guidelines are in use nowadays. As more than one guideline, sometimes quite different, are in use for one disease or condition, it is not an easy task to choose the »right» one. Since, according to definition, therapeutic guidelines have to consider local circumstances, we should develop guidelines for Croatia. The appraisal of therapeutic guidelines AGREE document is widely accepted. Aim and purpose, state holder involvement, rigour and development, clarity and presentation applicability, editorial independence of the guideline has to be assessed according to AGREE document.

Despite the fact that an enormous number of clinical therapeutic studies are published there is still a lack of evidence for statements in many therapeutic guidelines. Quality of evidence is graded as follows:

I. Evidence from ≥1 properly randomized controlled trial;

II. Evidence from ≥ well designed clinical trial, without randomization; from cohort or case-controlled analytical studies (preferably from >1 centre);

III. Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, of reports of expert committees.

Croatian Ministry of Health in collaboration with the Government of Netherlands has started development of therapeutic guidelines for antimicrobial therapy. At the moment a set of four guidelines are in final stage: MRSA infections; upper respiratory tract infections; surgical prophylaxis and urinary tract infections.

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# S9.2 The role of beta adrenergic blockers in therapy of hypertension

**J. BAGATIN**

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Abstract

# S9.3 Pharmacotherapy of hypertension and blood pressure control in Croatia

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Arterial hypertension (AH) is a significant health care problem in today's world. Improving the quality of hypertension care is a general priority that will result in a diminished number of patients with congestive heart failure, coronary artery disease, peripheral vascular disease, stroke, and renal insufficiency. AH prevalence in Croatia is 37.5%. Cardiovascular (CV) and cerebrovascular diseases were the cause of over 50% of deaths in Croatia in 2004. Adequate managing of AH, which includes antihypertensive drugs, leads to reduction of CV and cerebrovascular morbidity and mortality. According to the drug utilization data during the five-year period (2001-2005) total cardiovascular drugs utilization increased 64.65%. Group of drugs acting on the renin angiotensin aldosterone system had a steady but the largest share (about 32%). The most frequently used antihypertensives in Croatia were amlodipine, lisinopril and lisinopril+hydrochlorothiazide. Financial consumption data showed the highest increase of angiotensin II receptor blockers at the rate of 3.6 times comparing 2004 vs. 2002. The increase was also noticed in the same period for the combinations of ACE-inhibitors and diuretics (70.57%) and calcium channel blockers (62.86%). There is a slight decrease in the financial consumption of ACE-inhibitors (5.44%) and of old calcium channel blockers (17.73%). A cross-sectional study performed during December 2003 and January 2004 analyses hypertension control (BP<140/90 mmHg). This study which included 141 primary care physicians and 814 hypertensive patients from different parts of Croatia, showed that a controlled BP in this hypertensive population treated with antihypertensive drugs was in 23% of patients. The analysis of BP control according to risk factors showed that significantly related with higher levels of systolic or diastolic BP were the age (poorer SBP control in patients older than 60 years), left ventricular hypertrophy, changes of the eye retina, smoking and diabetes mellitus. Further, patients from towns closer to the hospital, from urban centers, with higher education and employed had significantly lower average BP. In conclusion, the usage of cardiovascular drugs including antihypertensives in Croatia increased considerably during the last six-year period especially comparing the period after 2003 to previous years. This can be explained by a legal change in Croatia (the new Health Insurance Act) which introduced supplementary health insurance. On the other side the outcome measured as hypertension control in Croatia is not satisfactory and there is a need to improve the quality of hypertension care. In the patients treatment the relationship between demographic and cardiovascular risk factors with poor BP control should be taken into account.

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Abstract

# S9.4 Drug prescribing – can it be better? Contributions of pharmacoepidemiology to clinical pharmacology

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Pharmacoepidemiology applies epidemiological methods to studies of the use of and the effects of drugs in large numbers of people. Its main purpose is is supporting the rational and cost-effective use of drugs in the population therby improving health outcomes. The main difference between drug utilization and pharmacoepidemiology is that the latter focuses to a greater extent on the quantitative benefits and risks of drug treatment in cohorts of patients, while drug utilization focuses more on drug exposure and differences in the quality and quantity of drug use in different countries, regions, and settings. Pharmacoepidemiologic studies supplement the information obtained through clinical drug investigation, prior to marketing. Potential contributions of pharmacoepidemiology may be devided into thre categories: information that supplements that available from premarketing studies – better quantification of the incidence of known averse and beneficial effects, new types of information not available from premarketing studies and general contributions of pharmacoepidemiology like drug safety and ethical issues. The effects of treatment in very strict premarketing clinical trials are not the same as those in every day patient lives. In daily practice drug treatment is not randomized within a homegenous patient group. Real patients differ substantially from patients involved in clinical trials. Premarketing clinical studies are necessary in drug approval process. Pharmacoepidemiologics studies contribute to clinical pharmacology because they evaluate the effects of drugs administered as a part of ongoing medical care in larger numbers of patients including those that are usually not included in premarketing studies, and for an unlimited period of time.

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# S9.5 Individualization of therapy in clinical practice – importance of pharmacogenomics

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Interindividual variability in response to the applied therapy represents a problem in everyday clinical practice. It can be represented as inefficiency or toxicity of drugs. Pharmacogenomic studies are attempting to define the impact that genetic polymorphisms have on positive and adverse reactions to drugs. Candidate genes for pharmacogenomic studies are those that encode proteins directly involved in the pharmacokinetics (drug–metabolizing enzymes, mostly cytochrome P-450, membrane drug transporters like P-glycoprotein, etc.) and pharmacodynamics (genes encoding drug targets: such as several receptors) of drugs. Results of these studies should improve the individualization and optimization of therapy. Warfarin is the drug widely prescribed in clinical practice, in the therapy and prevention of thromboembolic events. It is a drug with a very narrow therapeutic margin and complications in the form of bleeding in the cases when INR is above therapeutic margins and increased risk of new thromboembolic events in the cases when INR is below therapeutic margins. Dosage of the drug significantly varies in relation to CYP2C9 genotype as well as in relation to polymorphism for vitamin K epoxid reductase subunit 1 (VKORC1). Determination of only those two predictors in clinical practice can significantly improve individualization of therapy and reduce the complications of warfarin therapy, especially at the beginning of the therapy when the risk of development of complications is greatest. The CYP2C9 gene encodes the main metabolizing enzyme of coumarins, and the efficacy of warfarin is based on inhibition of VKOR activity which is encoded by the gene VKORC 1. Both of the genes show significant genetic variability. Individuals with different polymorhisms in those genes have a demand for different approach in dosing at the beginning of therapy.

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# S9.6 Clinical pharmacologist in therapeutic problem solving

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Clinical Hospital Osijek has 1200 hospital beds and between 1991 and 2004 had no specialist in clinical pharmacology. Two residents in clinical pharmacology stopped their residency in the last two years. In June 2006 at the Department of Internal Medicine an Ambulance for Clinical pharmacology is opened where the most frequent patients are pregnant women taking drugs, patients with allergy or adverse reactions to drugs. Therapeutic advices from clinical pharmacologist are most often requested from the colleagues from surgical hospital wards. Department for Neurosurgery and Department for Orthopedic surgery are having the most successful collaboration with clinical pharmacologist. Together we defined the guidelines for surgical prophylaxis, which resulted in better prophylaxis and in stopping using the drugs from the list of restricted antibiotics for surgical prophylaxis. In collaboration with the colleagues from the Department for Nephrology the guidelines for treatment of pregnant and lactating women are defined. Excellent collaboration with hospital microbiologist results in frequent team rounds in all hospital wards, helping solving therapeutic problems and pointing to mistakes in antimicrobial drugs use. Eight months ago amoxicillin/clavulanate was put to the list of restricted antibiotics because of its extremely high consumption and high bacterial resistance to it. This measure resulted in 15 times smaller consumption of amoxicillin/clavulanate, but also with smaller overall antibiotic consumption and immediate recovery of susceptibility of gram negative bacteria to amoxicillin/clavulanate.

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# S10.1 The renin-angiotensin system (RAS) in the regulation of vascular reactivity to dilator stimuli – the effects of increased oxidative stress

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There is an increasing body of evidence that normal regulation of the RAS plays important role in maintaining the normal vascular relaxation mechanisms that are subsequently impaired in hypertension, and possibly contributes to the maintenance of high blood pressure by an elevated total peripheral resistance. Newly emerging data have revealed that different strains of rats may exhibit substantial differences in vascular control mechanisms and in their susceptibility to hypertension. This emphasizes the importance of understanding the role of genetic factors in determining physiological control mechanisms. For example, impaired relaxation of resistance arteries in cerebral and skeletal muscle circulation in SS salt-sensitive rat strain (that exhibit chronically low plasma renin activity), and restored in consomic SS.13$^{BN}$ rats (that have chromosome 13, containing renin gene, introgressed from BN rat into SS genetic background). It is well recognized that the endothelium importantly determines vascular tone through the release of different relaxing and constricting factors that modulate contractile activity of the underlying smooth muscle. Increased oxidative stress has been recognized to have a very important role in the development of complex multifactorial diseases such as atherosclerosis, ischemia-reperfusion injury, diabetes and hypertension. In conclusion, results of numerous experimental studies suggest that hypoactive (as well as hyperactive RAS) may contribute to development and maintenance of hypertension due to the effects of angiotensin II on vascular reactivity. Increased oxidative stress could significantly modify NO-dependent and prostanoid- dependent vascular relaxation mechanisms in response to dilator stimuli.

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# S10.2 Fetal cerebrovascular response to chronic hypoxia – implications for the prevention of brain damage

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**Introduction**: Fetal cardiovascular responses to hypoxia, which include the redistribution of the cardiac output towards the vital organs, are considered the most important adaptive reactions responsible for maintaining fetal homeostasis. The redistribution of blood flow towards the fetal brain is known as »the brain sparing effect» and it can be precisely detected and quantified by the Doppler cerebral/umbilical ratio (C/U ratio = cerebral resistance index (CRI) /umbilical resistance index (URI)). **Objectives**: 1. to investigate the effects of chronic hypoxia on fetal cerebral circulation, 2. to determine whether prolonged fetal brain hyperperfusion (the brain sparing effect) is associated with the brain damage and a poor fetal outcome, 3. to estimate the value of the new vascular score, the hypoxia index in the prediction of brain lesions caused by fetal hypoxia. **Material and Methods:** Fetal cerebrovascular response to hypoxia were studied on an animal model and on human fetuses. In the animal model, pregnant ewes (n=14) were treated with cocaine (1 or 2 mg/kg) daily from midgestation until delivery, resulting in a significant fetal growth retardation and chronic hypoxia. The control group (n=7) received placebo injected intramuscularly daily in the same period. Cerebral flow responses and heart rate were measured at rest and during two acute hypoxic tests (cord compression and maternal aorta compression) at the cesarean delivery perfomed on day 134. In a prospective study, 29 growth-restricted and hypoxic human fetuses (29-38 weeks of gestation) were followed at least two weeks prior to delivery. The C/U ratio was determined in 48 hours intervals and the hypoxia index (HI) was calculated by summing the daily C/U ratio reduction (in % from the cut-off value of 1) over the period of observation. The C/U ratio and the HI were tested as potential predictors of the neonatal brain damage. Neonatal brain sonography and perinatal data were used as the outcome parameters. **Results**: Long-term exposure to cocaine induces the redistribution of blood floow towards the brain. During the hypoxic tests, the CRI and the C/U ratio decreased significantly less in the two cocaine groups ($p<0.05$). The fetal heart rate response was reduced significantly in the two cocaine groups ($p<0.05$). Pathohistological examination revealed the existence of hypoxic brain damage despite the maintained cerebrovascular reactivity and the brain sparing effect. During the period of surveillance of the human fetuses, hypoxia was detected in 22 fetuses and the neonatal brain damage were later found in 13 of these infants. In five hypoxic fetuses, cerebrovascular variability was lost for a minimum period of six days and the brain damage were later detected in all of them. Neonatal brain damage were also detected in eight hypoxic fetuses with the maintained cerebrovascular variability. The HI was identified as a predictor of the neonatal brain damage. The HI also showed the best correlation with biochemical parameters, such as umbilical venous pO2 ($r=-0.60$, $p<0.001$) and umbilical venous pH ($r=-0.66$, $p<0.001$). **Conclusion**: Our studies have demonstrated clearly that the brain sparing effect cannot prevent the development of perinatal brain damage in the case of severe or prolonged hypoxia. Brain damage can develop even before the loss of physiological cerebrovascular variability. The use of HI could allow for the first time a sensitive and reliable prediction and even the prevention of adverse neurological outcome in pregnancies complicated by hypoxia.

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# S10.3 Regulation of skeletal muscle blood flow

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At the onset of exercise bout as well as during sustained muscle activity there is substantial increase in blood flow directed to activated skeletal muscle. Concomitantly, sympathetic nerve activity toward vasculature that produces vasoconstriction is also increased. There are more data available in literature that support idea that vasodilatation at the onset of exercise occurs very rapidly. Exact mechanism(s) that are responsible for such rapid vasodilatation remain elusive. Proposed theories include: metabolic vasodilatation, neural mechanism, acetylcholine spill over, potassium, mechanical mechanism. There will be presentation of evidence that argues for and opposing mentioned mechanisms.

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Abstract

# S10.4 Exercise, oxidative stress and human endothelial function

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It is well recognized that people who exercise have better cardiovascular health than those who do not. While aerobic exercise clearly improves cardiovascular function, the benefits of resistance exercise are less certain. On the one hand, experienced weight lifters are known to suffer fewer post-exertion cardiac events thus weight lifting is commonly recommended for cardiovascular health and disease prevention. However, weight lifting is associated with large increases in arterial pressure and an inflammatory response that may be detrimental to vascular health. Recently, our laboratory has identified a differential effect of a single weight lifting session on vascular endothelial health (assessed by measuring flow-mediated dilation [FMD] of the brachial artery) in exercise conditioned individuals (EX) compared to sedentary subjects (SED). Despite similar elevations in arterial pressure in both groups, SED subjects had severely reduced endothelial FMD in their brachial artery but no change was observed in EX. Since brachial FMD is mediated by nitric oxide (NO) and correlates with coronary vascular function, this observation raises several important questions. How does exercise training protect against conduit artery endothelial dysfunction during acute resistance exercise? The answer may provide insight into the reported cardiovascular benefit of resistance exercise. Are other forms of exercise training like running also protective? Does weight lifting induce endothelial dysfunction in the microcirculation and what are the implications for tissue perfusion? The primary hypothesis is that chronic aerobic and resistance exercise protect against endothelial dysfunction following acute exertion. Athletes are protected from ROS generation and reduced NO bioavailability otherwise associated with a single weight lifting session. Our laboratory investigations involve *in vivo* and *in vitro* measurements of endothelial function and reactive oxygen species in humans designed to advance our understanding of the relationship between vascular stress (acute hypertension) and endothelial function, a determinant of the propensity for atherosclerosis.

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# S11.1 Perspectives in physiology of diving

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SCUBA diving is becoming a popular touristic attraction worldwide, and especially in Croatia. It is reported by Divers Alert Network that in 2000 there were over 9 millions registered dives. The characteristic of the population that is engaged in this activity is also changing. Formerly, diving was reserved mostly for young, fit males. Today women equally participate, and the diving population grows older and includes individuals who are relatively unfit. Decompression sickness (DCS), which represents the major health risk of diving, is not a single entity, but rather a syndrome that can be characterized by various clinical symptoms ranging from local (pain in the area of the shoulder, termed bends) to serious neurological abnormalities which can lead to death of the diver. Exact cause of DCS is not known, and the same is truth for the genesis of venous gas bubbles. Although, venous gas bubbles do not represent DCS a high number of bubbles is clearly linked to the high incidence of DCS. Exercising before, during or after diving is proscribed due to increased incidence of DCS. Our findings showed that carefully selected type of exercise performed in timely fashion before the dive or during decompression will reduce number of venous gas bubbles formed. Exercise after diving did not increase the number of bubbles. Nitric oxide seems to play a protective role.

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# S11.2 Can antioxidants protect scuba divers?

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People scuba dive for recreational and professional purposes. However, only recently there is evidence of the different cardiovascular changes that appear after each scuba dive. In most cases those changes are silent or subclinical, posing little or no threat to the health of divers, but is that always the case? A study carried out on a group of professional scuba divers before and after a moderate load scuba dive (a dive to a depth of 30 meters for 30 minutes, similar to those enjoyed by countless recreational divers) showed different alterations in cardiovascular function, including changes of arterial endothelial function. A single scuba air dive induced mild changes in cardiac function and a significant decrease in endothelial function. We thought to examine could these changes be influenced by oral ingestion of antioxidant vitamins C and E prior to diving, and can endothelial function, in particular, be preserved. We used two different approaches with implementation of antioxidant vitamins, first with acute administration of larger dose of vitamins two hours prior dive and chronic administration during four weeks of lower dose of vitamins. Both interventions showed a positive effect on vascular endothelial function, whereas other cardiac functional changes were unaffected. Although generally very safe, diving may be associated with serious, and sometimes fatal, consequences, which are usually related to decompression sickness. These new data raise the possibility that pre-dive intake of antioxidant vitamins may prevent some of the negative effects of diving on vascular function. The results of this study are of interest for those involved in all types of recreational and professional diving.

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# S11.3 Spleen as a part of diving response

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In 1988 Enzo Majorca established a remarkable record in breath hold diving reaching for the first time a depth of 100 m, under controlled conditions. His achievement attracted the interest of the physiologists, and stimulated new studies of breath-hold diving in the humans. The diving response elicited by breath-holding mainly consists of bradycardia, changes in cardiac output, arterial hypertension and redistribution of regional blood flows. These responses reduce oxygen consumption and thereby prolong duration of the dive. In the near past spleen has been recognized as an important part of the diving response. It is generally accepted that the spleen has the ability to autotransfuse a large quantity of red blood cells into the systemic circulation during times of stress by contracting, and thereby augmenting the blood's oxygen transport. Our finding showed rapid, probably active contraction of the spleen and its slow recovery in response to breath-hold diving in humans what can contribute to prolongation of successive, briefly repeated apnea attempts. Moreover we found that spleen volume was reduced for 20% as early as 3 seconds after the start of apnea indicating that splenic volume changes during apnea may be related to concurrent changes in sympathetic activation. Furthermore, we want to evaluate contribution of splenic contraction to changes in red blood cell volume (RBCV), white blood cell (WBC) and platelet (PLT) venous blood concentrations following repeated apneas and to investigate the impact of consecutive maximal breath holds on central hemodynamics in the post-apneic period. The RBCV and WBC venous concentrations increased in both trained apnea divers and untrained persons following repeated breath-holds apneas, while in splenectomized subjects there was no change in RBCV and a delayed increase in WBC concentration. None of the groups showed significant changes in PLT concentration. Increased right ventricular afterload and decreased cardiac output in postapneic period were associated with $CO_2$ retention and signs of peripheralization of blood volume. These results indicate that repeated apneas may cause prolonged hemodynamic changes after resumption of normal breathing, which may suggest what happens in sleep apnea syndrome.

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# S11.4 Muscle and cerebral oxygenation during breath-hold

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Elite breath-hold divers have considerably expanded the limits of the dive depth and duration during recent years. The current absolute record is 183 meters of sea water, set in August 2006 by Herbert Nitsch. Cardiovascular adaptations during breath-holding include bradycardia, decreased cardiac output, peripheral vasoconstriction and increased arterial blood pressure. These mechanisms accomplish the temporary reduction of peripheral tissue oxygen uptake resulting in increased diving duration, especially in trained breath-hold divers where the effect seems accentuated. The effects of maximal apneas on cerebral and muscle blood flow (using Doppler ultrasound) and oxygenation (using near infra-red spectroscopy) are unknown in humans. Our studies showed moderate reduction in cerebral oxygenation during the maximal static breath-holds in breath-hold divers, despite larger increases in cerebral blood velocity and similar cerebral oxygen delivery. Similar peripheral vasoconstriction was found during the apnea attempts in breath-hold divers and untrained controls. We found that despite large increases in cerebral perfusion and global cerebral oxygen delivery during, and especially at the end of the breath-hold, regional cerebral desaturation may become a factor limiting the maximal breath-hold duration. These studies showed that muscle oxygen saturation occurs, i.e. supply of oxygen to the muscle is reduced to a greater extent that can be explained by reduction in the arterial blood oxygen supply. It appears therefore that the diving reflex acts as an oxygen conserving effect at resting humans and that cerebral oxygenation is reduced relatively little at the expense of reduced peripheral blood flow and oxygenation, supporting the oxygen-conserving nature of the diving response.

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Abstract

# RT1 Medicinal products regulation in Croatia – the new pharmaceutical legislation

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Medicinal products play a crucial role in saving lives, restoring health, and preventing diseases and epidemics. But they also need to be safe, efficaceous, of good quality and used rationally. This means, that their production, import/export, storage, supply and distribution should be subject to government control through prescribed norms and standards and an effective regulatory system. Regulatory objectives in Croatia are achived through the Act on Medicinal Products adopted in June 2007 that replaced the former Medinal Products and Medical Devices Act from 2003. The Act stipulates different regulatory functions: licensing and inspection, product assesment and registration, quality control, monitoring of promotion and advertising, pharmacovigilance and clinical trials. The legislation recognises orphan medicinal products and introduces the Sunset clause, thus harmonysing further with the European Pharma Review. The Croatian Government established the Agency for Medicinal Products and Medical Devices in 2003 with the aim to ensure the quality and safety of medicinal products through the implementation of the relevant legislation by a competent workforce. The Agency is involved in manufacturing authorisation, marketing authorisation, pharmacovigilance, drug consumtion monitoring and quality control. The Agency is not responsible for clinical trial premissions. These stay with the Ministry of Health and Social Welfare. Likewise, the inspections are actually conducted by the Ministerial Pharmaceutical Inspectorate. However, in case of off-shelf quality control of products, the Ministry delegates the sampling to the Agency. Since substandard and counterfeit medicines proliferate at the global level, the new legislation stipulates, for the first time, anti-counterfeit provisions. The new legislation represents an overall rapprochement of the Croatian regulatory framework to the pharmaceutical acquis of the EU.

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Abstract

# RT2 Counterfeit medicines – impact on national healthcare system

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Counterfeit or fake medicines (according to definition of World Health Organisation) are medicines that are deliberately and fraudulently mislabelled with respect to identity and / or source. Counterfeiting can apply to both branded and generic products. Counterfeit products may include products with the correct ingredients or with wrong ingredients, without active ingredients, with insufficient active ingredients, or with fake packaging. The quality, efficacy and safety of counterfeit medicines are usually unacceptable and they can be dangerous to both: patient and the whole society. Patients who take the counterfeit medicines may be at risk of serious health problems e.g. unexpected adverse drug reaction, prolongation of a therapy, illness even death. Counterfeit medicines are manufactured and distributed by unauthorised persons towards their enlarged profit but endangering patients, damaging pharmaceutical industry and the national healthcare system. Even if their quality, safety and efficacy are appropriate we have to be aware that they are not produced according to accepted guidance, they are not authorised by regulatory drug agency and studies for proving their safety and efficacy are not performed. Counterfeit medicines can be found both in developed and developing countries. According to studies of the Food and Drug Administration more that 10% of worldwide medicines are counterfeited. The centre of counterfeiting is in some of the Asian and African countries where the legislation and control of medicines are still rather weak. In those countries life saving medicines like antibiotics, HIV/AIDS medicines are usually counterfeited whereas in developed countries more counterfeit medicines can be found among lifestyle medicines (hormones, steroids, medicines for erectile dysfunction etc.). The factors which also increase the counterfeiting are high prices of medicines, weak distribution and no penalty for counterfeiters. Croatian Agency is involved in combating counterfeit medicines at the international level. The Agency has also developed a rapid alert system to inform public in case of occurrence of counterfeit medicines in our legal distribution network. Counterfeiting is not just a problem of one country or institution but a global problem which demands integration of different stakeholders at national and international level. Regulatory and judicial authorities, police, customs, pharmaceutical companies, wholesalers, healthcare professional, patients associations, media, nongovernmental organisation, should work together on developing national strategy for preventing and suppressing the problem of counterfeiting medicines according to situation and available recourses.

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# RT3 Emerging issues in regulatory views on bioequivalence: highly variable drugs & drug products – how should they be handled?

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The term bioequivalence (BE) has emerged from the pharmacokinetic (PK) mileu and although it subsumes a relatively broad spectrum of meanings, it is typically used to describe a relationship between two drug products containing the same active pharmaceutical ingredient (API) in respect to their PK properties, i.e., delivery of the API to the site of action. One product (Test product, T) is said to be BE to the other product (Reference product, R) if it delivers API equivalently (i.e., no »worse« and no »better«). The concept of BE is applicable for various pharmaceutical forms, but typically it is used in the context of pharmaceutical forms intended for systemic API delivery by »non-i.v.« routes, i.e., by all routes of administration that include the process of absorption. BE has become an important concept in the drug regulatory environment since it has been recognized that, under certain circumstances, two drug products containing the same API and equivalently delivering the API to the site of the action (and being, therefore, bioequivalent) could be considered therapeutically equivalent. The major conditions that need to be met for such an interpretation of BE are that T and R contain qualitatively and quantitatively the same API and that they come in the same pharmaceutical form. Formally, T is said to be BE to R if it exhibits an equivalent rate and extent by which API becomes available at the site of action, when T and R are administered in the same dose and under the same conditions. It has been well recognized that in the case of drug products intended for systemic API delivery, systemic circulation is a valid surrogate of the »site of action«. Consequently, although there are different ways to estimate the presence of API at the site of action, typically one concludes on it based on the concentrations of API in the systemic circulation. In this respect, the »rate« and »extent« by which API becomes available at the site of action could be translated into the »rate« and »extent« of absorption. It has been accepted that maximum plasma API concentration achieved ($C_{max}$) and area under the API plasma concentration-time curve (AUC) are feasible indicators of the two processes, respectively. Highly variable drug product is a product which displays a high intra-subject variability of the rate and/or extent of absorption. In practical terms, highly variable products are defined by intra-subject coefficient of variation (CV, %) for $C_{max}$ and/or AUC that is $\geq 30\%$. Intra-subject variability of a drug product is thought to be due primarily to formulation inconsistency (ie., tablet-to-tablet variability), rather than to inherent property of the drug (i.e., API). When R product is highly variable, then a large number of subjects need to be included in a BE study in order to meet the formal criteria of BE – even if the T product is »truly BE«. This has been recognized as a practical and ethical problem and a search for acceptable alternative ways of demonstration of BE for highly variable drugs is underway. The potential solutions include arbitrary widening of the acceptance interval, widening of the interval based on a fixed sample size, scaling of the acceptance interval to the Reference intra-subject variability and widening of the acceptance interval on mixed criteria of a fixed sample size and scaling to the Reference variability. The present paper reviews these proposed methodologies and regulatory views on this problem.

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# RT4 Clinical trials in Croatia and the Central Ethics Committee

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A clinical trial is an experimental process of testing a new medicine or medical device on human subjects. Clinical trials will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with good clinical practice and the requirements of these Regulations. Clinical trials in Croatia are run in accordance with local laws (Drug law, and specific acts), which are in accordance with European legislation, and in some parts also in the process of current harmonization. For final regulatory approval given by the Ministry of Health, it is of the utmost importance that clinical trials have a positive, mandatory ethical approval from the Central Ethics Committee (CEC) for Clinical Drug Trials. CEC is an independent body consisted of medical and nonmedical members (13 regular and 13 deputy members). Members of CEC will have the sort of experience which will be useful in scrutinizing the ethical aspects of a research proposal. In preparing its opinion, the CEC is considering the relevance of the clinical trial and its design (evaluation of the anticipated benefits and risks), the protocol, the investigator's brochure, the suitability of the investigator and supporting staff, the quality of the facilities for the trial, the adequacy and completeness of the written information to be given, and the procedure to be followed, for the purpose of obtaining informed consent to the subjects' participation in the trial. Further, provision for indemnity or compensation in the event of injury or death attributable to the clinical trial, any insurance or indemnity to cover the liability of the investigator or sponsor, the amounts, and, where appropriate, the arrangements for rewarding or compensating investigators and subjects, etc. According to a defined procedure and discussion in 2004 (after May 01) CEC positive opinion was given to 42 clinical trials, 92 in 2005, 83 in 2006, and 44 till May 01, 2007. During the last three years 87 clinical trials per year in average have positive opinion from CEC. There is a time limit of 30 days for CEC to give an opinion. In conclusion, CEC in Croatia, as an independent body, consisted of multidisciplinary members, giving qualified ethical opinions during the last period. In the future some changes in the legislation in Croatia and the European Union will have certain impact on the ethics of clinical trials, and especially on the administrative aspects. CEC should be involved as partner in the future legal changes in the field of clinical trials.

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Abstract

# RT5 Drug consumption in Croatia in 2005

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The increasing interest for drug utilization research began in the 1960s. In 1996, WHO recognized the need to develop use of the ATC/DDD system as an international standard for drug utilization studies. Drug use evaluation is a system of continuous, systematic, criteria-based drug evaluation that provides the appropriate use of drugs. Drug consumption research does not necessarily ensure answers, but it contributes to state whether drug therapy is rational or not. Besides, drug consumption monitoring is a base for pharmacoeconomic evaluation and evidence-based medicine as an aid to formulary and purchasing decisions for medicines. The purpose of this overview is to present drug utilization in Croatia in 2005. Data on drug consumption were collected on the basis of reports submitted by pharmacies and hospital pharmacies. 76,24% of all pharmacies (public and hospital) deliver their reports and the data were extrapolated to total number of country pharmacies. Drug utilization data are presented in defined daily doses/1000 inhabitants/day (DDD/1000/day), and costs are expressed in pharmacy purchase price, in HRK. In this overview drug utilization data are presented for all ATC groups (1st level), first 25 therapeutic subgroups in rank (2nd level) and first 25 drugs in rank (INN 5th level). Drug consumption in hospitals and in state counties is presented respectively. In conclusion, drug use evaluation applied in an appropriate way can assess the quality of prescribing which has become an important issue in assessing the quality of health care.

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Abstract

# W1.1 The role of the health care professionals in adverse reaction reporting and pharmacovigilance system in Croatia

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Pharmacovigilance is the science and activity relating to the detection, assessment, understanding and prevention of adverse effects or any other medicine-related problem.The first part of the workshop will go through a short history of Pharmacovigilance, and will give an overview of the international collaboration (WHO, ICH, EU). The importance of adverse drug reaction (ADR) monitoring was recognized in Croatia already in 1974 when the National Centre for Adverse Drug Reaction was instituted in the University Hospital Zagreb. In March 2005, after new legislation came into force, the obligation of pre- and post-marketing drug safety surveillance was delegated to the Agency for Medicinal Products and Medical Devices (Agency) and the Agency's Pharmacovigilance Unit was than formed. The principles of pharmacovigilance will be given and the reporting method will be discussed; differences between adverse event reporting in post marketing phase (spontaneous reporting) and in clinical trials. Also the definitions of seriousness, expectedness and causality of ADRs will be given. The participants will be acquainted with new safety topics for particular drugs. In the second part of the Workshop the participants will have the opportunity to practice how to fill in the reporting form, how to decide whether the reaction was serious and how to assess the relationship between the drug and the reaction. The Workshop is intended for physicians and pharmacists.

# ABSTRACTS
# Poster sessions

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Abstract

# P1.1 Comparison of out patient utilization of psychopharmaceuticals between Zagreb and Scandinavian countries (2001–2006)

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**Introduction**: Neurotropic drugs account for 15% of total drug utilization in the City of Zagreb. The aim was to estimate outpatient consumption of psychotropic drugs in Zagreb in correlation with Scandinavian countries. **Methods**: Data on drug utilization in Zagreb were collected and analyzed for the 2001-2006 period. Data were obtained from Zagreb Municipal Pharmacy and extrapolated to the total number of pharmacies. All drugs were classified according to Anatomical-Therapeutic-Chemical (ATC) drug classification system. These data were used to calculate the number of defined daily doses (DDD) and DDD *per* 1000 inhabitants *per* day (DDD/TID). Data of the Nordic Medico-Statistical Committee were used for Scandinavian countries. **Results**: In Zagreb, total utilization of psychopharmaceuticals of 115.4 DDD/TID in 2001, 104.5 DDD/TID in 2002, 106.9 DDD/TID in 2003, 101.2 DDD/TID in 2004, 95.5 DDD/TID in 2005 and 93.15 DDD/TID in 2006 was lower than that recorded in Scandinavian countries. The use of these drugs increased in Finland by 9.9% and in Denmark by 10.1%. In Zagreb, a reduction of their consumption was recorded in 2006 relative to 2001 (19%). The utilization of psycholeptics (N05) was by 22% higher than in Denmark; in 2001 it was the same as in Finland whereas in 2006 it was by 28% lower than in Finland. In Zagreb, the utilization of psycholeptics (N06), 90% of them antidepressants, was 2.3 times lower than the lowest rate in Finland, and 4 times lower than the highest rate in Denmark. Anxiolytics accounted for 90% of psycholeptic utilization in Zagreb *versus* 29% in Denmark and 34% in Finland. The anxiolytic/antidepressant ratio decreased in Croatia by 35.19% (7.19 in 2001 and 3.83 in 2006), whereas in Scandinavian countries it showed a constant rate (0.7 in Finland and 0.4 in Denmark). **Conclusion**: In Zagreb, benzodiazepines as a symptomatic rather than etiologic therapy accounted for 86.9% of psychopharmaceutical utilization, pointing to the need of respective guidelines as a measure of rationalization.

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Abstract

# P1.2 Cross-sectional analysis of drug use in pregnancy in Zagreb's hospitals

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The aim of the paper is to investigate impact of drugs utility on pregnancy in the City of Zagreb. This one-month cross-sectional study was conducted in all four Zagreb maternity hospitals using a questionnaire administered to 893 pregnant women. The women used a mean of 2.6 drugs. The vitamin-mineral complex was the leading medicament used by the women during the study period (62.9%) and during pregnancy period. The leading drugs taken between hospital admission and delivery were metoclopramide (10.1%) and diazepam (6.0%). Utilization of diazepam is high during all pregnancy. According to FDA risk classification during pregnancy, the most drugs are in B class (88%), and in A class (77%). Percent of FDA C class is 16%. In the FDA classes with fetal risk, D class has 47, 5%, and X class, with only one woman using drug from this class has a 0, 1% of total utilization. In spite of some limitations of the study, the results pointed to the uneconomical, potentially harmful drug use during pregnancy and puerperium, obviously calling for therapy quality upgrading in this vulnerable period of life.

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# P1.3 How safe are antibiotics during pregnancy?

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Administration of antibacterial medicines during pregnancy may increase the risk of congenital malformations, functional disorders, frequency of spontaneous abortions and suppression of a childžs immune system. On the other hand, infection without introduced treatment sometimes may perhaps cause more serious embryo damages than medicines included within the therapy of certain disease. It is well known that pharmacoepidemiological studies dealing with prescription of drugs in pregnancy are numerous. Results showen, that only exceptionally drugs that used in pregnancy have been proven teratogenic. However, little is known about subtle effects of drugs on fetal development. The study was performed during 2001 at the Institute for Child and Youth Health Care in Novi Sad, at the Clinic of Gynecology and Obstetrics and at the Department of Pathology and Histology Clinical Centre in Novi Sad. The sources of the data were: the questionnaires for the pregnant women given by the doctor during the hospitalization at Clinic of Gynecology and Obstetrics or at Genetics Counseling Department; detailed physical examination of newborn baby in order to establish the presence of major or minor malformations, examination performed according to a standard protocol, done by trained pediatricians; pathophysical examination of fetuses performed according to a standard protocol, done by trained pathologists. Questionnaires filled 6099 pregnant women, malformations were found in 326 (5.35%) fetuses. According to the results 2013 (33.00%) pregnant women used medicines during the pregnancy, malformations were found in 113 (5.61%) cases. Antibiotics used 392 (19.5%) women, malformations were found in 20 (5.1%) fetuses. Antibiotics in the first trimester used 207 (44.6%) pregnant women while as much as 176 (37.9%) pregnant women used antibacterial drugs in the third trimester. The most frequently used antibacterial medications were from category B (297 or 75.8%), while 57 or 14.5% belonged to category D and 4 or 1.0% to category X. Penicillins (192 pregnant women) and cephalosporins (104 pregnant women) were the most frequently used antibiotics during pregnancy with malformations frequency of 5.73% (11 fetuses) for penicillins and 2.88% (3 fetuses) for cephalosporins.

Based on these results we cannot certainly declare what is the exact cause of identified malformations. If pregnant woman require certain therapy the most secure antibiotics from related group has to be included in any event. Stages of pregnancy that are critical with respect to possible harmful effects of medications on the developing fetus are still inexplicitly defined and potential risks of drugs for mother and the fetus are faintly understood, thus question of medication use during pregnancy still remains a chronic and everlasting problem. A comprehensive monitoring of the use of medicines in a particular environment over the longer period of time can decrease the risk of harmful effects of drugs during pregnancy through proper evaluation of pharmacotherapy during pregnancy, and if necessary, through appropriate educational measures towards improvement of pharmacotherapeutical practice.

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# P1.4 Consumption of the drugs ATC a in Clinical hospital Osijek during the period of 2004–2006

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This retrospective analysis shows the consumption of the drugs ATC A in Clinical hospital Osijek during the period of 2004–2006. Clinical hospital Osijek has 23 wards/ clinics. ATC A group of drugs shows the use of 3% for each year during the analyzed period, compared to total use of all other drugs. Analysis shows 5% increase in use of the drugs ATC A from 2004–2005 and 17% increase from 2005-2006. ATC A02 group of drugs has the highest increase of 60% in analyzed group, compared to total use of the drugs ATC A in analyzed period. ATC A02 drugs shows increase from 2% to 8% during the period 2004–2006; reduction in use of the drugs ATC A02BA is significant (H2-receptor antagonists) from 37% to 29% and increase in consumption of the drugs ATC A02BC (proton pump inhibitors) from 30% to 37%. Lansoprazole (ATC A02BC03) has the lowest participation in group ATC A02BC, with 13% in 2004 and only 1% in 2006; omeprazole (ATC A02BC01) participates in overall consumption with 20% in 2004, 11% in 2005 and 5% in 2006. Pantoprazole (ATC A02BC02) has the highest consumption in group ATK A02BC, it oscillates from 80% in 2004, 85% in 2005 and it drops in use to 70% in 2006. Esomeprazole (ATC A02BC05) participates with 10% in total use of the drugs group ATC A, and 24% compared to use of the drugs group ATC A02BC. Increase in use of the ATC A group is the result of increased prescribing of pantoprazole and esomeprazole, after their introduction to the Croatian Institute for Health Insurance Drug list.

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# P1.5 Reporting adverse drug reactions occurring during anaesthesia

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**Introduction**. Adverse drug reactions are one of the biggest problems that can occur during anaesthesia. Anaesthetics, muscular blocking agents, opioid analgesics, antibiotics and many other drugs are administered during anaesthesia. Using so many different drugs in such a short period of time can produce significant number of adverse reactions. Anaphylactic reactions and drug interactions are of special concern. In that situation, many adverse reactions in anaesthesia are not recognized, or are not reported, and therefore not evidenced in official statistics. That is especially true for minor, clinically less important reactions. On the other hand, severe, life threatening reactions are often not recognized as drug related. Many studies show differences in prevalence of adverse drug reactions between different countries, or even regions. System design. We are designing an auxiliary system for adverse drug reactions occurring during anaesthesia reporting. Our objective is to create a system that is simple and easy to use. Gathered information must be easily accessible for viewing and processing. The system is web based. The core of the system is relational data base. Users will be given user name and password by system coordinator. After authentication, they can use a web form to input data on adverse drug reactions. Patient's personal information neither information about other participants in the event will not be not entered. Software gives a unique key for every form. The only person that knows unique combination key-patient-adverse-reaction is the person that entered that data. His identity is known to the system coordinator. This is the way to avoid using expensive and complicated software and hardware for protecting personal information. All data in the data base is anonymous. Searching the data base is achieved using another web form. **Conclusion**: Development of this project is collaborative effort of personnel that already work together in developing a Wiki-based web project . The primary goal of this system is to address specific problems and situations occurring during anaesthesia in our geographic region.

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# P1.6 Peripheral serotonergic markers after total thyroidectomy: the effect of antihypertensive medications

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**Background**: Previous studies have shown some evidence that hypothyroid status could be associated with altered serotonin (5-HT) responsiveness. Platelet serotonin (5-HT) levels and platelet monoamino oxidase (MAO B) activity might be used as limited peripheral markers of the central 5-HT system. Objective: The aim of the study was to determine a) the relationship between thyroid state and peripheral serotonergic markers (platelet 5-HT and MAO B) and b) the effect of antihypertensive drugs (adrenergic blocking agents) on platelet 5-HT levels and MAO B activity in hypothyroid patients and euthyroid control subjects. **Material and Methods:** The study included 41 hypothyroid patients in postoperative follow-up after total thyroidectomy due to papillary thyroid carcinoma and 67 age and sex matched euthyroid healthy controls. All hypothyroid patients had increased TSH serum level that is achieved by the exclusion of levothyroxin hormone replacement therapy. All subjects were free of concomitant psychiatric and endocrine illnesses. Subjects taking different medications (with the exception of antihypertensive therapy) were excluded from the study. 16 hypothyroid patients and 19 control subjects were taking different antihypertensives including alpha 1 and beta adrenoceptors blockers in combination with angiotensin converting enzyme II antagonists and calcium channel blockers. Blood sampling in all subjects was done in the morning after an overnight fasting, prior to physical and nuclear medicine examinations. Platelet 5-HT and MAO B activity were determined using spectrofluorimetric methods. Total thyroxin (T4) and thyroid stimulating hormone (TSH) levels were determined using commercially available radioimmunoassay and immunometric kits. **Results**: As expected, T4 levels were significantly ($p<0.05$) decreased and TSH levels increased in hypothyroid patients as compared to hormone values in control subjects. Platelet 5-HT values were significantly ($p<0.001$) lower in hypothyroid patients compared to healthy controls. There was no significant difference in MAO B activity between patients and control subjects. A positive correlation between 5-HT levels and MAO B activity was found but only in the control subjects. A significantly lower ($p=0,025$) 5-HT levels were observed in the subgroup of control participants taking antihypertensive medications compared to those without medications. There was no significant difference in platelet 5-HT value between thw two subgroups of hypothyroid patients depending on the presence of antihypertensive medication. Antihypertensive therapy did not influence platelet MAO B activity in patients and control subjects. **Conclusion:** Our results show a positive relationship between severe hypothyroidism and decrease in peripheral 5-HT concentration. The findings suggest that the pharmacological features of alpha 1 and beta adrenoceptor antagonist could be relevant to the psychopharmacotherapy. Further studies are needed to elucidate the interaction between the thyroid function, serotonergic system and antihypertensive medications in healthy controls and patients with mental disorders.

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# P1.7 Clinical significance of the interaction between antihypertensive and antirheumatic drugs

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Non-steroidal anti-inflammatory drugs (NSAIDs) may increase blood pressure (BP) and blunt the effects of many antihypertensive agents. It seems that different antihypertensive drugs and NSAIDs vary in their propensity to such an interaction. **Methods**. A prospective, parallel group clinical trial in a family practice setting in Split, Croatia, included 88 treated hypertensive subjects of either gender above 55 years of age; 39 controls and 49 taking NSAIDs for osteoarthritis. After clinical evaluation and informed consent the examinees were allocated following a factorial design. Compared were two antihypertensives: lisinopril/hydrochlorothiazide combination (L/H) and amlodipine (AM), with three NSAIDs: paracetamol (PC), ibuprofen (IB), and piroxicam (PX). The antihypertensive treatment was unchanged during this 3-month study, while the NSAID medication was split in 3 monthly blocks (IB or PX – PC – the first NSAID again). **Results**. In the control group there were 17 female and 22 male examinees, aged 68.9±6.7 years, weighting 82.0±12.4 kg, and their standing BP at the inception of this study was 148.6±10.6/89.2±7.1 mm Hg. In the study group there were 33 female and 16 male patients, aged 69.1±6.8 years, weighting 82.1±13.9 kg, and their baseline standing BP was 149.3±9.8/88.7±6.8 mm Hg. In the L/H subgroup IB and PX elevated systolic BP (SBP) by 7.7-9.9% ($p<0.001$), which during PC period decreased by 6.9-9.4% or to 0.3-0.9% above baseline ($p<0.001$), increasing again by 7.0-7.7% ($p<0.001$) during the second exposition. In the AM subgroup IB or PX increased SBP by 1.1-1.6% ($p>0.290$) only, with insignificant changes during the PC or the second IB or PX period. Similar alterations were observed in diastolic BP as well. In the control group BP did not fluctuate appreciably during this study. **Conclusions**. NSAIDs indeed blunt the effects of antihypertensive drugs but not to the same extent; PX marginally surpassed IB, while PC was almost inert. L/H was much more affected by the interaction than AM. Implications for clinical practice are self-evident.

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# P1.8 Suicidity and platelet serotonin concentration in alcoholism

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**Introduction**: Alcoholism has become a serious and global health problem, a chronic disease refractory to treatment, associated with high comorbidity, and frequently with suicidal behavior. Suicide is a major complication of different psychiatric disorders including alcoholism. Suicidal behaviour carries devastating effects on patients and their families, and financial burden on the whole society. The neurobiological basis of alcoholism, or suicidal behavior, involves the altered functions of different neurotransmitter systems including serotonin (5-hydroxytryptamine, 5-HT). Blood platelets might be used as limited peripheral model for the central 5-HT neurons, since platelets and 5-HT synaptosomes share similarities (in uptake, storage and release of 5-HT, in platelet monoamine oxidase (MAO) type B, 5-HT transporters, 5-HT2 and $a_2$-adrenergic receptors, and binding sites for $^3$H-imipramine, $^3$H-paroxetine, and $^3$H-LSD), and differences are in the synthesis and function of 5-HT. Platelet 5-HT concentration was reported to be decreased or unchanged in alcoholic patients, and reduced platelet 5-HT concentration has been found in suicidal behaviour in different psychiatric disorders. The hypothesis of this study was that alcoholic patients with suicidal behavior will have altered platelet 5-HT concentration when compared to non-suicidal alcoholics. Since we have previously shown that comorbid depression affected platelet 5-HT concentration in female alcoholic patients, alcoholic patients were subdivided into those with and without comorbid depression. **Methods**: Platelet 5-HT concentration was determined fluorimetrically in medication-free 252 male and 62 female ethnically homogenous medication-free subjects with alcoholism (diagnosis made using SCID based on the DSM-IV criteria), subdivided further according to the past history of suicidal attempts into suicidal and non-suicidal patients, and subdivided into those with or without comorbid depression, and these values were compared with platelet 5-HT concentration in corresponding subjects (control healthy subjects and depressed patients). **Results**: Platelet 5-HT concentration was significantly lower in 252 male and 62 female alcoholic patients than in corresponding 147 male and 140 female control subjects. Sex differences in platelet 5-HT concentration were found, showing significantly lower values in female compared to male (control or alcoholic) subjects. Suicidality did not affect significantly platelet 5-HT concentration within male alcoholics, while female suicidal alcoholics had significantly higher platelet 5-HT concentration than nonsuicidal alcoholic women. Both suicidal male or female alcoholics had significantly lower platelet 5-HT concentrations than corresponding healthy subjects. Suicidal depressed patients had significantly lower platelet 5-HT concentration than the corresponding nonsuicidal depressed patients, or control subjects. The presence of comorbid depression in alcoholic patients did not affect platelet 5-HT concentration in male, but increased these values in female alcoholic patients. **Conclusion**: Platelet 5-HT concentration was reduced in all alcoholic patients. The presence of suicidal behaviour and/or comorbid depression increased platelet 5-HT concentration which was reduced in female alcoholic patients. In male alcoholic patients neither suicidality nor depression affected significantly platelet 5-HT values. The results from the present study did not confirm the hypothesis of a reduced platelet 5-HT concentration in suicidality across different psychiatric entities, indicating that a search for other valid peripheral biochemical marker(s) of suicidality must be continued for the screening or prediction of the suicidal risk in alcoholic patients.

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# P1.9 Drug-induced disturbances of serum glucose and lipid profile during treatment with antipsychotic and antidepressive drugs

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**Introduction**: The second-generation antipsychotics (SGAs) and antidepressives (AD) are of great benefit to a wide variety of people with psychiatric disorders. Treatment with those drugs has been linked with increased rates of the metabolic syndrome (dislipidemia, obesity and hyperglycemia). The etiology of increased risk for metabolic abnormalities is uncertain but their prevalence seems correlated to an increase in body weight often seen in patients taking an SGA and AD. Although the data are limited the available evidence suggests that changes in serum lipids are concordant with changes in body weight. Among those disturbances serum lipids are less investigated. The role of lipid metabolism in disease of psychotic illnesses has been a point of extensive research. The relative contribution of cholesterol from any of these source is dependent upon genetic predisposition, diet, drug therapies, interplay of enzymatic up- and down regulations and other potential factors. Cholesterol is needed for proper function of serotonin receptors in the brain. Low cholesterol levels have been linked to aggressive and violent behaviour, depression and suicidal tendencies. These adverse conditions are closely linked and their prevalence appears to differ depending on the SGA used. The clozapine and olanzapine produce the greatest weight gain and associated with greatest increases in total cholesterol, LDL cholesterol, triglycerides and with decreased HDL cholesterol. Amisulpride or ziprasidone are associated with decrease in body mass index and total cholesterol whereas HDL cholesterol increased. The aim: The aim of this study is determined effects of SGA and AD treatment on metabolic profile in psychiatric patients. **Patients and Methods:** We conducted study in 30 patients in order to compare metabolic profile during six month treatment and more with SGA, AD and both drugs. According to therapy we divided patients in three groups (SGA, AD and SGA+AD). The levels of fasting blood glucose, total cholesterol (TC), triglycerides(TG),LDL- cholesterol, HDL- cholesterol and BMI were assessed. **Results**: Our results showed disturbance of lipid profile in patients who are treated with SGA and AD. The concentrations of total cholesterol (76% patients), triglycerides (50% patients), LDL-cholesterol (60% patients) and BMI (73% patients) were increased in all group of patients. The level of fasting glucose was normal in 84% patients in all groups. HDL- cholesterol was normal in all group of patients. The significantly differences of measured parameters (TC, p=0,749; TG, p=0,470; LDL, p=0,855; HDL, p=0,076; GUK, p=0,164; BMI, p=0,503) between all three groups of patients were not found (p> 0,05). **Conclusions**: Our results of disturbance of lipid profile are important with regard to the increased risk for cardiovascular events in patients who are treated with SGA and AD. The existence of serious health risks in patients taking SGA and AD should receive appropriate baseline screening and ongoing monitoring.

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Abstract

# P1.10 Monoamine oxidase type B polymorphism in combat related posttraumatic stress disorder

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**Introduction**: Neurobiological basis of posttraumatic stress disorder (PTSD) involves the alternations in different neurotransmitter systems such as noradrenalin, dopamine and serotonin. Monoamine oxydase (MAO) is an enzyme responsible for the degradation of different endogenous and exogenous amines. Endogenous amines involve neurotransmitters (noradrenaline, dopamine and serotonin) and therefore MAO plays a central role in the metabolism of monoamine neurotransmitters. There are two isoforms of MAO, MAO-A and MAO-B. These subtypes are encoded by two different genes, placed near each other on X chromosome (region Xp11.23-11.4). In platelets MAO exists in MAO-B isoform, and it has been proposed to be a biomarker for different personality characteristics and psychiatric disorders. Platelet MAO or MAO-B activity is under influence of various factors, such as smoking, gender, age, ethnicity, some neurodegenerative diseases and lithium or haloperidol treatment. One of the factors assumed to influence platelet MAO activity might be a polymorphism of *MAO-B* gene on the polymorphic region of the intron 13. The molecular basis of this polymorphism is A/G substitution 36 bp upstream from the intron 13-exon 14 boundary. Aim of the study was to determine the distribution of the MAO-B genotypes in Croatian war veterans with PTSD, in war veterans who were exposed to the similar combat experience but did not develop PTSD, and in healthy control subjects. **Methods**: The study included medication-free male Caucasian subjects who did not have neurodegenerative diseases: 91 war veterans with PTSD, 36 war veterans without PTSD and 100 healthy control subjects. DNA was isolated from their blood samples, and the method used for genotyping was a real-time PCR using Taqman –based allele –specific polymerase chain reaction assay. Results: The results showed no differences in the distribution of A allele or G allele of the *MAO-B* gene between healthy control subjects, war veterans without PTSD, war veterans with PTSD with psychotic symptoms and war veterans with PTSD with non-psychotic symptoms. **Discussion**: Therefore, no association was found in the allele frequencies of the MAO-B genotype in PTSD. In agreement with our data, no significant association between the allele frequency and A/G polymorphism in intron 13 of the MAO-B gene was found in schizophrenia, schizophrenia with or without tardive dyskinesia, schizophrenia with aggressive behavior, migraine, Parkinson's disease, or depressed state. Since our data did not confirm the hypothesis that the appearance of A allele or G allele is associated with the etiology or development of PTSD, further studies should evaluate other polymorphisms, presumably related to MAO-A gene, to determine functional significance and associations with different traits or disease.

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Abstract

# P1.11 Color reproductions of psychopharmacs registered in Croatia

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Poster presents color photographs of most psychopharmacs (psychoactive drugs: groups N02 to N07) registered in Croatia in July 2007. One photo contains all forms of a single original or generic compound. This is the very first and widest large scale color reproduction of drugs used in everyday psychiatric clinical practice in Croatia. We are strongly convinced that this poster will help in several aspects of routine work in both psychiatric and family medicine practices, as well as in internal medicine and other intensive care units. We hope it to be of assistance to medical students and scholars in familiar and close fields. The aim of the authors is to update the color reproductions of registered psychopharmacs in Croatia periodically.

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Abstract

# P1.12 Allelic variants of the multidrug resistance gene (MDR1/ABCB1) and response to corticosteroid therapy in patients with inflammatory bowel disease

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**Background/aims:** Steroid dependency is an important problem in managing patients (pts) with inflammatory bowel disease (IBD). This study examined the association of single nucleotide polymorphisms in the MDR1 gene of 124 IBD pts (40 ulcerative colitis (UC) and 84 Crohn's disease (CD) pts) with respect to response to corticosteroid therapy. **Patients and Methods:** According to European evidence-based consensus on the diagnosis and management of CD, IBD pts in this study were characterized as steroid-dependent pts (n=71) and good responders to corticosteroids (n=53). Analysis of G2677T polymorphisms in exon 21 and C3435T in exon 26 of MDR1 gene was performed by PCR-RFLP method. **Results**: Genotype frequencies of the 2677GG, 2677GT and 2677TT MDR1 exon 21 in the sample were 48, 54 and 22, respectively and of the 3435CC, 3435CT and 3435TT of MDR1 exon 26 genotypes were 31, 57 and 36, respectively. Test result for linkage disequilibrium between loci was found to be significant in good responders, steroid dependent pts and total sample. Pair-wise comparisons of the allele and genotype frequency among different groups of responders revealed no statistical differences for both loci in total sample and CD pts analyzed separately. Analysis in UC patients revealed statistically significant difference in genotype distribution (p=0.025) and in distribution of exon 21 G2677T allelic variants showing overrepresentation of the 2677T allele in the group of steroid dependent pts (p=0.003). In UC patients, statistical differences were found in distributions of the estimated haplotypes between good responders and steroid dependent pts (p=0.006). Subsequent analysis showed G2677/3435T haplotype to be overrepresented in the group of good responders compared to steroid dependent pts (p=0.012) and 2677T/3435T haplotype to be overrepresented in group of steroid dependent pts compared to good responders (p=0.003). In CD patients no statistical differences were found in distributions of estimated haplotypes between good responders and steroid dependent pts in the sample. **Conclusions**: Results in this study indicate that G2677T polymorphisms in exon 21 and C3435T in exon 26 of MDR1 gene have significant influence on development of steroid dependency in UC. Overrepresentation of G2677/3435T haplotype in the group of good responders and 2677T/3435T haplotype in steroid dependent pts suggests that polymorphisms of MDR1 gene could contribute to development of steroid dependency in pts with UC. Such correlation was not shown for CD pts.

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Abstract

# P1.13 Azathioprine-induced allergic hepatitis associated with thiopurine methyltransferase (TPMT) genotype; case report

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**Introduction**: Azathioprine (AZA) is well established treatment for steroid-dependent and refractory inflammatory bowel disease. However, the use of azathioprine is limited by both its long onset of action and drug toxicities. The likelihood and type of adverse effect may relate to thiopurine methyltransferase (TPMT) enzyme activity and genotype. According to available data bone marrow suppression and toxic hepatitis (but not pancreatitis or allergic reactions) are related predominantly to the activity of TPMT. Hepatoxicity is a rare complication with the unknown mechanism of hepatic injury, registered more frequently among those with higher TPMT activity. Case: A 54-year-old man with steroid dependent ulcerative colitis presented with high grade fever 24 hours after being started AZA (1 mg/kg/day) and acute hepatitis (AST 236U/l, ALT 278U/l, GGT 277U/I). Fever rapidly resolved one day after AZA was discontinued and aminotransferases normalized after six weeks. In that time we had no possibility for TPMT genotyping. Recently, the allelic variants of the TPMT gene (*2, *3A,*3B,*3C) were analyzed by polymerase chain reaction-based assays. Genotyping revealed that our patient was heterozygous for TPMT*3A allele (mutations G460A and A719G), which correlates with a low activity phenotype. **Discussion / Conclusion:** The TPMT-deficient individuals probably account for most of the AZA-intolerant patients previously considered to have »idiosyncratic« toxic effects. Genotyping for the major TPMT variant alleles may be a valuable tool in preventing severe AZA toxicity and optimization of immunosuppressive therapy. Not only homozygous patients may be at highest risk for severe toxicity and therefore alternative therapy should be considered.

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Abstract

# P1.14 Thalidomide for the treatment of multiple myeloma

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Thalidomide is a synthetic glutamic acid derivative which was initially developed in the early 1950s as an anticonvulsant for the treatment of epilepsy. Following a lack of sufficient efficacy as an anti-epileptic, it was eventually marketed as a sleep aid and was also widely used as an anti-emetic during pregnancy. Use of this agent during the first trimester of gestation led to alarming rates of phocomelia, defects in long bones, absence of auricles, cleft lip, and cardiac and gastrointestinal anomalies. While thalidomide had not received approval by the Food and Drug Administration (FDA) in the United States (US) at this time due to concerns of neurotoxicity, the widespread international use of this drug as a sedative was eventually halted during the early 1960s. More recently, thalidomide was approved for the treatment of multiple myeloma in May 2006, and has reported efficacy in a wide spectrum of malignant and non-malignant diseases.The use of IMiDs in the treatment of multiple myeloma has recently emerged as the standard of care following multiple reports of efficacy in both front-line therapy and in relapsed or refractory disease. In May 2006, thalidomide was approved by the US FDA for the treatment of newly diagnosed multiple myeloma in combination with dexamethasone. Thalidomide, a first in-class immunomodulatory orally bioavailable drug, was found to have significant single agent activity in relapsed refractory multiple myeloma.Extensive preclinical studies elucidated several novel biologic mechanisms of action: direct pro-apototic effects and G1 growth arrest of multiple myeloma cells; down-regulation of binding of multiple myeloma cells to bone marrow stromal cells (BMSCs), which confers cell adhesion-mediated drug resistance (CAM-DR); and inhibition of multiple myeloma growth factors including IL-6, TNF, and vascular endothelial growth factor (VEGF). Antiangiogenic effects, mediated via inhibition of VEGF and beta fibroblast growth factor (FGF), are also an important component of its activity in multiple myeloma.Especially noteworthy are its immunomodulatory effects, evidenced by upregulation of natural-killer cells through the release of interferon gamma and IL-2, in both preclinical studies and in patients on clinical protocols. Importantly, thalidomide and dexamethasone are synergistic *in vitro* and have impressive clinical activity in relapsed refractory multiple myeloma patients, even in patients in whom either agent alone has failed. Thalidomide has been shown to be effective in approximately 30% of patients with refractory or advanced multiple myeloma (MM). Here we report a case of patient treated with thalidomide for refractory MM showing dedifferentiation of the neoplasm. In this case thalidomide treatment – despite reduction of M-component – was followed by disease progression and a very poor clinical outcome which was paralleled by bone marrow plasmacytosis showing marked signs of dedifferentiation, inducing us to speculate on a potential role of thalidomide on dedifferentiation of myeloma cells. In our opinion, a possible dedifferentiation of MM should therefore be taken into account in MM patients treated with thalidomide when clinical course deteriorates despite reduction of M-component.

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Abstract

# P1.15 Therapy of temporomandibular joint displaced disc according to anxiety

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**Introduction**. Disc displacement (DD) of temporomandibular joint (TMJ) is the most frequent form of temporomandibular disorders (TMD) with the aethiopathogenesis not being sufficiently clarified. Occlusal splint is the most frequent reversible and unspecific form of initial treatment of patients with DD, but also of persons with parafunctional oral activity – bruxism. Initial treatment includes various types of supportive therapies, like pharmacologic therapy. The main goal of TMD treatment is directed toward the reduction of pain. The nonsteroidal antiinflammatory drugs are helpful with most temporomandibular pains. The purpose of this study is to determine the success in eliminating clinical symptoms in patients with DD after occlusal splint therapy, possibly depending on a level of anxiety. Material and methods. DD was diagnosed in 40 patients (mean age 35.5, 76% women) using Research Diagnostic Criteria for Temporomandibular Disorders Axis I and was confirmed by magnetic resonance imaging of the TMJs. Pain intensity was rated on a visual-analogue scale (VAS 0-10). The control group consisted of 25 asymptomatic volunteers (mean age 23.4, 72% women). Bruxism was diagnosed based on case history and clinical findings. The anxiety was confirmed by Spielberger's State-Trait Anxiety Inventory (STAI). **Results**. By applying occlusal splint a reduction of pain was achieved in 61% of TMJs. 40% of patients had bruxism and tooth wear was significantly more correlated in patients ($p<0.05$) than in the asymptomatic volunteers. A higher level of anxiety was determined for all examined patients without statistically significant difference ($p>0.05$) with respect to the asymptomatic volunteers. Also, success of splint treatment was not dependent on anxiety ($p>0.05$). Including only patients with determined anxiety resulted in 62.5% of patients with anxiety according to the STAI 1=42.84, and 72.5% of patients with anxiety according to the STAI 2=44.20. Distribution of patients according to diagnosed bruxism, and scores in STAI 1 and STAI 2 test was significant ($p<0.001$). Statistically significant differences between patients with lower (<5) and higher (=5) degree of pain were rated on a VAS for age ($p=0.004$), passive mouth opening ($p=0.013$), STAI 2 ($p=0.012$), and duration of splint therapy ($p=0.011$). **Conclusion**. There was no correlation between success of occlusal splint therapy and anxiety. Psychological factors are considered to play key roles for the development of bruxism behaviour and temporomandibular pain. However, patients with DD experience a higher level of anxiety and bruxism behaviour.

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Abstract

# P1.16 Nicotine and periodontal health of adolescents

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**Introduction**: Cigarette smoking and theirs active ingredient nicotine, is well known and proven risk factor for beginning and progression of periodontal disease. Nicotine, which is transformed in the organism to its active metabolite cotinine, has been found in serum, saliva and gingivocervicular fluid of smokers. It is still not known the exact negative effects it has on periodontal tissue, but is known its immunosuppressive effect. Also nicotine decreases revasculation of hard and soft tissues, decreases oxygen pressure in mouth what allows anaerobic bacteria to grow better and it enhances bacterial adherence on periodontal epithelial cells. Aim of this study was to determine association between cigarette smoking and periodontal health of adolescents, to determine are there any differences in periodontal status among smokers and non-smokers, and define treatment need for both. **Materials and Methods:** Study was carried out on 517 high school students. Data regarding age, gender and smoking habits were taken. Periodontal health was examined with CPI index. **Results**: There were 34.6% of smokers among high school population, evenly percentage of male (35.8%) and female (37.1%) students. More then half examinees 55.3% smoked 1-10 cigarettes per day (daily), and 38.8% smoked 11-20. Periodontal health differs between smokers and non-smokers. Bleeding after probing was found more frequently among non-smokers, while supergingival calculus was found more among smokers. Consequently non-smokers treatment need (TN)was instruction in oral hygiene and correctly teeth brushing method, while smokers treatment need was instruction in oral hygiene and teeth brushing method together with initial periodontal therapy. **Conclusion**: The study has indicated the influence of smoking on periodontal health even in this young age population, therefore it is very important to inform young smokers about the negative consequences of cigarette smoking.

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Abstract

# P1.17 Case report: Could the oral contraceptive pills be the cause of the internal jugular vein thrombosis during the perioperative period?

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A 22-year-old female patient was scheduled for elective tympanoplasty due to a chronic otitis media of the left ear. Other than that she was healthy. According to the anamnesis she stopped taking oral contraceptive pills (OCPs) a month before the surgery. She did not receive thromboprophylactic therapy before the surgery because it was estimated that there is a low risk for thromboembolic incident. Several hours after the surgery she was still not responding properly to external stimulus and there was no verbal contact. An urgent CT scan of the head and neck revealed thrombosis of the left internal jugular vein. She was admitted to the ICU and heparin therapy started. After a few days she was fully recovered. OCPs were the only risk factor for thrombosis.

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Abstract

# P1.18 Therapeutic drug monitoring of mycophenolic acid in patients with kidney transplant

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Mycophenolate mofetil (MMF) is an immunosupressive drug used in prophylaxis of rejection of transplanted solid organs in combination with cyclosporine A (CyA) and steroids. It is metabolised in the liver to the active component mycophenolic acid (MPA), an irreversible noncompetitive inhibitor of cell enzime 5´-monophosphate (IMP) dehydrogenase. The aim of study was to determine the concentrations of MPA, CyA and creatinine in patients with kidney graft and find out if there is correlation between standard daily MMF dose and MPA concentrations, a correlation between MPA and CyA concentration, a correlation between MPA concentrations and serum creatinine (surogat marker of kidney function), and a correlation between CyA level and creatinine. MPA was determined on 42 kidney transplant patients aged 11-60 years. All were receiving MMF in combination with CyA and low dose steroids. After stabilisation of kidey function, more than 6 months posttransplant, mean doses of MMF and CyA were 915± 206 mg/m$^2$ and 2,74± 1,24 mg/kg, respectively. MPA concentration was measured by HPLC method on the Chromsystems column with 1,0 mL/min flow and UV detector ($\lambda$=215 nm). CyA concentration was determined using immunoassay method (Dimension, Dade Behring). Mean serum concentration (C-0) of MPA was 8,56± 6,25 $\mu$mol/L, and mean CyA concentration (C-0) in whole blood was 111,16± 41,93 $\mu$mol/L. Mean creatinine value was 135±67 $\mu$mol/L. No correlation was observed between MMF daily dose (mg/m$^2$) and MPA trough concentration in serum ($\mu$mol/L) ($P< 0,05$). Also there was no correlation between MPA and CyA concentrations ($P<0,05$) and CyA and creatinine ($P<0,05$). Slight positive correlation was established only between MPA and creatinine ($r^2=0,35$). The absence of correlation between the stated parameters indicates the need to monitor therapeutic concentrations of MPA in order to optimize therapy, similarly to regular monitoring of CyA concentrations.

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Abstract

# P1.19 Oral valganciclovir is as effective and safe as IV ganciclovir for the treatment of cytomegalovirus infection in renal transplantation

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**Introduction.** Cytomegalovirus (CMV) infection and disease are major complications of renal transplantation. We compared the efficacy and safety of oral valganciclovir (VGCV) versus intravenous (IV) ganciclovir (GCV) for treatment of CMV infection in adult renal transplant recipients. **Methods**. Patients (n=98) who developed CMV infection proved by positive CMV DNA, were randomized to receive either 900 mg daily oral VGCV (n=49) or 5 mg/kg twice daily IV GCV (n=49) for 21 day; then all patients received 900 mg once daily VGCV maintenance therapy for an additional 70 days. Drug doses were adjusted for renal function. CMV viral loads were determined by quantitative PCR at days 0, 14, 21, 91 and then compared between VGCV and IV GCV treated patients. **Results**. Demographic and baseline characteristics were similar between the two groups. Viral clearance did not differ between treatment groups at day 21 (VGCV: 46.7% vs. GCV: 49.1%) and at day 91 (VGCV: 97.1% vs. GCV: 93.7%). Both treatment s were well tolerated and there was no difference in the adverse event profile. The median time to viral eradication was similar in both treatment arms (VGCV 21 days vs. 19 days GCV). **Conclusions**. The most important factor influencing time to eradication was the initial viral load. These data clearly demonstrate that oral valganciclovir and intravenous ganciclovir provide equivalent viral clearance in the treatment of CMV infection in renal transplant recipients. These findings have major implications for the treatment strategies of CMV infection for patients, physicians and health-care providers.

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Abstract

# P1.20 Soluble P-selectin in hypertensive patients might be a marker of disturbed hemorheology

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**Introduction**: Endothelial dysfunction seems to have a central role in the pathogenesis of hypertension-induced atherosclerosis. The adhesion of activated platelets to the dysfunctional endothelial cells plays an important role in the early develpment and progression of atherosclerosis. Apart from its origin from endothelial cells, soluble form of adhesion molecule P-selectin is mainly a product of activated platelets. The aim of this study was to assess whether plasma levels of soluble P-selectin is raised in hypertensives, compared with normotensives and whether its plasma levels depend on the severity of hypertension. **Methods**: Soluble P-selectin was measured in plasma of 113 older subjects, using commercially available enzyme immuno assays. There were 39 patients with severe hypertension (sistolic blood pressure =180 mmHg and diastolic =110 mm Hg), 40 patients with mild-moderate hypertension (sistolic blood pressure 140-179 mm Hg and diastolic 90-109 mm Hg) and 34 sex- and age- matched normotensive subjects of a control group. The relationships were also assessed, using univariate and multiple regression analyses, of the levels of soluble P-selectin with: 1.other risk factors, including Body mass index, fasting glucose, total cholesterol and triglycerides, 2. markers of inflammation, including total leukocyte count and C-reactive protein, 3. markers of renal dysfunction (as an example of target-organ disease), and 4. Red Blood Cell sedimentation rate, conventional marker of inflammation, indicating Red Blood Cell aggregability. Multiple regression analyses were perfomed on both the whole group of 113 subjects and on the hypertensive patients particularly. **Results**: There were no significant differences in plasma levels of soluble P-selectin, across the groups. Parameters selected in multiple regression analyses were: Red Blood Cell sedimentation rate ($\beta$=0.27, p=0.005) and total leukocyte count ($\beta$=0.217, p=0.022) – on pooled subjects, and Red Blood Cell sedimentation rate ($\beta$=0.278, p=0.016) and total cholesterol ($\beta$=0.23, p=0.044) – on all hypertensives. **Conclusions**: Raised levels of soluble P-selectin may not be merely the consequence of high blood pressure. Rather, the hypothesis might be proposed about soluble P-selectin as an integral component of pathogenic condition associated with enhanced aggregability and adhesivity of blood cells, known as disturbed hemorheology. Disturbed hemorheology affects peripheral vascular resistance and blood flow and may represent cyclic feedback mechanism, leading gradually to the elevation of blood pressure and an enhancement of metabolic disorders.

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Abstract

# P1.21 Toxic epidermal necrolysis after linezolid administration

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Clinic for skin disease, This is a case report of a 66-year-old patient, who presented with a serious adverse cutaneous reaction after linezolid administration. The patient was admitted to the intensive care unit (ICU) after an urgent craniotomy due to a spontaneous cerebral haematoma. His medical history showed that he had COPD and psoriasis, a penicillin allergy, and was also a smoker and a chronic alcoholic. Several years ago he had undergone a prostatectomy due to a prostatic adenoma. Before his ICU admission, the patient was treated in another institution, where meticillin resistant Staphylococcus aureus (MRSA) colonisation of the upper airway was established. An infectious complication developed during the postoperative period, which aggravated the patient's general and psycho-neurological condition. Treatment with vancomycin and meropenem was started, due to the suspicion of postoperative meningitis. Neither meningitis nor another sepsis focus were confirmed. Despite intensive care the patient's condition was not improved, and MRSA was isolated repeatedly from the tracheal aspirate. A pneumonic infiltration was later established, so linezolid was introduced instead of vancomycin, since vancomycin was not efficient with the MRSA eradication. Six hours after the first dose (600 mg) of linezolid, purpuric eruption appeared on the extensor surface of both arms and legs. These petechial lesions were considered a sepsis manifestation. After the second dose of linezolid, which was administrated 24 hours after the first one, numerous confluent purpuric macules, bulles and erosiones appeared at the place of the earlier petechial eruption. Patient's clinical condition aggravated dramatically, with clinical signs of circulatory shock and MODS. Due to the suspicion that the skin lesions were related to linezolid treatment, linezolid was omitted from further therapy. Despite the intensive therapeutic measures the patient died on the 29th day of hospitalisation. Autopsy was not performed, but the posmortal skin biopsy suggests a toxic epidermal necrolysis. According to the distinctive time sequence between drug application and side effect, we speculate this adverse event to be linezolid related.

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Abstract

# P1.22 Oxygen as a drug – a presentation of method

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Oxygen is the most prevalent and most important element on earth. It is ubiquitarious and inevitable for life. In the form of hyprebaric oxygen, it is called a drug. The most natural of all drugs. As a drug, oxygen has its farmacokinetics and farmacodynamics, according to physiology of oxygenation and gas exchange. Hyperbaric medicine is rather new and, therefore, controversal medical branch. Even a lot of medical doctors are very poorly informed about its basic principles, mechanisms, effects and practical appliance, which often results in misunderstandings in practise. Hyperbaric oxygenation is medical method of breathing 100% $O_2$ under pressure greater that found on earth's surface at sea level. That can arise the amount of oxygen dissolved in blood about 15-20 times. That increase produces several positive physiological effects in various patological states- acute or chronical- triggered by hypoxia. On that base, the indication list has been made, approved by the Undersea and Hyperbaric Medical Society. They are very limited and rely on the proof of efficacy of HBO by controlled studies. As well, hyperbaric oxygen-as-a-drug has its contraindications, complications and states completely inert for its appliance. Another 'drug characteristic' of hyperbaric oxygen is dosage – a recommended regime for each indication (oxygen pressure, exposure duration, total number of expositions, breaks), as well as proper timing of treatment. Also, the certain complications should appear during HBO treatment, some side effects and interaction with other drugs. Furthermore, oxygen toxicty has still been a miscellaneous and doubtful chapter in hyperbaric research for determining the safety margins of HBO practise /- to predict an effectivness of HBO treatment, controling its duration./ To resume all, regarding all these physiological, pharmacological and clinical determinations, hyperbaric oxygenation is very safe, pleasant and efficient medical method, suitable even for improvement of general condition status, but also for entirely healthy men. So, why is this method still so extremely low spreaded? And why is it still treated as doubtful, controversal, suspicious? The answer should sound pretty hereticaly and provocatively…First, it is cheap! Second, it has been promoted mostly by non-institutional, non-clinical physicians, declearing often like divine Hoffman's drops!

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Abstract

# P1.23 Effect of leptin levels and selective leptin resistence on hypertension and consequence myocardial hypertrophy in obese individuals

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Hypertension and cardiovascular disease are strongly associated with obesity. Leptin is a product of ob-gene, produced primarily by adipocytes and secondary in the skeletal muscle, heart, vessels and brain. It promotes weight loss by reducing apetite and food intake and by increasing energy expenditure. Concentrations of serum leptin level is proportional to body mass and it is notable increased in obesity. Obesity is charachterised with hyperleptinemia and selective metabolic leptin resistence, with preservation of its sympathoexcitatory actions. Leptin posses cardio-renal actions wich contributing to obesity-related hypertension including generalized symapthoactivation. Hypertension leads to myocardial hypertrophy, but till now it was not well understood, it can be result from increased hemodynamic stress and humoral factors. Hyperleptinemia could be one of the factor who potentially contribute to myocardial hypertrophy. The research was carried out on 42 hypertensive patients (21 male and 21 female) with myocardial hypertrophy and on control group of 32 hypertensive patients without myocardial hypertrophy (16 male and 16 female), with BMI in range 25–30 kg/m$^2$. All patients were with similar waist/hip ratio as well as normal values of blood glucose level. Height, weight and circumference of the upper arm and forearm, waist, hips, upper and lower leg were measured in the midlle of each body part. Interventricular septum and left ventricular wall thickness as well as ejection fraction were determined by ultrasound. Glucose in blood, creatinine clearance and lipidograme were determined by biochemical blood analysis. Blood samples were taken from all subjects between 7 and 7:30 a.m. Serum leptin level was measured with RIA CT method, it is radioimmunoassay with polyclonal antiserum (Biosource, Belgija). Results showed different values of blood leptin levels in men and women with myocardial hypertrophy. In addition, study gave better insight into adipocyte metabolism and its relation to arterial hypertension and consequential myocardial hypertrophy.

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Abstract

# P1.24 Association between interferon gamma gene polymorphism and cyclosporine a dose requirement in kidney transplant patients

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**Aim**. Cyclosporin A (CsA) is a potent and selective immunosuppressant widely used for the prevention of transplant rejection and for the treatment of various autoimmune diseases. The aim was to examine the relationship between interferon gamma (IFN-γ) gene polymorphism (GP) and requirements for cyclosporine A (CsA) dose in kidney transplanted patients. **Patients and Methods.** The study included 50 patients (21 males, 29 females), mean age 45±13 years (min. 16, max. 62), who underwent the first cadaveric kidney transplantation at least 1 year ago (median 4 years, 1-16)), with creatinin clearance 1,19 ± 0,48 ml/s. IFN-γ gene polymorphism (PCR. SSP, One Lambda Inc USA), daily dose and serum concentration of cyclosporin A (C0) were determined. C0/CsA dose ratio was calculated. **Results**: There were 12 patients with TT genotype (high producers of IFN-γ), 26 patients with TA genotype (intermediate producers of IFN-γ) and 12 patients with AA genotype (low producers of IFN-γ). Mean CsA dose was 135,4 ± 40 mg, and mean CsA concentration was 77,61 ± 26,167 $\mu$l. The C0/CsA dose ratio significantly differed between the subgroups (Chi-square= 7,450, p=0,024, Kruskal-Wallis test). Post-hoc Mann-Whitney test revealed the significant difference between TT and AA subgroups (z=-2,598, p=0,009) and between TA and AA subgroups (z=-2,355, p=0,019). **Conclusion**: Genotype AA for IFN-γ (low producers) was related to the higher CsA requirement. IFN-γ could possibly interfere with CsA pharmacokinetics by inhibiting its metabolizing. This study supports the possibility that the genotype IFN-γ might be an important factor related to the CsA therapy especially in relation to various CsA side effects as it was shown previously for IL-1A gene polymorphism..

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Abstract

# P1.25 The influence of alpha-melanotropin and tetracosactid on ethanol-induced gastritis in rats

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**Introduction**: $\alpha$-MSH (alpha-melanotropin) and $^{1-24}$ACTH (tetracosactid) peptides have anti-inflammatory effects in several *in vitro* and *in vivo* disease models. In this paper, the model of ethanol induced gastritis in rat was used to test the efficiency of $\alpha$-MSH and $^{1-24}$ACTH treatment. **Materials and Methods:** The tested substances were given intraperitonealy one hour prior to the gastritis provocation. The animals were sacrificed one hour after the intragastric application of 70% ethanol (1 ml). Hemorrhagic gastric lesions were measured macroscopically (detecting the lesion surface – a quantitative analysis) and microscopically (detecting the lesion severity – a qualitative analysis). **Results**: In animals treated with $\alpha$-MSH (0.125-2.0 mg/kg) the lesions were significantly smaller than in the control animals ($p<0.05$). This protective effect was also noticed when the $^{1-24}$ACTH was applied (0.225-3.6 mg/kg). Animals treated with $\alpha$-MSH (2.0 mg/kg) and $^{1-24}$ACTH (3.6 mg/kg) had less submucous edema, and no hyperemia, bleeding and superficial epithelium damage, when compared to the control group. **Conclusion**: Contrary to $^{1-24}$ACTH, $\alpha$-MSH does not influence glucocorticoid secretion, so its administration is not followed by the characteristic side-effects. This is due to the lack of $^{14-24}$ACTH region. Other $\alpha$-MSH characteristics (inflammatory modulation, low toxicity and good tolerance) indicate that this molecule might be a promising agent for the inflammatory and autoimmune diseases.

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Abstract

# P1.26 Prostaglandins are involved in the regulation of renal tubular glucose co-transport

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Renal prostaglandins play some role in the regulation of ion transport and solute balance. It is still not enough clear whether the prostaglandins act by direct epithelial effects or mediated by receptors to some secondary phenomena. Therefore, by using electrophysiological method, the experiments were performed *in vivo* on rat kidney, to obtain some insight into possible prostaglandins effects on cellular transport phenomena. The luminal or the peritubular perfusate of proximal tubule contained alternatively one of the prostaglandins ($PGE_2$ or $PGF_{2\ alpha}$) and additionally D-glucose (5 mmol/L) in the tubular lumen. Since glucose is co-transported with $Na^+$ across the brushborder membrane, glucose perfusion elicited a sudden cell depolarization indicating rheogenic co-transport, which was followed by a slower secondary cell depolarization, indicating the adjustment of the cell to a new steady state of transport. The results with prostaglandins showed that the rheogenic glucose response specific changed, although none of the prostaglandins significantly affected the control cell membrane potential. It was found that $PGE_2$ (1 mmol/L) increased the glucose-induced cell depolarization by + 27% (n= 6), while $PGF_{2\ alpha}$ (1 mmol/L) decreased the response of glucose by – 32% (n=6). At lower concentrations of $PGE_2$ (0.1 and 0.01 mmol/L) the overall increase was less pronounced ed. 21% and 13.5% respectively. After preperfusion of tubular lumen with $PGE_2$ the effects were additionally more pronounced, depending from the time. The opposite effects observed with $PGE_2$ and $PGF_{2\ alpha}$, by changing the first phase of cell depolarization, indicates specific involvement on proximal tubular glucose cotransport through the luminal membrane. The alteration of the secondary phase of Na-dependent substrate response suggests that different ion transport mechanisms for $Na^+$, $K^+$, $Ca^{++}$, $H^+$, $HCO_3^-$ or $Cl^-$ in apical or basolateral membrane might be also affected. The exact nature of prostaglandins by regulatory transport mechanisms is yet not quite clear but they are involved in maintaining renal cell homeostasis.

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Abstract

# P1.27 Altered expression of insulin receptor in hippocampus of streptozotocin intracerebroventricullary treated rats

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**Introduction**: Ethiopathogenesis of sporadic Alzheimer disease (SAD) is associated whit changes in brain glucose metabolism particularly in the brain regions connected with cognition and memory. Glucose metabolism in the brain is partly controlled by insulin in the brain which binds to the neuronal insulin receptor (IR) demonstrating signal transduction mechanism similar to the one at the periphery. Following insulin binding, IR becomes autophosphorylated and binds insulin receptor substrate which transduces the signal downstream the phosphatidylinositol-3 kinase pathway inactivating glycogen synthase kinase (GSK-3). GSK-3a isoform is involved in the regulation of metabolism of amyloid-ß peptides and GSK-3ß isoform is involved in tau-protein phosphorylation. Therefore, changes in the phosphorylation/dephosphorylation homeostasis of IR signal cascade are capable of causing alterations in amyloid precursor protein metabolism and tau hyperphosphorylation, both known as the major hallmarks of SAD. In the human post mortem brain tissue of SAD patients IR density was found increased while IR mRNA and protein expression as well as IR tyrosine kinase were found decreased. Brain insulin system has also been investigated in experimental AD model, rats treated intracerebroventricularly (icv) with a betacytotoxic drug streptozotocin (STZ). STZ-icv rat model demonstrates brain glucose metabolism changes and deficit in learning and memory that resemble those found in AD patients. We have recently reported altered expression of enzymes downstream the IR signaling pathway; decreased phosphorylated GSK-3/non-phosphorylated GSK-3 (p-GSK-3/GSK-3) ratio and increased level of p-tau protein in the hippocampus of STZ-icv treated rats, three months following the drug (1 mg/kg) icv treatment. In these rats increased expression of IR protein was found in hippocampus. We were investigated the changes of IR protein expression in the hippocampus of rats treated with a higher STZ-icv dose three month after the drug icv treatment. **Material and Methods:** Three-month-old male Wistar rats (Department of Pharmacology, School of Medicine, University of Zagreb) were given general anaesthesia (chloralhydrate 300 mg/kg, ip), followed by STZ (3mg/kg), dissolved in 0.05M citrate buffer pH 4.5) injection bilaterally into the lateral ventricle (2 μL/ventricle). Control animals were given vehicle icv. Cognitive functions were tested by Morrris Water Maze Swimming Test. Animals were sacrificed three months after the drug treatment. IR protein expression was measured by SDS electrophoresis followed by a Western blot analysis. Data were analysed by Mann-Whitney U test ($p<0.05$). **Results**: Cognitive deficits in learning and memory were found 3 months following the STZ-icv treatment ($p<0.05$). These cognitive deficits were followed by decreased levels (-23%) of IR found in hippocampus of STZ-icv treated rats in comparison to the control ones ($135.67\pm5.4$ vs. $175.33\pm5.5$) ($p<0.05$). **Conclusion**: Decreased IR expression in hippocampus of rats treated with a higher STZ-icv dose is in line with SAD human data suggesting that STZ-icv treated rats are useful tool in modelling of SAD.Acknowledgement:

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Abstract

# P1.28 Cardioprotective effects of 2,3-butanendione monoxime against the Vipera ammodytes ammodytes venom in the isolated rat heart

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**Introduction**: The nose-horned viper, *Vipera ammodytes ammodytes*, the most venomous snake in Europe is widespread in its southeastern part, including Croatia. The venoms of *Viperinae* family cause local tissue damage and systemic symptoms, including cardio circulatory disturbances. The aim of this study was to examine the cardioprotective effects of 2, 3-butanendione monoxime (BDM), a potent negative inotropic and vasodilatatory agent that causes excitation-contraction uncoupling in muscles. The effects of BDM were compared with effects of cyclooxygenase inhibitor (indomethacin), lipoxygenase inhibitor (nordihydroguaiaretic acid (NDGA)), inhibitor cytochrome P450 enzymes (clotrimazole), L-type $Ca^{2+}$ channels blocker (verapamil) and phospholipase A2 inhibitor (quinacrine), agents that have been shown to have cardioprotective effects against different snake venoms. **Materials and Methods:** Wistar rat hearts (n=70) were isolated in the Langendorff mode and perfused with modified Krebs-Henseleit solution, at the constant pressure of 55 mm/Hg and temperature of 37 °C. Dried venom was dissolved in the Krebs-Henseleit solution and infused into the heart for 10 minutes at the rate of 1% of coronary flow allowing final concentration of 90 $\mu$g/ml. The hearts were divided into 7 groups of 10 hearts each: 1. venom, 2. venom + indomethacin (10 $\mu$mol/L), 3. venom + clotrimazole (1 $\mu$mol/L), 4. venom + NDGA (5 $\mu$mol/L), 5. venom + verapamil (1 $\mu$mol/L), 6 venom + quinacrine (2$\mu$mol/L) and 7. venom + BDM (20 mmol/L). The drugs were injected ten minutes before and during venom injection. Left ventricular pressure (LVP), coronary flow (CF), heart rate (HR), atrioventricular conduction time (AVCT) and $O_2$ consumption were continuously measured. Biochemical indicators of myocite damages, enzymes (LDH- lactate dehydrogenase, CPK- creatine phosphokinase, AST- aspartate aminotransaminase) and troponin I, were collected from coronary effluent after 5 and 10 minutes of venom administration. **Results**: The venom caused irreversible damage of the myocardium, resulting in decreased developed LVP from 101.31±3.57 mm/Hg to 13,0±2.61 mm/Hg, CF from 10,71±0.71 ml/min to 7,70±0.34 ml/min, and increased LDH, CPK, AST (U/L), and troponin I (mg/L) from 0,27 ±0,20, 3,36 ±0,96, 4,13 ±2,97and 0.01 ±0,00 to 93,45 ±10,27, 93,73 ±13,04, 335,24 ±35,79 and 6,18 ±2,68, respectively. This was accompanied with arrhythmias and inefficient increase in $O_2$ consumption. In comparison with other agents, BDM provided the most effective cardioprotection against venom. CF recovered completely and LVP to 81,2±3,2% of the initial values. Induction and duration of arrhythmias significantly decreased and no increase in the enzyme levels was observed. **Conclusion**: Under *in vitro* conditions BDM proved to be very effective protector against cardio toxic effects of the nose-horned viper venom. Further studies under *in vivo* conditions are warranted.

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Abstract

# P1.29 Effect of simvastatin treatment on MDA level in rat plasma measured by spectrophotometric and HPLC-MS analysis

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**Introduction**. Statins, inhibitors of hydroxy-methylglutaryl coenzyme A reductase, are considered first-line therapy for treatment of hypercholesterolemia. However, beneficial effects of statin treatment on cardiovascular morbidity and mortality has been not entirely explained by the reduction in low density lipoproteins (LDL) level but also in antioxidant activity of statins that contribute to their protective cardiovascular effects. There is an extensive evidence that links hypercholesterolemia with increased lipid peroxidation and increased oxidative stress. Several studies correlate levels of LDL oxidation with circulating malondialdehyde (MDA) which is secondary oxidation product. Mainly level of MDA in animal plasma and tissue was determined by the thiobarbituric method which is based on condensation of MDA with two molecules of 2-thiobarbituric acid (TBA) and method was widely used to measure the extent of oxidative deterioration of lipids in various biological systems. The absorbance of the complex is usually measured by spectrophotometry or by spectrofluorometry directly or by specific techniques, also based on TBA adduct, where HPLC separation with spectrophotometric or spectrofluorometric or MS detection was employed. The aim of the present study was to examine effect of simvastatin (SIMV) treatment on LDL oxidation based on the measuring of the rat MDA plasma level as parameter of oxidative stage by two different analytical techniques. Comparison of results between these analytical approaches would contribute in decision which technique is more reliable and accurate for further investigation on statin antioxidant action. **Material and Methods.** Forty six male Wistar rats (weight 170-200 g), were divided in two control groups (n=7 each) and four experimental groups (n=8 each). Experimental groups were on SIMV treatment which was given orally in the doses 10 and 50 mg/kg/day and for the period of 3 weeks. At the end of drug treatment animals from first two groups were sacrificed and blood samples were taken and other two groups were left additional 9 days and than sacrificed and samples were taken. The level of MDA was measured using the spectrophotometrically or by reverse phase HPLC-MS method. MDA levels are expressed as $\mu$mol concentrations using calibrating curves and expressed as percentage of decrease of MDA level comparing to negative control. **Results**. SIMV in both doses decreases MDA level in plasma. Spectroscopic analysis of MDA level after SIMV application have shown decrease in plasma concentration by 9,7% and 16,1% after administration of 10 mg/kg/day and 50 mg/kg/day dose respectively after 21 day of application. When the same doses and same application period was applied but blood was taken after 30 days, 32,2% and 64,5% decrease of MDA level were recorded. However, HPLC-MS analysis has shown 12,2% and 17,3% decrease of MDA level after 10 mg/kg/day and 50 mg/kg/day of SIMV dose after examining the same plasma samples. **Conclusion**. Our results have shown that SIMV shows antioxidant action which was indicated by lowering one of the oxidative markers – MDA. We suggest that both methods, spectroscopic or HPLC-MS gives results that have similar direction, but with difference in magnitude or sensitivity.

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Abstract

# P1.30 Wistar: Zagreb-5HT rat: further progress in development of the model

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Serotonergic system possess a peculiarity as compared to the other neurotransmitter/neuromodulator systems that consists in the peripheral expression of three relevant synaptic proteins on accessible blood elements – transporter (5HTt), postsynaptic receptor (5HT-2A) and metabolising enzyme (MAO-B). By selective breeding of animals for the extremes of transporter velocities, two sublines of rats markedly differing in platelet 5HT transporter kinetics were developed. The model was termed Wistar:Zagreb-5HT rats (WZ-5HT rats). The obtained constitutional dysregulation of platelet 5HTt was paralleled with analogous dysregulations of its central counterpart (i.e. presynaptic membrane transporter mediating 5HT reuptake), as strongly indicated by neurochemical, behavioural and molecular indicators. Taking into account a pivotal role which 5HTt holds in the regulation of serotonergic transmission, the mentioned dysregulation provoked behavioural alterations, as expected. On the other hand, alterations in neurochemical and molecular synaptic parameters that have also been expected, were not so easy demonstrable under physiological conditions. Results of both sets of experiments will be presented.

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Abstract

# P1.31 Correlation between serum butyrylcholinesterase activity and serum lipids concentration in rats treated with different antagonists of the adrenergic system

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Our earlier results showed that the nonselective $\beta$ adrenergic receptor antagonist oxprenolol significantly increased the butyrylcholinesterase (BuChE) activity in rats of both sex. Because the serum/plasma triglyceride (TG) and total cholesterol ((TC) concentration was altered in these experiments, it was concluded that the increase of enzyme activity was rather due to the altered lipid metabolism caused by oxprenolol than direct effect of oxprenolol on the enzyme. Based on the fact that antihypertensives that are adrenergic receptor antagonists can change serum lipid concentration and that it has been suggested that BuChE is involved in lipid metabolism, different adrenergic blocking agents were administered to rat to evaluate their ability to influence BuChE activity. The correlations between BuChE and serum lipids were investigated. **Materials and Methods:** Six groups of male Fischer 344 rats (9 rats each) were treated during 6 weeks with adrenergic antagonists that act on different sites in adrenergic system: oxprenolol, atenolol, doxazosin, doxazosin and oxprenolol, doxazosin and atenolol, or guanethidine. The doses of antagonists were 2-9 times higher than maximum recommended human doses (mg/kg body weight). Antihypertensive agents were given to the animals orally, mixed in the commercial diet for laboratory animals. Control group received only commercial diet. BuChE activity (U/L) in serum was determined with kinetic colour test using butyrilthiocholine as a substrate. Concentrations of TC and TG in serum were determined by enzymatic colourimetric tests. The concentration of HDL cholesterol (HDL-C) was determined by enzymatic colourometric tests in the supernatant after the precipitation of VLDL and LDL with polyethylene glycol. The concentrations of serum lipids were expressed as mmol/L. Data were analyzed by Kruskal-Wallis test. Spearman's correlation coefficient ($\rho$) was used to identify relationship between BuChE activity and the concentration of TC, TG or HDL-C. Results revealed that oxprenolol and doxazosin (given alone or in combination with atenolol or oxprenolol) increased BuChE activity in serum. The increase of BuChE activity was higher than 30% in these groups. The positive correlations between serum BuChE activity and TC ($p<0.01$) or HDL-C ($p<0.05$) was found in group treated with oxprenolol. In atenolol treated group positive corelation was found ($p<0.05$) between BuChE activity and TG concentration. Simultaneous administration of oxprenolol and doxazosin revealed negative correlation between BuChE activity and TG concentration ($p<0.05$). When all group were evaluated together, positive correlation ($p<0.01$) was obtained between TC, HDL-C concentration and BuChE activity. In contrast, BuChE activity negatively correlated ($p<0.05$) with TG concentration. **Conclusion**: Obtained results showed that BuChE activity in rat serum correlated with different serum lipids and that correlation depended on the type of adrenergic blockade. Although examined adrenergic antagonist did not influence serum lipid concentration, the increase of BuChE activity and correlation with lipid serum concentrations suggest that the increase of this enyzme activity might be the first sign of altered lipid metabolism.

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Abstract

# P1.32 The effects of statins on rats butyrycholinesterase

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**Introduction**. Butyrylcholinesterase (BuChE) is an enzyme with an unknown physiological function. It is synthesised in the liver and released into serum immediately following its synthesis. The clinical importance of BuChE lies in its pharmacological property of hydrolysing suxamethonium and ester type of local anaesthetics. BuChE activity is increased in certain metabolic disorders, such as hypercholesterolemia, hypertension or obesity. In such patients, BuChE activity correlates strongly and positively with serum levels of low density lipoproteins (LDL)-cholesterol and triglycerides (TG) and inversely with serum high density lipoprotein (HDL)-cholesterol. All these observations suggest a relationship between BuChE activity and lipoprotein metabolism, but the explanation for this connection is unclear. Statins are very often used for treatment of certain type of hyperlipoproteinemia. Because statins lower plasma triglycerides (TGs) rather than cholesterol in rats, it was of interest to investigate the effects of simvastatin (SIMV) and atorvastatin (ATOR) on catalytic activity of BuChE in serum and liver of normolipidemic rats and to determinate if any relation between BuChE activity and plasma TGs exists. Material and Methods. Forty six male Wistar rats (weight 170-200 g), were divided in two control groups (n=7 each) and four experimental groups. Two experimental groups were on SIMV treatment and the remaining two groups (n=8) on ATOR treatment. Both agents were given orally in the same doses (10 and 50 mg/kg/day) and for the same period of 3 weeks. At the end of drug treatment animals from all groups were sacrificed. BuChE activity was measured using the spectrophotometric method of Ellman *et al*. with butyrylthyocholine as a substrate. Enzyme activities are expressed as $\mu$mol of substrate hydrolysed per min per ml of serum or of g the tissue wet weight. Concentrations of TGs in the serum were determined by enzymatic colorimetric test, and were expressed as mmol/L. Data were analyzed by Kruskal-Wallis test and results were considered as significant with $p<0.05$. **Results**. SIMV and ATOR in both doses increased the catalytic activity of BuChE in plasma and liver. In the case of SIMV, the increase in plasma and liver were 28% and 36% after administration of low dose and 14% and 68% ($p<0.05$) in plasma and liver after administration of high dose. After administration of ATOR, the increase of BuChE in plasma and liver were 31% and 48% at low dose, and 85% ($p<0.05$) and 123% ($p<0.05$) at high dose. In the same time plasma TGs leves were in average lower for about 10% after SIMV administration in both doses, or for about 15% after ATOR administration in both doses. **Conclusion**. We didn't find any relation between the plasma BuChE activity and the plasma TGs level neither in the case of SIMV nor in ATOR. But, our results have shown that both statins increase the liver BuChE activity. In other words statins have a stimulating effect on BuChE synthesis in the liver. Consequently, the plasma BuChE activity has also been increased. Epidemiological studies indicate that statins might confer protection against demention, where a reduction of acetylcholine exists. In clinical trials simvastatin and pravastatin were not demonstrably protective. Our results observed in normolipidemic rats, have shown that both statins increase BuChE activity, which mens, that they are not protective in this disorder.

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Abstract

# P1.33 The opposite effects of simvastatin and atorvastatin on serum and liver paraoxonase activity in normolipidemic rats

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**Introduction**. Paraoxonase (PON1) is serum esterase which is synthesized in the liver, and has unknown physiological function. PON1 has a clinical importance, because it plays a protective role in case of organophosphates intoxication. According to the many experimental data PON1 is included in lipid metabolism. It is suggested that PON1 is associated with high density (HDL)-cholesterol, and that HDL stimulates PON1 secretion from the liver. Also it has been shown that PON1 prevents the formation of oxidized LDL and protects phospholipids in HDL from oxidation. Hyperlipidemia and oxidative stress reduce PON1 activity. It has been suggested that statins which are used for the treatment of hyperlipidemia, can decrease or increase serum PON1 activity, and that this is the consequence of another, independent effect of statins, i.e. their antioxidant action. Because the most of these results were goten from human and animals in conditions of altered lipid's metabolism, it was of interest to investigate the effects of simvastatin (SIMV) and atorvastatin (ATOR) on PON1 activity in serum and liver of normolipidemic rats. **Material and Methods.** Forty six male Wistar rats (weight 170-200 g), were divided in two control groups (n=7 each) and four experimental groups. Two experimental groups were on SIMV treatment and the remaining two groups (n=8) on ATOR treatment. Both agents were given orally in the same doses (10 and 50 mg/kg/day) and for the period of 3 weeks. At the end of drug treatment animals from all groups were sacrificed. PON1 activity in plasma and liver was measured by the spectrophotometric method using synthetic diethyl-p-nitrophenyl phosphate (paraoxon) and moderator CaCl2. The activity toward paraoxon was determined by measuring the initial rate of substrate hydrolysis to p-nitrophenol. The plasma or liver enzyme activity was calculated from E405 of p-nitrophenol and expressed as $\mu$mol of substrate hydrolysed per min per ml of serum or g of the tissue wet weight. Concentration of HDL-cholesterol and TGs in plasma were determined by enzymatic colorimetric methods and expressed as mmol/L of plasma. Data were analyzed by Kruskal-Wallis test and results were considered as significant with $p<0.05$. **Results**. SIMV administration in both doses reduced serum PON1 activity by 21%. Low dose of SIMV decreased liver PON1 activity by 37% and high dose by 56%. But, in any case the decrease of PON1 activity was not significant. High dose of ATOR caused the increase of serum PON1 activity by 18% ($p<0.05$), and both doses caused the increase of liver PON1 activity by 28% (10 mg/kg/day), and 43% (50 mg/kg/day) ($p<0.05$). Both doses of SIMV had no significant influence on plasma HDL-cholesterol. In opposite, HDL-cholesterol concentration was significantly increased after low dose of ATOR. Both drugs didn't markedly reduce plasma triglycerides. **Conclusion**. Our results have shown that SIMV and ATOR have the opposite effects on PON1 activity. It was mentioned that ATOR shows antioxidant action. We suggest that ATOR reduces oxidative stress in a grater manner, and that the significant increase in PON1 activity by ATOR may be the result of its better antioxidant action in comparison with that of SIMV. Consequently, ATOR does not cause the inactivation in PON1 activity, than its increase.

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Abstract

# P1.34 Long-lasting antinociceptive effect of botulinum toxin type a in experimental diabetic neuropathy

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**Introduction**: Up to 10 % of diabetic patients suffer from painful neuropathy. All available treatments are short-lasting. Recently we have found that in rats with the surgical neuropathy botulinum toxin type A (BTX-A) reduces thermal and mechanical hypersensitivity for 15 days or even longer (Bach-Rojecky *et al*., J Neural Transm 2005; 112: 215-219). Here we investigate the possibility that BTX-A has antinociceptive effect in experimental diabetic neuropathy. **Materials and methods:** Adult male Wistar rats were made diabetic by a single subcutaneous (s.c.) injection of alloxan (140 mg/kg b.w.). Control animals were injected s.c. with the same volume of saline. Animals with a tail-vein blood-glucose concentration >15 mmol/l were considered diabetic. Measurements of mechanical sensitivity by the paw-pressure test were first performed 3 weeks following alloxan or saline injection. Diabetic animals with nociceptive threshold lower for at least for 25% compared to control group were considered neuropathic and were than subjected to the peripheral (in the plantar surface of the hindpaw) BTX-A (7 U/kg) or saline treatment. In the time-course experiment, mechanical sensitivity to the pressure was tested on day 5, 10, 15, 22 and 27 following the BTX-A injection. Sensitivity to chemical stimuli was measured once, on day 10, by the formalin test. **Results:** Significant antinociceptive effect was observed 5 days following the BTX-A application, i.e. the mechanical hypersensitivity was reduced compared to the saline-treated diabetic controls (91.3±2.8 g for BTX-A *vs.* 58.7±2.0 g for diabetic control; $p<0.001$, $n=6$-7). The antinociceptive effect of the toxin was significant till the day 15 (90.3±3.5 g for BTX-A *vs.* 70.3±2.5 g for control; $p<0.05$) and than started to decrease. BTX-A also reduced chemical hypersensitivity, i.e. number of flinches/shakes of the formalin injected paw (302,4±17 for BTX-A *vs.* 478±6 for diabetic control; $p<0.001$). **Conclusion**: In comparison with other available treatments here we demonstrate that BTX-A might have a long-lasting antinociceptive effect in diabetic neuropathy. Supported by Croatian Ministry of Education, Science and Sport (Project No. 108-1080003-0001 and 108-1080003-0020) and Deutscher Akademischer Austausch Dienst (DAAD).

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Abstract

# P1.35 Effects of exploration behaviour, locomotor activity and emotionallity on paw withdrawal latencies in Hargreaves test

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We have recently reported that single training session significantlly decrease baseline rat paw withdrawal latencies (PWL) in Hargreaves tests. Thus, we hypothesized that the first exposure to testing device induces behavioral reaction(s) to a new environment which might interfere with pain response. Since PWL remained stabile from the second exposure to the end of testing period, we supposed that animals quickly habituate to a new environment.. To test this hypothesis, we examined the open field behavior in enclosure of testing device used for Hargreaves test, throughout the period of 3 subsequent days. Also, the effect of repeated testing on PWL in Hargreavdes test was reexamined during the same period. Ten male Wistar rats (Charles River, Italy), were used during entire testing period. Single testing session occurred once a day, during light phase of the day. Each rat was first exposed to the open field test and then to the Hargreaves test. The size of the floor of Plantar test device used for the open field test was 22 cm x 17 cm. The wals were 14 cm high. Open field behavior was monitored, during 5 minutes using web camera (Logitech, Quick cam Pro 5000, USA). Any maze softver (Stoelting, USA) was used for tracking and analysis of the open field data. The following behavioral parameters were obtained: rearings (the number of times the animals moves verticaly, with hind limbs on the floor, and forelimbs on the walls of Plantar test enclosure); grooming (the number of times the animal wash its face with forepaws, or washing, licking and scratching the various part of body); distance travelled (distance that rat travells in Plantar test enclosure by walking on all four feet); time mobile (time in seconds fom the start to the end of walking on all four feet); time immobile (time in seconds from the start to the end of the motionless period). PWL were measured using Hargreaves test, as it is described elsewhere. Two PWL values were obtained alternatively from each hind paw. Individual PWL values were determined as the mean of for measurements on both paws. PWL means for 3 consecutive testing sessions were analyzed using one way ANOVA and Bonferroni post hoc multiple comparison test. Open field data were analysed using nonparametric Kruskal Wallis and Mann Whitney U tests. In all cases $P < 0.05$ was used as the criterion for significance. Significant decline in PWL on the second and third testing sessions, when compared to the first testing session was detected (ANOVA, $P< 0,01$; Bonferroni $P< 0,05$). From the second to the third exposure to Plantar test device, there was no change in PWL. The animals displayed significantlly more grooming behavior during the first and the second retesting sessions, as compared to the first testing session (Kruskal Wallis $P< 0,01$; Man-Whitney U test $P< 0,05$). Besides, the number of rearing responses significantlly decreased from the testing day one to testing day two and testing day three (Kruskal Wallis $P< 0,001$; Man-Whitney U test $P< 0,001$). Locomotor responses in open field test remained stabile during entire testing period. The effect of repeated testing on PWL in Hargreaves test was accompanied by alterations in the open field behavior. It seems that behavioral reactions to a new environment could modify pain behavior in Hargreaves test.

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Abstract

# P1.36 Behavioral and morphological changes in rat DRG following lidocaine injection

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**Introduction**: Segmental nerve root blocks with local anesthetics have been used for diagnostic purposes in patients with chronic back pain, as well as to manage cancer pain and back pain. Injury of peripheral nerve and dorsal root ganglion (DRG) as a result of accidental direct injection can be considered as a serious complication with insufficiently described consequences. It has been demonstrated that local anesthetic deposited at the DRG causes pain and hyperalgesia when the effects of the local anesthetic have dissipated, but nothing more than that has been published on the subject. We performed this study to evaluate possible morphological changes in DRG following intraneurial and intraganglionar lidocaine injection. Our hypothesis was that observed behavioral changes following direct injection are result of inflammatory response. **Materials and Methods:** We used four groups of male Sprague-Dawley rats: first two groups underwent minimal laminectomy and injection of 4uL lidocaine into the L5 nerve or L5 DRG; the third group underwent the same surgical procedures, but was injected with 0.9% NaCl. The fourth group contained control animals. Once before and three times after the surgery we did pin-prick behavioral testing. The results of behavioral tests were statistically analyzed. After behavioral tests the animals were sacrificed and DRGs and corresponding peripheral nerve segments harvested. The ganglia and nerves were fixed, cut with cryotome and immunostained with Glial Fibrillary Acid Protein (GFAP) primary antibody. Stained tissue was photographed with digital camera and number of immunoreactive cells was counted in multiple visual fields. The number of immunoreactive cells was divided with the number of non-stained cells to get a percentage of immunoreactive cells in the tissue. **Results**: We found that pain-related behavioral response increases during first and second postoperative testing on the ipsilateral paw. We observed similar effect, but less pronounced, at the contralateral paw. All the tissues from control animals had no immunoreactive satellite cells in the DRG. In the group of animals injected into the right L5 nerve, contralateral (L5) ganglia had 3%, the lidocaine-injected animals had 23% and sham animals had 10% of immunoreactive satellite and microglia cells. In the tissue of rats injected into the L5 DRG, contralateral (L5) ganglia had 6%, the lidocaine-injected animals had 61% and sham animals had 33% of immunoreactive satellite and microglia cells. **Conclusion**: Our results show that injection of lidocaine into the spinal nerve or DRG induces a pain related behavior and results in marked inflammatory response in the DRG. The same changes, but in a lesser degree, were observed when rats were injected with saline. We suspect that saline induces these changes due to the mechanical effects of the injected volume. With lidocaine, this effect is augmented with the neurotoxicity of a local anesthetic. From this we can conclude that the nerve or DRG injury is a complication of the segmental nerve root blocks that the patients and clinicians are justly afraid of and that every effort should be minimize the risk of these neural injuries.

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Abstract

# P1.37 Therapeutic effect of ropinirol on polysomnographic characteristics of sleep in patients with PLMD/RLS

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**Introduction**: Periodic Limb Movement Disorder (PLMD), usually accompanied by Restless Legs Syndrome (RLS), is a motoric disturbance appearing specifically at the time of falling asleep and during sleep. Periodic motoric activity causes insomnia because of difficult falling asleep and fragmented sleep, the consequences of which are daily fatigue and sleepiness. The illness is relatively frequent, according to some studies its prevalence is over 10% in adult population, but in our country it is poorly known and diagnostically neglected. It is treated successfully by substitutional dopaminergic therapy or dopaminomimetics such as ropinirol (Requip). **Material and Methods:** in the present investigation the author analyzed the characteristics of sleep recorded in the Center for Sleep Disorders on MEPAL polysomnograph with automatic analysis in nine patients with PLMD before and after the therapy with ropinirol. **Results:** the improvement of all parameters was registered, median periodic LM index (the number of leg movements per hour) before therapy was 128, after the therapy 23. Median sleep latence in minutes was at the first recording 126 minutes, after the therapy with ropinirol 78 minutes, total sleep time without therapy 215 minutes, after the beginning of treatment 277 minutes. The efficacy of sleep (sleep time / recording time) was first 49%, then at the control recording 66%, the number of partial and full awakenings 39, after the beginning of therapy 21. Deep sleep also improved to a certain degree, with no therapy the phases 3 and 4 made about 3% of total sleep time, and after introducing ropinirol their share was 8%. REM did not improve, before the recording its median was 2%, after the therapy 1%, which can be ascribed to the small sample, because in four patients REM after the treatment was over 10%. **Conclusion:** is that treatment with ropinirol undoubtedly improves continuity and the structure of sleep in the patients with PLMD/RLS.

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Abstract

# P1.38 Association study of therapeutic response with sert, MDR1 and 5-TH2c gene polymorphisms in female schizophrenic patients

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**Introduction**: Interindividual differences in treatment response to second generation antipsychotics point out that genetic factor may be relevant. The aim of this study was to investigate the relationships between variants of serotonin transporter gene (SERTPR and SERTin2), serotonine receptor (5-HT2c-759C/T) and multidrug resistant gene (MDR1-2667G/T, 3435C/T) with initial symptomatology and treatment response in 106 female schizophrenic patients treated with olanzapine for up to 3 months. Afterwards, we compared allele, genotype and haplotype distributions between those patients and 108 control female subjects. **Methods**: Genotyping was performed by PCR-RFLP, and Real-time PCR methods. To assess and evaluate therapeutic response all patients were rated using PANSS. Overall, presence of SERTPR-S allelic variant and SS genotype was associated with significantly more weight gain in subjects who were non-obese at the time of admission ($p= 0.02$). The presence of SERTPR-L variant was associated with significantly better treatment response measured with total PANSS and general PANSS subscale ($p<0.04$), while the presence of SERTin2-l variant determined better treatment response only in several items. We found significant associations with lower initial PANSS, and MDR1-2667G/T genotype. 2677T allele and TT genotype were associated with significantly worse treatment response. Also overrepresentation of G2677/3435T haplotype in schizophrenic female patients compared to controls was significant ($p=0.025$). Test result for linkage disequilibrium between two MDR1 loci was found to be significant. These findings identify genetic factors associated with olanzapine- treatment response in female schizophrenic patients.

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Abstract

# P1.39 Effect of pioglitazone in the lithium-pilocarpine model of status epilepticus in rats

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Introduction. A role of inflammation in the brain, both in human epilepsy and in animal epilepsy models, has been recently recognized. Agonists of the peroxisome proliferator-activated receptors gamma (PPAR-γ) have been shown to be neuroprotective in models of various neurological diseases such as Parkinson's disease, Alzheimer's disease, multiple sclerosis, focal cerebral ischemia, mostly through their anti-inflammatory actions. The aim of our study was to investigate the effects of a synthetic PPAR-γ agonist pioglitazone on the 24 hrs-survival rate, as well as on the COX-2 and HSP70 protein expressions in brains of rats with lithium plus pilocarpine-induced status epilepticus (SE). **Materials and Methods.** SE was induced by administration of pilocarpine hydrochloride (30 mg/kg, i.p.) 20-24 hrs after LiCl (127 mg/kg, i.p) and 30 min after methylscopolamine nitrate (1 mg/kg, s.c.) in 80 to 90 days old male Wistar rats. The onset of SE was defined as uninterrupted generalized motor seizures lasting over 5 min or occurring with intervals lesser than 2 min. Rats were administered i.p. either pioglitazone (1; 3 mg/kg) or equivalent volume of the vehicle (DMSO), 10 min after the SE onset. SE was interrupted 2 hrs after its onset by an injection of diazepam (10 mg/kg, i.p.). Control animals received LiCl, saline instead of pilocarpine hydrochloride and methylscopolamine nitrate, DMSO and diazepam. Rats were sacrificed 24 hrs after the SE onset and Western blotting analyses were performed in different rat brain regions. **Results**. Pioglitazone treatment increased the rate of 24 hrs-survival in rats with lithium-pilocarpine induced SE, in a dose-dependent manner from 27% (vehicle treated rats) to 45.8% (pioglitazone 1 mg/kg) and 55% (pioglitazone 3 mg/kg). Western blotting analyses showed a strong induction of the COX-2 expression in animals with SE, as well as its attenuation in pioglitazone treated rats. Additionally, pioglitazone treatment led to an overexpression of the HSP70. **Conclusion.** The results of our study suggest a protective role of a single dose of pioglitazone in the lithium-pilocarpine model of SE in the rat. Namely, these data indicate that the inhibition of the post-SE COX-2 overexpression and an enhancement in the HSP70 expression may be involved in the protection mechanisms underlying the prominent increase of the 24 hrs-survival rate of rats in this epilepsy model. Supported by Grants 062-0620529-0519 »Epilepsy and traumatic brain injury: damage mechanisms and pharmacotherapy« and 0062049 »Excitotoxicity and neuroprotection in epilepsy and brain ischemia« from the Ministry of Science, Education and Sports of the Republic of Croatia.

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Abstract

# P1.40 Pioglitazone limits the cortical oxidative damage following traumatic brain injury in the rat

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**Introduction**. The tissue and cell damage induced by traumatic brain injury (TBI) results from primary damage and from a complex secondary cascade of events including oxidative stress, inflammation, increased vascular permeability, mitochondrial dysfunction and excitotoxic damage. There is no standard pharmacological treatment that blocks the progression of this secondary injury and the current management of TBI is mainly supportive. The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a member of nuclear receptor superfamily involved in different cellular processes such as regulation of oxidative stress and inflammatory response. Agonists of PPAR-γ have already shown the beneficial effects in the stroke and the spinal cord injury models. This work was performed in order to determine the effects of pioglitazone, a PPAR-γ agonist, on the level of antioxidant enzyme activities and the lipid oxidative damage in the parietal cortex 24 hrs after the induction of TBI in the rat. **Materials and Methods.** Experiments were performed on adult male Wistar rats. TBI of moderate severity was induced using the lateral fluid percussion (LFP) brain injury model. Briefly, rats were placed in a stereotactic frame and surgically prepared for LFP brain injury or sham operation as previously described (McIntosh *et al.,* 1989). A 5-mm craniotomy was performed over the left parietal cortex, between lambda and bregma sutures, leaving the dura mater intact. A hollow female Luer Lock fitting was positioned over the craniotomy and held in place with dental cement. Animals were attached to the LFP device and brain injury was induced by a rapid injection of a pressure pulse of saline. Animals were i.p. injected with either pioglitazone (1 mg/kg) or vehicle 10 min after the TBI. Sham-operated, vehicle-treated animals were used as the control group. Rats were sacrificed 24 hrs after the TBI induction. Activities of antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPX) were determined by standard spectrophotometric measurements. Levels of the brain lipid peroxidation were determined by thiobarbituric acid reactive substances (TBARS) assay. **Results**. TBI caused the significant increases of the GPX activity and the TBARS level, and a slight decrease of the SOD activity in the injured parietal cortex. Pioglitazone treatment resulted in the additional increase of GPX activity and in the decrease of the TBARS level in the examined cortical region of injured rats. **Conclusion**. Our findings demonstrate the activation of the antioxidant enzymatic system and the intense oxidative damage of lipids in ipsilateral parietal cortex following TBI. These results also suggest a protective role of pioglitazone in the brain oxidative damage in our experimental conditions. Supported by Grant 062-0620529-0519 »Epilepsy and traumatic brain injury: damage mechanisms and pharmacotherapy« from the Ministry of Science, Education and Sports of the Republic of Croatia.

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Abstract

# P1.41 Development of transmantle pressure gradient in cats with acute aqueductal blockage

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**Introduction**: It is not known whether pressure gradient of cerebrospinal fluid (CSF) between brain ventricles and cortical subarachnoid space (transmantle pressure gradient) is necessery for development of hydrocephalus. Some results in patients with noncommunicating hydrocephalus show that such a transmantle pressure gradient is not present. We have investigated in cats with acute occlusion of aqueduct whether transmantle pressure gradient can be developed. **Material and Methods:** In chloralose anaesthetized cats the aqueduct was totally occluded by implantation of plastic cannula with tip covered by cyanoacrylate glue through a small tunnel in vermis of cerebellum and the CSF pressure recorded in both isolated ventricles and cisterna magna after hermetic reconstruction of skull. The head of cat was fixed in stereotaxic holder and CSF pressures were recorded under control conditions and during infusion of arteficial CSF (aCSF) either in isolated ventricles or in cisterna magna. **Results**: In control condition without infusion of aCSF, the CSF pressure in isolated ventricles and cisterna magna were not different from control value (about 10 cm $H_2O$) and cerebral transmantle pressure gradient did not develop over 120 min. Infusion of aCSF in cisterna magna at two rates (13 and 52 $\mu$l/min; n=4) during 5 min increased the CSF pressures in both cisterna magna and isolated ventricles without development of transmantle pressure gradient. However, when aCSF was infused (rates of 13 and 52 $\mu$l/min; n=4) for 5 min in isolated ventricles the transmantle pressure gradients were developed. **Conclusions**: Acute occlusion of aqueduct does not cause transmantle pressure gradient during 120 min, while a such gradient develops very soon during infusion of aCSF in isolated ventricles. During infusion of aCSF in cisterna magna the CSF pressure increase in cranial subarachnoid space is immediately transmitted across brain parenchyma to isolated ventricles preventing development of any pressure gradient. Thus, it would appear that transmantle pressure gradient in noncommunicating hydrocephalus can develop only in pathological conditions which cause an acute increase of intraventricular pressure.

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Abstract

# P1.42 Mechanism of absorbtion of the cerebrospinal fluid

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**Introduction**: It is generally accepted that volume of cerebrospinal fluid (CSF) is secreted in brain ventricles and flows to subarachnoid space to be absorbed into dural venous sinuses or/and into lymphatics *via* perineural sheats of cranial nerves. **Methods and Results:** Since 99% of CSF volume is water, in experiments on cats $^{3}$H-water was slowly infused into lateral ventricle and found that it does not flow to subarachnoid space but that it is rapidly absorbed transventricularly into periventricular capillaries. When $^{3}$H-water was infused in cortical subarachnoid space, it was absorbed locally into cerebral capillaries *via* pia mater. On the contrary, when macromolecule $^{3}$H-inulin is applied in CSF, it is very slowly eliminated in bloodstream, and, with time, is carried by systolic-diastolic pulsations and mixing of CSF bidirectionally along CSF system. **Conclusions**: These data indicate that CSF volume (water) is absorbed rapidly into adjacent cerebral capillaries while inulin is distributed bidirectionally due to its long residence time in CSF. Previously, the macromolecules have been used to study CSF volume hydrodynamics and with this misconception of CSF physiology arose.

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Abstract

# P1.43 Evaluation of perfusion method for measuring of cerebrospinal fluid secretion

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**Introduction**: The aim of the study was to evaluate is cerebrospinal fluid (CSF) formation rate (Vf) calculated according to the equation of Heisey *et al.*, truly showing the secreted cerebrospinal fluid. **Material and Methods:** For this reason Vf was simulated by infusion of mock CSF (40.6 $\mu$l/min) in a plastic cylinder and the evaluation was done by comparing the results obtained between the calculated Vf and the simulated one. In both cases the result should be the same (40.6 $\mu$l/min). Other types of experiments were carried out by ventriculocisternal perfusion (92.4 $\mu$l/min) on anaesthetized and sacrificed cats. If the equation is correct, the calculated Vf for sacrificed animals should be zero, because there is no secretion of CSF in dead animals. **Results and Conclusion:** The fact that the calculated Vf in the plastic cylinder (46.5 $\mu$l/min, $n = 4$) was different from the simulated one (40.6 $\mu$l/min, $n = 4$, $p<0.001$) and that Vf was calculated even for dead animals (3-5 $\mu$l/min) clearly shows the that perfusion method may not be a suitable method for determination of CSF secretion.

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Abstract

# P1.44 The effect of body position on intracranial and intraocular pressure in cats

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**Introduction**: There are contradictory reports about relationship of intracranial and intraocular pressure (IOP). Intracranial pressure is measured invasively in patients with intracranial hypertension, while IOP is determined by less precise noninvasive methods. For this reason we compared intracranial pressure and IOP measured by invasive tehniques in new experimental model in cats during changes of body position. **Material and Methods:** Experiments were performed on adult cats anaesthetized with chloralose (100 mg/kg i.p.; n=10). The stainless steel cannulae (o.d. 0.9 mm) were introduced into lateral ventricle (LV), cortical (CSS) and lumbar (LSS) subarachnoid spaces, and in anterior ocular chamber of right eye. The cannulae were connected to pressure transducers, and intraural line was taken as reference zero (0 cm $H_2O$) pressure. The animals and measuring instruments were fixed on board and pressures were measured in horizontal and vertical position. **Results**: In horizontal position pressure in eye (IOP = 18.5 ± 0.6 cm $H_2O$) and pressures in cerebrospinal fluid (LV = 17.4 ± 0.9; CSS = 17.2 ± 0.7; LSS = 17.8 ± 1.2 cm $H_2O$) were similar and did not differ significantly ($p>0,05$). In vertical position lumbar pressure increased (LB = 33.5± 2.3 cm $H_2O$), pressures in cranial cavity fell to subatmospheric value (LV = -4.1± 0.9 cm $H_2O$; CSS = -4.8± 0.5 cm $H_2O$) while intraocular pressure showed small decrease (IOP =14.3± 0.1 cm $H_2O$). **Conclusion:** It appears that change of body position from horizontal to upright position causes drastic changes of pressures in different parts of cerebrospinal fluid system, while pressure change in ocular fluid is relatively small. Our results indicate that in animals without intracranial hypertension, the intraocular and intracranial pressures are not importantly interconnected.

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Abstract

# P1.45 Absence of association between genetic polymorpisms in CRFR1 and PTSD

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One of the main mechanisms in the activation of the hypothalamo– pituitary–adrenocortical (HPA) axis is the release of corticotropin releasing hormone hormone (CRH) from the hypothalamus and it's binding to the corticotropin releasing hormone receptor (CRHR1) in the pituitary gland. Recently it was reported that two haplotype tagging SNPs (htSNP) in CRHR1 are associated with patterns of human alcohol drinking and could potentially contribute to the development of alcohol dependence. Aiming to see whether same genetic variations are associated with potential predisposition for the development of post-traumatic stress disorder (PTSD) we have investigated association of these two haplotypes of CRHR1 and PTSD. DNA was isolated from blood of 200 patients with diagnosed PTSD and 200 matching control individuals. TaqMan Pre- designed SNP genotyping assays were used to genotype two htSNPs (rs242939 corresponding to T to C exchange at position 44371356 and rs1876830 corresponding to C to T exchange at position 44386772 of Chromosome 17). Contrary to the situation observed in alcoholism, we were not able to find any association of CRFR1 genotype with PTSD since frequencies of all alleles were nearly the same in both studied populations. Interestingly, the observed frequency of the major allele at rs242939 corresponded to the frequency reported to correlate with low alcohol consumption, while the observed frequency of the major allele at rs1876830 was between the reported frequencies for low and high alcohol consumers.

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Abstract

# P1.46 Does positive history of allergic drug reactions or atopy justify testing for allergy to local anesthetics?

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**Introduction**: Although no more than 1% of adverse reactions to local anesthetics (LAs) are thought to be immunologicaly mediated, many patients are referred to allergy clinics for allergy work-up. The aim of our study was to evaluate the appropriateness of allergy testing to LAs in patients with history of drug allergy other than LA or atopy. **Matherials and Methods:** We retrospectively analysed medical records of 112 consecutive patients referred to our Department for allergy testing to LAs in a nine-year period (1996-2005). Intradermal tests with diluted (1:10) LAs were performed to identify patients at risk for IgE mediated hypersensitivity reaction. The odds for being test-positive were calculated in regard to defined risk factors (atopy, history of allergy to LAs or other drugs, underlying autoimmune disease). **Results**: Eleven out of 112 patients (9.8%) were tested positive for allergy to LAs. Atopy, history of allergy to LAs or other drugs and underlying autoimmune disease did not increase the odds for being test-positive. The prevalence of mentioned risk factors (except history of allergy to other drugs) was higher among patient tested positive as compared to patients tasted negative [4/11 (36.4%) vs. 23/101 (22.8%); 7/11 (63.6%) vs. 51/101 (50.5%); 6/11 (54.6%) vs. 78 (77.2%); 3/11 (27.3%) vs. 19/101 (18.8%), respectively]. The prevalence of multiple drug allergies, IgE values and eosinophil count were not significantly higher among the patients tested positive, as compared to patients who tested negative. **Conclusion:** We found no increased odds for being test-positive for IgE mediated hypersensitivity to LAs in patients with atopy, history of allergy to LA and/or history of drug allergy other than LA and underlying autoimmune disease. The results of our study indicate that previous drug allergy other than to LAs or atopy do not increase odds for IgE mediated hypersensitivity to LAs.

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Abstract

# P1.47 Quantitative determination of gabapentin and vigabatrin by HPLC in the serum of patients with epilepsy

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**Introduction**: Gabapentin (Gabalept®, Katena®, Neurontin®) and vigabatrin (Sabril®) are chemical compounds that represent new-generation antiepileptic drugs. Gabapentin (GBP) has been designed as a structural analogue of GABA, and has been demonstrated to increase GABA concentration. The mechanism of action is related to events modulated through its interaction with a receptor thought to be associated with L-system amino acid carrier protein. Vigabatrin (VGB) is a synthetic GABA derivate. It is an enzyme-activated, irreversible inhibitor of GABA transaminase that resulted from a systematic search into possible ways of increasing GABAergic inhibition through interference with GABA metabolism. GBP and VGA are eliminated renally completely unchanged and the pharmacokinetic varability is more predictable. **Material and Method:** A simple method was developed for the routine clinical monitoring of the antiepileptic drugs GBP and VGB in the serum of patients with epilepsy using high- performance liqid chromatography (HPLC) with fluorescence detector set at $\lambda_{exc}$=235 nm and $\lambda_{em}$=435 nm. After protein precipitation with acetonitril compounds are pre-column derivatized by o-phthaldehyde (OPA). Separations were carried out at 28° C on Nucleodur $C_{18}$ 100-5 column (*dp*=5μm) from Macherey Nagel. The mobile phase was isocratic, consisting of 0.02 M phosphoric acid and acetonitril (45:55, v/v) with flow-rate 0.6 ml/min. **Results:** The method was fully validated and linear calibration curves were obtained in the concentration ranges from 21.9 to 146.0 μmol/L for GBP and 57.9 to 386.5 μg/ml for VGB. The limit of detection (LOD) was 0.047 μmol/L for GBP, and 1.724 μmol/L for VGB. The limit of quatitation (LOQ) was 0.123 μmol/L for GBP, and 3.718 μmol/L for VGB. The within-day precision expressed as relative standard deviations (RSD) for two different concentration levels (n = 10) were < 10% for both compounds, and day-to-day RSD at two different concentration levels during 3 separate days were < 5% for GBA, and < 10 % for VGB. **Conclusion:** The described method was simple, fast, highly sensitive and reproducible, while endogenous compounds did not interfere with the assay. It was used for simultaneous analysis of both antepileptic drugs in therapeutic drug monitoring.

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Abstract

# P1.48 Therapeutic drug monitoring: quantitation of oxcarbazepine and its active metabolite in human serum

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**Introduction**: Oxcarbazepine (Trileptal®) is a keto-derivative of carbamazepine. It was developed by introducing minimal changes in the structure of carbamazepine, which altered the metabolism to avoid the production of epoxide metabolite. Oxcarbazepine is rapidly metabolized to a pharmacologically active monohydroxy metabolite (MDH; 10-hydroxy-carbazepine, licarbazepine). 10-hydroxy-carbazepine has independent anticonvulsant properties and is mainly responsible for the effect of oxcarbazepine. The aim of this study was to develop and validate a HPLC method for measuring oxcarbazepine and 10-hydroxy-carbazepine simultaneously with other antiepileptics (lamotrigine, carbamazepine, phenobarbitone, phenytoin) which are already in routine use in our laboratory. **Materials and Methods:** Serum sample (250 μL) pretreatment was based on liquid extraction with ethylacetate/hexane, evaporated and desolved in 200 μL mobile phase. The separation was obtained on reverse-phase column ($C_{18}$, 250X4,6 mm I.D. 5μm) using a phosphate buffer/acetonitrile/methanol mixture (pH 6,8) as a mobile phase, at the flow rate of 1.3 ml/min and DAD detector at 237nm; total time for chromatographic separation was 13 min. **Results**: The calibration curves were linear for both analytes (r= 0.9975) in the expected therapeutic range. Intermediate precision (inter-day) expressed as relative standard deviations was less than 3% for 10-hydroxy-carbazepine and oxcarbazepine at two concentration levels. Accuracy, expressed as percent error, ranged from 94.5-103.8% for 10-hydroxy-carbazepine and from 88-100% for oxcarbazepine. The limit of quantitation was 2.07 μmol/L for 10-hydroxy-carbazepine and 0.08 μmol/L for oxcarbazepine. **Conclusion**: Based of analytical parameters (linearity, precision, accuracy, limit of quantitation), the presented method is suitable for pharmacokinetic studies and therapeutic drug monitoring. The method shows good specificity with other prescribed drugs. While less pronounced than for carbamazepine, pharmacokinetic variability for oxcarbazepine was still considerable, and it can be affected by age, pregnancy, concurrent disease and drug-drug interaction. These characteristics suggest that therapeutic drug monitoring may be of value in treatments with oxcarbazepine.

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Abstract

# P2.1 Analysis of biologically active polyphenols in wines from Croatia

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**Introduction**: Polyphenols are secondary plant metabolites that attract great interest due to their biological activities, such as antioxidant activity, anti-inflammatory, antimicrobial and antifungal action. There is evidence that some of them have positive effect against a large variety of degenerative processes, such as cancer, atherosclerosis, cardiovascular disorders, age-related macular degeneration and cognitive decline in aging. Wines contain large amount of polyphenols such as phenolic acids, flavones, flavonols, catechins, anthocyanins, procyanidins and resveratrol. Considering the importance of biologically active polyphenols in wine, it is necessary to develop an accurate and rapid method for their analysis. **Materials and Method:** Twelve commercial wines, vintage 2002, from three different wine-growing Sub-regions of Croatia were analyzed. Total polyphenols were analyzed according to the Folin–Ciocalteu method. Samples for chromatographic analysis were prepared by extraction of wines with diethyl ether at pH 2. A reversed-phase high performance liquid chromatographic (HPLC) method that uses gradient elution and diode array detection (DAD) to determine 7 phenolic acid and eight flavonoids in wine extracts was developed. The chromatographic separation of these polyphenols was performed in a single run with flow rate at 1 ml/min and UV-visible spectra were recorded between 250 -500 nm and compared with those of standards. Wavelengths used for quantification were 280 nm and 360 nm. **Results**: Analysis of total polyphenols by Folin–Ciocalteu method implied that wines produced in Mediterranean part of Croatia have a highest polyphenolic content. White wines contain significantly lower amounts of total polyphenols (400.8 mg/L) compared with red wines (1665.131 mg/L). Applied HPLC method allows the separation of 19 polyphenols with antioxidant properties from wine extracts in a single run. The most abundant polyphenol is gallic acid (0.7 – 26.9 mg/L). Biologically most active compounds, catechin and *trans*-resveratrol were present in each of analyzed wine. Highest concentrations of those compounds, together with one of the most potent antioxidant – quercetin, were determined in wines from Dalmatia. Since the vintage of the examined wines was identical, the observed quantitative differences are related to the grape variety, geographical origin, growing and winemaking procedures. **Conclusion**: The present study, which utilized new HPLC method for simultaneous determination of fifteen polyphenols and analysis of total polyphenols, demonstrates that the wines made from Croatia contain a significant amount of biologically active polyphenols and certainly, moderate and regular consumption of those wines (in particular red wine) can reduce risk of some diseases. However, wines made in Dalmatia contain a highest level of polyphenols as a result of dry mediterranean clime with high cluster sun exposure, so those wines could have highest positive effects on human health.

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# P2.2 Effects of flavonoids on production of reactive oxygen species in differentiated THP-1 cell line

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Flavonoids are a large group of polyphenolic compounds that occur commonly in plants. In addition of being one of the essential nutrients of numerous herbivores and omnivores (including humans), flavonoids exert a remarkable array of biochemical and pharmacological activities. The biological activities of flavonoids are thought to be due mainly to their antioxidant properties, which are displayed by limiting the production of reactive oxygen species (ROS) and/or scavenging them. High levels of reactive oxygen species can disrupt the normal redox state and shift cells into the state of oxidative stress, hallmarked by intracellular increase in products of lipid peroxidation, hydrogen peroxide, and elevated damage to other biomolecules. The aim of our study was to investigate the effect of 35 structurally diverse flavonoids on production of reactive oxygen species using appropriate cell culture model. THP-1 cells (human acute monocytic leukemia cell line) were differentiated into macrophage-like cells, and the induction or inhibition of ROS production by flavonoids was measured using dichlorofluorescein (DCF) assay *(1)*. The nonfluorescent fluorescin derivatives (dichlorofluorescin, DCFH), after being oxidized by various oxidants, will become DCF and emit fluorescence, and in such way it is possible to quantify the production of ROS. The cells were incubated with chosen substances (30 $\mu$M) for 30 minutes and after that time period production of ROS was measured. The same procedure was repeated with 1% hydrogen peroxide as a positive control. Also, cells were pre-incubated with flavonoids for 24 hours and 1% hydrogen peroxide, as a standard ROS for the DCF assay, was added to the same cells. This experiment was performed to establish the effect of flavonoids on antioxidant defense system of the cells. Cell viability during given time periods was determined using bioluminiscent cytotoxicity assay that quantitatively measures the release of adenylate kinase from damaged cells. None of the tested flavonoids exhibited cytotoxic effects in used cell line, but effects on ROS production were variable. Only few of the tested flavonoids (apigenin, diosmetin, flavone, hesperetin, daidzein, formononetin, genistein) exhibited pro-oxidant activity (after 30 minutes of incubation they increased the production of ROS more than 120% comparing to the non-stimulated cells). After 24 hours of incubation and after the addition of $H_2O_2$, the following flavonoids decreased the fluorescence for more than 50%: apigenin, diosmetin, fisetin, isorhamnetin, kaempferide, chrysin, chrysin-dimethylether, quercetin, luteolin, rhamnetin, tamarixetin and tangeretin. Considering that apigenin and diosmetin exhibit dual effect, these compounds were shown to be the most interesting for further investigation.

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Abstract

# P2.3 Antioxidative and vasodilatatory effects of phenolic acids from wine

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**Introduction**. Beneficial effects of moderate wine intake on human health have been attributed primarily to the phenolic compounds in wine. Although phenolic acids represent a significant fraction of wine phenolics, their biological effects have been scarcely investigated. The aim of the present study was to determine the antioxidative and vasodilatatory properties of 9 phenolic acids from wine and possible correlation between these two effects. **Materials and Methods.** The following phenolic acids, as pure compounds, were studied: sinapic, ferulic, vanillic, syringic, *p*-hydroxybenzoic, caffeic, protocatechuic, *p*-coumaric and gallic acid. Antioxidative capacity of the acids (1 mmol/L) was determined by two methods, ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC). Their vasodilatatory activity *in vitro* was determined on isolated rings (n = 12 per acid) from rat aorta. Four rings from each animal were mounted in 20 ml organ baths containing modified Krebs-Henseleit solution. Each ring was pre-constricted with norepinephrine (NE, $10^{-7}$ mol/L) and cumulatively exposed to one phenolic acid ($10^{-6} – 10^{-2}$ g/L). **Results:** Antioxidative capacity, measured by FRAP method, was 1.26, 0.99, 0.37, 1.47, 0, 1.11, 1.02, 0.12 and 2.32 mmol/L Trolox equivalents, while values obtained by TEAC method were1.96, 1.80, 0.87, 1.36, 0.06, 1.42, 1.22, 1.09 and 2.79 mmol/L Trolox equivalents for sinapic, ferulic, vanillic, syringic, *p*-hydroxybenzoic, caffeic, protocatechuic, *p*-coumaric and gallic acid, respectively. There was a positive correlation between FRAP and TEAC values for antioxidative capacity of phenolic acids ($r = 0.8859$, $p = 0.0015$). Maximum vasodilatatory activity *in vitro* was approximately 21.7, 27.0, 38,0, 21.7, 26.0, 22.0, 12.0, 25.6 and 5.9% relaxation of norepinephrine-induced vasoconstriction for sinapic, ferulic, vanillic, syringic, *p*-hydroxybenzoic, caffeic, protocatechuic, *p*-coumaric and gallic acid, respectively. There was a significant negative correlation between antioxidative capacity (measured by FRAP method) and maximal vasodilatatory effect of the phenolic acids ($r = – 0.7348$, $p = 0.0241$). **Conclusion**. This study indicates that phenolic acids are potent antioxidants but weak vasodilators. Moreover, there is a negative correlation between these two effects of wine phenolic acids.

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Abstract

# P2.4 Free resveratrol monomers in varietal red and white wines from Dalmatia (Croatia)

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**Introduction**. Stilbenes are phenol-based plant metabolites widely present in nature and implicated with different human health benefits. Among stilbenes, the phytoalexine resveratrol (*trans*-3,5,4'-trihydroxystilbene) has attracted immense attention due to its numerous significant biological properties. Resveratrol is considered to be one of the major antioxidant constituents in red wine. In this contribution we investigated the presence of and relation between *trans*- and *cis*-resveratrol monomers in the most characteristic varietal red and white wines from Dalmatia (Croatia), produced according to the Croatian appellation of origin system. **Materials and Methods.** We analyzed 20 samples of red and 15 samples of white wines of various grape varietes (Red: *Plavac mali, Merlot, Cabernet sauvignon, Babić, Plavina, Trnjak, Vranac, and Lasin*; White: *Cetinka, Pošip, Maraština, Debit, Kujundžuša, Malvasija Dubrovačka, Vugava, Medna and Zlatarica*), vintage 2004. Wine samples were analyzed in June 2005, after the wine processing had been completed and the wines prepared for market. Standard methods of analysis for general wine components were used for preliminary control of selected wines quality. To determine the phenolic composition of wines, total phenol content, phenol index, as well as flavonoid and anthocyanin (unpolymerized pigments) content were analyzed. The separation and quantification of *trans*- and *cis*- resveratrol were done by HPLC on reverse phase. The stock solution of *cis*-isomer was prepared by UV irradiation of *trans*-resveratrol. **Results**. Significant differences in the phenolic composition, even between the wines produced from same grape varieties, but originating from different localities, were found. The concentration of free resveratrol monomers (*cis*- and *trans*-) in white and red wines ranged from 0.11 – 1.04 mg/L (mean 0.43) in white wines, and from 0.5 – 8.57 mg/L (mean 2.98) in red wines. The average relative amount of *cis*-resveratrol in white wines was almost 2-fold higher in comparison with red wines. A significant impact of grape variety on resveratrol content in wine was confirmed. Besides wine Merlot, high concentrations of free resveratrol monomers were found in red wines Dingač and Postup produced from autochthonous grape variety *Plavac mali*. Among white wines, highest concentrations were detected in wine Zlatarica. **Conclusion**. According to the obtained results some red wines from the region of Dalmatia could be a good dietary source of resveratrol.

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Abstract

# P2.5 Antioxadative capacity and vasodilatatory activity of strawberry leaves extract

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**Introduction**. Consumption of strawberries has been associated with several beneficial effects of human health. Most studies have been focused on the strawberry fruit, due to its high content of bioactive phytochemicals, most notably polyphenols. However, strawberry leaves as a potential source of bio-potent polyphenols has been largely overlooked. The aim of this study was to determine the antioxidative and vasodilatatory activities of strawberry leaves (*Fragariae herba folium*) extract. **Materials and Methods.** Dried, milled strawberry leaves (15 g) were added into 150 mL of boiled deionised water and left at room temperature for 30 min without additional heating. The infusate was filtered and evaporated to dryness, yielding 1.44 g of dried extract, which was then dissolved in distilled water to final concentration of 6 g/100 ml. Antioxidant capacity and total phenolic content of the extract were determined by ferric reducing antioxidant power (FRAP) and Folin-Ciocalteau method, respectively. *In vitro* vasodilatatory activity of the extract was studied on the isolated rat aortic rings and the isolated guinea pig hearts after Langendorff. Isolated vascular rings (n = 15) were pre-constricted with norepinephrine (NE, 0.1 μmol/L) and cumulatively exposed to the extract (0.06 – 60 mg/100 ml). By use of L-NAME (100 μmol/L) and indomethacin (10 μmol/L) and by removal of vascular endothelium, role of nitric oxide, cyclooxygenase products and endothelium – independent effects of the extract were determined (n = 8 per group). The isolated guinea pig hearts (n = 12) were exposed to 4 doses of the extract (6, 18, 60 and 180 mg / 100 ml) added into the perfusate at the rate of 1 % of coronary flow, allowing final concentrations of 0.06, 0.18, 0.6, and 1.8 mg/100 ml, respectively. Each dose was perfused for 3.5 minutes, with 15 minutes of washout period between doses. **Results**. Total phenolic content of the extract was 8.2 g/L gallic acid equivalents, and the antioxidant capacity was 47.7 mmol/L Trolox equivalents. A dose – dependent vasodilatation of pre-constricted rat aorta was observed, with maximum vasodilatatory response of 72 ± 4%. The vasodilatation was endothelium – dependent, and mediated by both nitric oxide and prostacyclin. In the isolated heart, a dose-dependent increase of coronary flow was also observed, with the maximal response of 45% above the control value. This was accompanied by simultaneous dose – dependent reduction of oxygen extraction, up to 34% from the control value. **Conclusion**. This study indicates that strawberry leaves are valuable source of bioactive phytochemicals with potentially beneficial effects on cardiovascular system.

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# P2.6 Modification of red wine vinification process: addition of white grape seeds, skins and ethanol causes changes in biochemical properties and vasodilatatory activity *in vitro*

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**Introduction**. Beneficial effects of moderate red wine intake on human health have been attributed primarily to the high content of polyphenols (like catechin), strong antioxidants in red wine. White wine contains significantly less polyphenols than red wine, due to different wine making technique. Skins and seeds of white grapes are excluded from fermentation, and white grape juice is the only source of polyphenols in white wine. Technological and biological potential of white grapes skins and seeds is mainly overlooked and insufficiently exploited. The aim of this study was to determine how addition of white grape seeds, skins and ethanol to the red grape marc before and during fermentation, might influence biochemical properties and vasodilatatory activity *in vitro* of the obtained beverages. **Materials and Methods.** Basic red wine was made with grapes of Merlot variety. Chardonnay variety was used as a source of white grapes skins and seeds. Five different beverages were produced: 1. control red wine (CRW), from 20 kg of Merlot grapes by standard enological practices, with no additional intervention; 2. modified wine (W40), by adding skins and seeds from separately crushing and pressing of 40 kg of Chardonnay grapes to CRW prior fermentation; 3. modified wine (W40+E), the same as W40, with addition of ethanol to halt fermentation and to increase extraction of polyphenols into the wine. The final ethanol concentration was 25% vol.; 4. modified wine (W80), the same as W40, with doubled amount of added skins and seeds (from 80 kg of grapes); 5. modified wine (W80+E), the same as W40+E, but with doubled amount of added skins and seeds (from 80 kg of grapes). All five beverages were analyzed for their total content of ethanol, sugars, phenols, catechins, flavonoids, anthocyanins, antioxidant capacity, and *in vitro* vasodilatatory activity on isolated rings (n = 15 per beverage) from rat aorta. **Results**. Addition of white grape seeds and skins resulted in related changes of total phenolic content (1.01, 1.35, 1.32, 1.75 and 1.77 g/L gallic acid equivalents) and total catechins content (388, 610, 452, 625, 681 mg/L) for CRW, W40, W40+E, W80 and W80+E, respectively. Antioxidant capacity of the beverages, measured by ferric reducing antioxidant power (FRAP), was 5.89, 7.77, 6.66, 8.79, and 8.43 mmol/L Trolox equivalents for CRW, W40, W40+E, W80 and W80+E, respectively. FRAP values generally correlated with the phenolic content of the beverages. All modified beverages were more potent vasodilators than CRW but did not differ between each other. **Conclusion**. This study confirms that white grapes skins and seeds are valuable unused plant materials with significant biological potential. Modification of red wine vinification by adding white grape seeds and skins might prove useful from economical and biomedical point of view.

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Abstract

# P2.7 Chemical composition and antioxidant capacity of selected spice volatile aglycones in two lipid model systems

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The antioxidant capacity of volatile aglycones from basil (*Ocimum basilicum* L.), laurel (*Laurus nobilis* L.) and clove (*Syzygium aromaticum* L. Merill et Perry) were examined. The volatile aglycones were extracted from their nonvolatile glycosidically bound compounds by enzymatic hydrolysis with *β*-glycosidasae enzyme. The chemical composition analyses of volatile aglycones were run on coupled system gas chromatography-mass spectrometry (GC-MS) with two different polarity columns. All volatile aglycone fractions have complex chemical compositions with identified aliphatic monoterpenic, sesquiterpenic, phenylpropanoid and norisoprenoid alcohols, ethers, acids, esters and carbonyl compounds. The major volatile aglycones are phenylpropanoid compounds. The major basil volatile aglycones are eugenol (44,0%) and chavicol (29,5%). Of laurel it is benzyl alcohol (63,4%), while the major clove volatile aglycone compound is eugenol (80,5%). The antioxidant capacity of isolated volatile aglycone fractions were tested by two lipid model systems: TBARS method (Method with Thiobarbituric Acid Reactive Species) and Rancimat method (Determination of Oxidative Stability of Fat). These methods were chosen because they are both bases on real lipid media for measurement. That is very important for real evaluation of antioxidant capacity. The results were compared with those for frequently used commercial antioxidants BHT (betahydroxytoluene) and vitamin C. The results show that the basil and laurel volatile aglycones have low antioxidant capacity or have no antioxidant capacity, tested by TBARS method. Contrary, the clove volatile aglycones antioxidant capacity was better: comparable with vitamin C capacity and lower than BHT capacity. Similarly results were obtained for basil and laurel tested by Rancimat method. Clove aglycone fraction antioxidant capacity is comparable or better than BHT capacity, but lower than vitamin C capacity. This is another confirmation of clove's significant role among spice plants, a guideline to its usage in dieting and replacing commercial antioxidants with natural ones.

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Abstract

# P2.8 *In vitro* and *in vivo* antitumor activity of thymoquinone and thymohydroquinone

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The aim of the study was to investigate antitumor activity of the putative pharmacologically active constituents of *Nigella sativa* L. volatile oil thymoquinone (TQ) and thymohydroquinone (THQ). In the *in vitro* experiments, L929 mouse fibroblasts normal cells lines were used as a control and two tumor cell lines (squamous cell carcinoma (SCC VII) and fibrosarcoma FsaR)) were used. The cells were cultured with 0.1 and 0.01 mg/ml TQ and THQ for 24 h, and cytotoxicity assay was performed using crystal violet staining technique. In the *in vivo* experiments two murine tumor models (fibrosarcoma (FsaR) and squamous cell carcinoma (SCC VII)) were used. The used dose was equal for both substances. Antitumor affect of 4 intratumoral injections of TQ and THQ at the dose of 5 mg/kg was evaluated by comparison of tumor growth kinetics between treated and control animals. *In vitro* study showed that TQ and THQ exhibit statistically significant antitumor activity ($p>0.01$). The antitumor activity was dose dependent and more expressed against tumor cells than against L929 fibroblasts. The result of antitumor activity of TQ and THQ *in vivo* reached TGI of 52% and it was statistically significant ($p < 0.05$). The results indicate that THQ antitumor activity may be improved with further increase in dose.

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Abstract

# P2.9 Selective cytotoxicity of diazenecarboxamides towards human leukemic cell lines

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**Introduction**: The search for anti-cancer and anti-leukemic agents lead to the synthesis of new drugs. Diazenecarboxamides (diazenes) are new compounds that are cytotoxic towards various tumor cell lines, including mutidrug-resistant tumor cells. However, their efficacy was not examined on haematopoietic cells. In this study the cytotoxicity of 16 diazenes was tested on human leukemic cell lines. These compounds belong to the three subclasses: diazenecarboxamides (11 compounds SB-681, LK-34, UP-39, LK-26, JK-1197, UP-11, LK-95, LK-79, JK-279, JK-835, MG-19), diazenedicarboxamides (4 compounds SB-409, SB-672, SB-411, SB-410) and alkyl aminocarbonyl-diazenecarboxylate (only SB-166). **Materials and methods:** Four leukemic cell lines NALM-1, JURKAT, HL-60 and K-562. were treated with 5 concentrations of each compound for 3 days. The cytotoxicity was determined by MTT-test. The selective cytotoxicity of diazenes was tested towards resting and Con-A stimulated human peripheral blood mononuclear cells (PBMC) and towards mouse 3T3 fibroblasts. **Results**: 15 out of 16 tested diazenes were cytotoxic towards the leukemic cell lines: 11 with high efficacy ($IC_{50} < 50\ \mu M$) and 4 with medium efficacy ($IC_{50} > 50\ \mu M$). Ten out of these 11 diazenes have common structure and belong to the subclass of diazenecarboxamides. Five diazenes (SB-681, LK-34, UP-39, JK-1197, UP-11) were highly cytotoxic ($IC_{50}$ values 3.3 – 38.9 $\mu M$) towards all four leukemic cell lines. Diazenes cytotoxic towards leukemic cells, did not affect the viability of the resting PBMC suggesting selectivity of their action, and eight of them (LK-34, JK-1197, UP-39 UP-11, LK-79, JK-835, JK-279, SB-410) affected neither normal dividing cells (Con-A-stimulated stimulated) nor mouse 3T3 fibroblasts. **Conclusion**: 15 out of 16 examined diazenes were cytotoxic towards leukemic cells but did not affect normal resting human peripheral blood mononuclear cells. Eight of them affected neither normal or dividing cells (Con-A-stimulated PBMC and mouse 3T3 fibroblasts). Thus, the observed selective cytotoxicity of these diazenes makes them promissing new potential agents for the treament of leukemic patients.

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Abstract

# P2.10 Altered cell-cell adhesion in cisplatin-resistan human carcinoma cells: a link between beta-catenin/plakoglobin ratio and cisplatin resistane

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**Introduction.** The majority of cancer patients are still treated with classical chemotherapy. In this respect, cisplatin is one of the most effective and commonly used agents for the treatment of a wide spectrum of solid tumors. Nevertheless, resistance to this drug is a major problem that limits the effectiveness of its use in cancer treatment. Greater insight into the molecular mechanisms regarding modulation of the cellular response to cisplatin should help to develop and optimize therapeutic strategies. We have developed cisplatin resistant human laryngeal carcinoma cells, which exhibited altered formation of cell-cell junctions comparing to their parental HEp2 cells. The aim of the present study was to examine molecular basis of this phenomenon. **Materials and methods.** We analyzed and compared the basal and cisplatin-induced expression of cell-cell junctional proteins in parental laryngeal carcinoma HEp2 cells and its cisplatin resistant $CA3_{ST}$ and CK2 sublines:E-cadherin, N-cadherin, beta catenin, plakoglobin, p120 catenin and desmoglein. The expression of proteins was determined by Western blot assay and the expression of mRNA by semiquantitative RT-PCR. Immunocytochemical analysis was performed to estimate the subcellular distribution of selected junctional proteins. Coimmunoprecipitation method was used to examine cellular cadherin-catenin complex-interaction. Cell sensitivity to cisplatin was assayed by MTT spectrophotometric assay. Plasmid tranfection of $CA3_{ST}$ CELLS with plakoglobin gene was done by the standard procedure with lipofectamine transfection reagent. **Results***:* In $CA3_{ST}$ and CK2 cells, N-cadherin, desmoglein and p120-catenin were expressed at the similar level, while none of the cell lines expressed E-cadherin. The expression of plakoglobin in cisplatin resistant cells was down-regulated (on both, mRNA and protein levels), and β-catenin up-regulated (only on protein level). Immunopre- cipitation of cadherin-catenin complex established that upregulation of β-catenin results from its stabilization through interaction with N-cadherin. While increase of *β*-catenin expression has been found in cisplatin resistant cell lines from different origin but not in vincristine resistant human laryngeal carcinoma cells, we speculate this could be a general phenomena accompanying cisplatin resistance. Transfection of plakoglobin-expressing plasmid vector in $CA3_{ST}$ cells reconstitutes beta-catenin/plakoglobin ratio of parental HEp-2 cells, but does not restore sensitivity to cisplatin. The shift in the composition of cadherin-catenin complexes was not induced by a single treatment with cisplatin. **Conclusions.** It appears that ß-catenin and plakoglobin are not involved in the resistance mechanism, implying that the observed alterations are an outcome of slowly generating process, which is presumably a secondary event of vital cellular response triggered by cisplatin toxicity.

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Abstract

# P.2.11 The influence of hyperthermia and chemotherapy on the tumour growth in mice

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**Aim**: The main goal of this study is to investigate antitumour activity of newly synthesized compounds from N-sulfonyluracil and benzothiazole group. These types of compounds showed potent inhibitory activity on the growth of human tumour cell lines *in vitro*. In this study we have investigated antitumour activity of sulfonyluracil derivatives and benzothiazoles on the growth of transplantable mouse tumours (mammary carcinoma, fibrosarcoma, squamous cell carcinoma, and melanoma) *in vivo*. These compounds have been applied alone or with local hyperthermia (LTH). **Material and Methods:** Mouse tumour cells ($10^6$) were injected into the mouse footpad of the right hind leg. Tumour bearing mice have been treated with new compounds as a single agent or in combination with hyperthermia (43, 0 °C/60 min). The end point was tumour growth time (TGT). TGT is the time needed for tumour volume to grow five times over the treated volume measured by calliper and calculated by the formula AxBxCx_/6. Sulfonyuracil derivatives have been synthesized at Ruđer Bošković Institute and benzothiazoles have been synthesized at Faculty of Chemical Engineering and Technology, University of Zagreb. **Results**: The obtained data show that examined sulfonyluracil compounds have suppressed the growth of mammary carcinoma in comparison to control group. But when this compound was combined with local hyperthermia, antitumour activity of this derivative was enhanced. Benzothiazole compounds have shown good antitumour activity against melanoma B16 and fibrosarcoma as well as against SCCVII carcinoma. **Conclusion**: The obtained data show that new antitumour compounds (*N*-sulfonyluracil and benzothiazole derivatives) can reduce tumour growth time in mice when applied as a single agent but much more when applied in combination with hyperthermia.

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Abstract

# P2.12 Effect and mechanism of thermoimmunotherapy with OK-432 on the tumour growth and lung metastases in mice

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**Introduction**. OK-432 (Picibanil), an inactivated and lyophilized streptococcal preparation, is an immunomodulatory agent extensively investigated as adjuvant therapy for cancer. The local administration of OK-432 in murine tumour enhances the response of tumour and normal tissue to elevated temperatures. Tumour growth is not affected by local administration without hyperthermia. Likewise without effect is the systemic administration (i.p. or i.v.) combined with local hyperthermia. Further studies were conducted to disclose the mechanism of OK-432 induced thermal enhancement. **Materials and Methods.** Animals were 8-week-old C3Hf/Sed mice. The tumours were fourth generation isotransplants of non-immunogenic fibrosarcoma Fsa-II which arose spontaneously in a C3Hf/Sed mouse. OK-432 was donated by Chugai Pharmaceutical (Tokyo, Japan). A dose of 2.5 KE in 25 mL PBS was injected into tumour 3 h before hyperthermic treatment. Hyperthermia was given by immersing animal feet, or the 100-mL glass bottles containing tumour cell suspensions, in a water bath maintained at a desired temperature by heater circulator. The anti-mouse T cell globulin, silica, trypan blue, cyclophosphamid and anti-(asialo GM1) globulin were used. The incidence of lung metastases was determined by counting the colonies formed on the surface of each lobe under a dissecting microscope. Each experiment was repeated at least twice, and if no differences, the results were combined. **Results**. Low direct cytotoxic activity against tumour cells *in vitro* indicated the involvement of the host-mediated mechanisms. The whole body irradiation had no effect on thermal enhancement suggesting that radiosensitive cells were not involved. Anti-mouse T-cell serum did not have any effect, as expected. In addition, administration of reticuloendothelium system blockers (silica, trypan blue) also did not reduce the OK-432 induced thermal enhancement. Further, local intratumour injection of normal or activated macrophages was without any effect on tumour growth as well. However, administration of anti-(asialo GM1) globulin dramatically diminished the thermal enhancement. Moreover, the administration of cyclophosphamide completely abolished the OK-432 thermal enhancement. Both results indicate that augmentation of mouse natural killer (NK) cell activity by combined hyperthermia and OK-432 was responsible for the thermal enhancement. In the second part of the investigation we examined the effect of thermoimmunotherapy with OK-432 on development of spontaneous lung metastases. Sham hyperthermia, i.e. restraining the mice in holders, as well as local injection of saline solution, increased metastases incidence. Yet, intratumour injection of OK-432 given alone and particularly with hyperthermia strongly reduced tumour cell dissemination. The incidence of lung metastases was lower than in control, non-treated animals. **Conclusion**. The local administration of OK-432 enhanced tissue response to hyperthermia. The treatment reduced the incidence of spontaneous lung metastases as well. The mechanism of antitumour thermal enhancement mediated by OK-432 is the augmentation of NK cell activity.

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# P2.13 Cytotoxic effects of new synthesized bisbenzimidazole derivatives on different tumour cell lines

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Since there is a constant need for new, more effective and less harmful potential anticancer drugs we tested antiproliferative activity of three novel benzimidazoyl hydrochloride analogues on different cancer cell lines. The cytotoxic effects of tested compounds were tested by colorometric MTT assay. A panel of 5 leukaemia (K562, RAJI, JURKAT, HL-60 and MOLT-4) and 6 carcinoma cell lines (AGS, HEp2, Caco-2, HeLa, HT-29, MiaPaca) were used. Investigated compounds showed a great variation in antiproliferative effect on tumour cell lines, depending on the cell line as well as on the dose applied. Analogues 2,5-bis[5-(*N*-isopropylamidino)benzimidazo-2-yl)]-3,4-ethylenedioxythiophene dihydrochloride (MB12) and 2,5-bis[5-(2-imidazolino)benzimidazo-2-yl]-3,4-ethylenedioxythiophene dihydrochloride (MB13) strongly inhibited the growth of leukemia cells, K562, RAJI and MOLT-4, for 50 – 80% at concentration of $10^{-4}$ and $10^{-5}$ M, but had no influence on JURKAT and HL-60 cells. At the same concentration 2,5-Bis[2-(5-amidinobenzimidazoyl)]-3,4-ethylenedioxythiophene dihydrochloride (MB11) inhibited proliferation of JURKAT and HL-60 cells for 30 – 40%. Lower concentration ($10^{-6}$ and $10^{-7}$ M) of all tested compounds had no effects on proliferation of leukaemia cells. The best inhibitory effects against carcinoma HeLa and HEp-2 cells at relatively low concentration of $10^{-6}$ M displayed MB12 compound. MB11 and MB13 were less effective on these cell lines. MiaPaca cell line was sensitive on MB11 and MB12 compounds at concentration of $10^{-5}$ M. Investigated compounds had no effect on Caco-2 and AGS cells. According to obtained results we can conclude that cytotoxic efficiency of tested benzimidazoyl hydrochloride analogues is more pronounced on solid tumours cell lines compared to effects on leukaemia cells.

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# P2.14 The role of the nuclear protein kinase B/AKT activation in HL-60 leukemia cells

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**Introduction**: At the cell membrane, phosphoinositide 3-kinase(PI3K)/Akt signaling pathway plays a crucial role in mediating growth factor-induced cell survival and proliferation. However, previous studies have demonstrated a progressive increase in the level and activity of PI3K in nuclei of HL-60 cells differentiated in the presence of all-trans-retinoic acid (ATRA). The aim of the present study is to investigate the changes in subcellular distribution and activities of Akt during agonist-induced differentiation and particular phase of the cell cycle and to determine the role of the nuclear Akt in differentiation and proliferation of HL-60 cells. **Materials and methods:** HL-60 cells (ECCACC, UK) are maintained in exponential growth and differentiated in the presence of 1μM ATRA, 1,25% DMSO or 500 nM phorbol miristate acetate (PMA). The expression of CD11b is determined by FACS analysis. Cell lysates and nuclei are analyzed for the presence of phosphorylated and total Akt by Western blot analysis or subjected to Akt kinase assay using Crosstide as the substrate *(Matkovic et al, Leukemia, 20:941-951,2006*). Akt protein is down-modulated by commercially available siRNA (Upstate, USA). Cells are synchronized by incubation in the presence of aphidicolin. **Results**: The Akt-activity is found to be increased in the nuclei and lysates of HL-60 cells incubated in the presence of 1$\mu$M ATRA for 4 days. The kinase assay show no increase in the activity of Akt immunoprecipitated from either postnuclear membranes or cytosol. Time-course study of nuclear Akt-activity in HL-60 cells show progressive increase in the levels of phosphorylated Akt that correlates with an increase in the expression of differentiation marker (CD11b). Down-modulation of Akt protein by siRNA inhibits the expression of Akt protein and decreases the level of CD11b. No increase in the level of the nuclear Akt is observed in HL-60 cells treated with other granulocytic inducers (DMSO) or strong antiproliferative agonists (phorbol miristate acetate, PMA). Preliminary data show a progressive increase in the level of phosphorylated Akt in lysates of aphidicolin-synchronized HL-60 cells. **Conclusion**: Our results reveal that ATRA increases activity of nuclear Akt in HL-60 cells, and that the activity of Akt is necessary for ATRA-mediated differentiation

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Abstract

# P2.15 The effects of phosphoinositide 3-kinase/Akt inhibitors on two retinoid-responsive leukemia cell lines

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**Introduction**: All-trans retinoic acid (ATRA)-based treatment of acute promyelocytic leukemia (APL) patients is the first example of therapy by differentiation. The classical view of the action of ATRA in APL holds that ATRA releases corepressors from the chimeric PML-RARa protein and thus allows the growth inhibition, differentiation and apoptosis in t(15;17) APL cells. The pharmacological inhibitors of phosphoinositide 3-kinase(PI3K)/Akt pathway have been proposed in the treatment of leukemia based on their antiproliferative effects. However, recent studies *(Matkovic et al, Leukemia, 20:941-951,2006)* demonstrated Akt-activation in nuclei of ATRA-treated HL-60 cells raising the possibility that PI3K/Akt –inhibitors may block antitumor properties of retinoids. The aim of this study is to compare the sensitivity of two different leukemia cell lines to commercially available PI3K/Akt-inhibitors. NB4 cell line, established from an APL patient, is a typical AML-M3 carrying t(15;17). HL-60 cells, isolated from AML-M2 patient, do not display specific t(15,17) translocation but share the responsiveness to ATRA and are widely used to demonstrate the differentiative activity of ATRA and to predict its efficiency *in vivo*. **Materials and Methods:** HL-60 cells and NB4 cells were maintained in exponential growth and differentiated in the presence of ATRA (1μM). The Akt activity in different cell fractions was determined by Western blot analysis and kinase assays using Crosstide. The following inhibitors were tested: Akt inhibitor I (1L-6-hydroxymethyl-chiro-inositol 2(R)-2-*O*-methyl-3-*O*-octadecylcarbonate, Calbiochem), Akt inhibitor II (SH-5, Calbiochem), LY 294002 (Calbiochem) and MEK-inhibitor PD 98059 (Calbiochem). The number of viable cells was quantified using a hemocytometer and trypan blue exclusion. The expression of CD11b and the cell cycle distribution of propidium iodide-labeled cells were determined by FACS analyses. **Results**: Both HL-60 and NB4 cells show an increase in the activity of Akt in nuclei and lysates after ATRA-mediated differentiation. PI3K inhibitor LY 294002 and MEK-inhibitor PD98059 reduced the number of viable cells and the expression of differentiation marker in both cell lines. The presence of Akt inhibitors inhibited the growth of both control and ATRA-treated NB4 and HL-60 cells and reduced the expression of CD11b in ATRA-treated NB4 cells. In contrast, Akt-inhibitors had no inhibitory effects on the expression of CD11b in ATRA-treated HL-60 cells, but increased the percentage of control cells expressing CD11b. The inhibition of Akt altered the life span of differentiated HL-60 cells as the number of events in sub-$G_1$ measured at 7 days after the addition of ATRA was higher in samples treated with the combination of Akt inhibitors and ATRA that in the cells treated with ATRA alone. **Conclusion**: Two retinoid-responsive leukemia cell lines respond differently to the presence of PI3K/Akt inhibitors; the expression of differentiation marker is reduced in ATRA-treated NB4 cells, there are no inhibitory effects on ATRA-induced differentiation of HL-60 cells and the percentage of control HL-60 cells expressing CD11b is increased. These results suggest that the combination of retinoids with Akt-inhibitors could be exploited to improve retinoid therapy in selected cases. In addition, data further suggest that ATRA may mediate a number of nontranscriptional changes in cell signaling by mechanisms apart from the direct effects of the nuclear receptor on transcription.

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# P2.16 Two distinct peaks of nuclear PI-PLCbeta$_{1b}$ activity occur in serum-stimulated HL-60 cells

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**Introduction**: The activity of phosphatidylinositol-specific phospholipase C (PI-PLC) increases in nuclei of cells at temporally distinct phases of the cell cycle. In HL-60 cell line, previous studies demonstrated a PI-PLC inhibitor-sensitive increase in the level of the nuclear DAG at 8 h after aphidicolin block ($G_2$-phase) and an early (30 min) increase in the PI-PLC activity in serum-starved HL-60 cells stimulated with IGF. Our recent study demonstrated two peaks of an increase in the nuclear PI-PLC activities in nocodazole-synchronized HL-60 cells that occur at 1 h and 8.5 h after $G_2$/M-block (*Lukinovic-Skudar et al., Biochim Biophys Acta 1733:148-156, 2005*). To further prove that the late increase in the PI-PLC activity observed in nocodazole-synchronized cells is a late $G_1$-phase-related event, the PI-PLC activity was measured in serum-starved cells after the re-addition of serum. **Materials and Methods:** HL-60 cells (ECCACC, UK) are maintained in exponential growth. For synchronization, exponentially growing cells were washed and maintained in medium containing no serum for 24h and then stimulated with FBS (10%, v/v). Cells were labelled by propidium-iodide or BrdU FITC set (556028, BD, USA) and the cell cycle analysis or BrdU incorporation is determined by using FACSCalibur system. Cell fractions were isolated as described *(Lukinovic-Skudar et al., Biochim Biophys Acta 1771:514-521, 2007)* and PLC activity was determined using [$^3$H] (PtdIns(4,5)$P_2$) as a substrate. PLCß$_{1b}$ was immunoprecipitated from nuclear fractions and immunoprecipitates were subjected to Western blot analysis using anti-P-serine antibodies or anti-PLCß$_{1b}$ antibodies. **Results:** In HL-60 serum-stimulated cells, two distinct peaks of the nuclear PI-PLC activation were detected at 30 minutes and 11 h with no parallel increase in PLC activity in total cell lysates, cytosol and postnuclear membranes. All the peaks of the nuclear PI-PLC activities were completely abolished in the presence of PI-PLC inhibitor ET-18-OCH$_3$ and MEK inhibitor PD98059. The increase in the activity correlates with a PD98059-sensitive increase in the serine phosphorylation of b splicing variant of PI-PLCbeta$_1$ and no change in the amount of PI-PLCbeta$_{1b}$ was detected in nuclei isolated at any time points. The number of cells entering the S-phase was reduced after the addition of inhibitors either immediately or 6 h after the re-addition of serum. **Conclusion**: Two distinct peaks of nuclear PI-PLCbeta$_{1b}$ activity occur in serum-stimulated HL-60 cells and both early and late $G_1$-phase increases in the nuclear PI-PLC are important for the progression of cells into the S phase.

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# P2.17 Expression of organic anion transporter Oat3 in rat liver is gender-dependent

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In the mammalian liver, elimination of endogenous and exogenous organic anions (metabolic products, various drugs, environmental toxins) is mediated by various organic anion transporters (OATs), transmembrane proteins that belong to solute carrier family Slc22. The rat liver organic anion transporter 3 (rOat3, Slc22a8) has been cloned, characterized as a dicarboxylate exchanger, and defined as »male predominant« due to much higher expression of its mRNA in males (M) than in females (F). However, cellular localization and the levels of rOat3 protein expression in rat hepatocytes are unknown. This study was designed to examine the expression of rOat3 in the rat liver at the level of: a) mRNA in the tissue using RT-PCR, and b) protein in isolated total cell membranes (TCM) using Western blotting (WB) and in tissue cryosections using immunocytochemistry (IC). For this purpose we used the following experimental groups of rats: a) intact adult M and F, b) castrated M treated with oil (O; control) or with oil solutions of testosterone (T), progesterone (P) and estradiol (E), and c) adult M treated with O, E and P. The RT-PCR data confirmed the male-predominant expression of rOat3 mRNA in the rat liver. Castration markedly decreased the expression of Oat3 mRNA; the treatment of castrated rats with T restored it to the level of intact M, while the treatment with E and P had no effect. However, the treatment of adult M with E and P strongly downregulated the expression of rOat3 mRNA as compared to that in O-treated M. By WB in TCM prepared in non-reducing conditions, a polyclonal anti-rOat3 antibody labeled a single peptide-blockable protein band of ~50 kDa. In various experimental conditions the density pattern of this protein resembled the pattern of rOat3 mRNA. In IC studies, the anti-rOat3 antibody strongly stained some intracellular organelles in the M but not F liver. The double staining studies with organelle-specific marker antibodies (plasma membranes, endocytic vesicles, mitochondria, peroxisomes, lysosomes) indicated that rOat3 might be localized in lysosomes. We conclude that rOat3 in the rat liver exhibits strong gender differences (M>F) at both mRNA and protein levels due to testosterone stimulation and estradiol and progesterone inhibition, and may be localized in lysosomes.

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Abstract

# P2.18 Immunolocalizaion of $Na^+$ – independent sulfate transporter Sat-1 (Slc26a1) in rat kidney and gastrointestinal tract

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The $Na^+$-independent sulfate (S) transporter Sat-1 has been localized to the basolateral membrane (BLM) of proximal tubules in the rat kidney (K). The same transporter may also mediate S transport in the gastrointestinal tract (GIT) and liver (L), but its presence and the exact cellular localization in these organs has not been described. In this work we used a monoclonal antibody to Sat-1 protein (Sat-1-Ab) and performed localization studies by immunofluorescence cytochemistry (IC) and Western blotting (WB) in the L and in various GIT segments in rats. The Sat-1-Ab was verified in the K by achieving a positive staining of BLM in the proximal tubule S1 and S2 segments, and by labeling a single, ~85 kDa protein band in BLM isolated from renal cortical homogenate. In the L, the Sat-1-Ab strongly stained the sinusoidal membrane; the canalicular membrane and other structures in hepatocytes remained unstained. However, the double staining in various GIT segments with the Sat-1-Ab and a polyclonal antibody to cell adhesion molecule 105 (CAM-105) indicated the localization of Sat-1 in an intracellular vesicular compartment. In the tested GIT segments, the intensity of intracellular Sat-1 staining showed the following pattern: esophagus < duodenum < jejunum < ileum < colon < stomach. The localization of Sat-1 in endocytic vesicles was disproven by an *in vivo* assay of FITC-dextran uptake in the colon cells, whereas the double-staining for Sat-1 and the mitochondrial marker cytochrome C in the colon and stomach revealed a complete colocalization of these proteins in mitochondria of enterocytes (colon) and oxyntic cells (stomach). However, in WB of total cell membranes, isolated from the L and various GIT segments, the Sat-1-Ab labeled weakly the ~40 kDa protein band, whereas the K-specific 85 kDa protein band was not labeled at all in these membranes. We conclude that in the rat L and GIT cells, the Sat-1 protein is different from that in the K and may exist as a shorter (truncated) isoform. In various GIT cells, the transporter may be localized in mitochondria.

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Abstract

# P2.19 Renal expression of organic anion transporters Oat1 and Oat3 is downregulated after treating rats with cadmium, mercury and cisplatin

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Previous studies have shown that treating rats or mice with some nephrotoxic metals (NTM) inhibited renal transport/secretion of organic anion (OA) p-aminohippurate (PAH) and its accumulation in incubated kidney cortex slices. Moreover, nephrotoxicity of these metals was prevented by probenecid, a compound that inhibits the transport of various OA, including PAH, thus indicating a possible interaction of NTM and OA at the level of membrane transporters. Major PAH transporters in the mammalian kidney have recently been cloned and characterized, and include Oat1 and Oat3 proteins localized to the basolateral membrane (BLM) of proximal tubule (PT) cells. The interaction between NTM and Oat1 and Oat3, that inhibits PAH secretion, has not been clarified. The aim of this study was to establish if cadmium (Cd), mercury (Hg), and cisplatin (cisPt) affected the expression of PAH transporters Oat1 and Oat3 at the protein and mRNA levels. We compared the expression of these transporters in vehicle-treated male rats (control) with that in rats treated with $CdCl_2$ (2 mg Cd/kg b.m., s.c., daily for 2 weeks), $HgCl_2$ (a single dose of 1 mg Hg/kg b.m., s.c., daily for 2 days) or cisPt (a single dose of 5 mg cisPt/kg b.m., i.p., 5 days before sacrifice). The methods applied were immunobloting (IB) in isolated total cell membranes (TCM) from the kidney cortex, immunocytochemistry (IC) in frozen kidney tissue sections, and RT-PCR with renal cortical tissue mRNA. IB and IC studies were performed using specific polyclonal antibodies, whereas RT-PCR was performed using commercial mRNA probes for specific transporters. In IB studies, the abundance of Oat1 and Oat3 proteins in TCM from the cortex of NTM-treated rats significantly decreased (Cd>Hg>cisPt). In IC studies, both transporters were localized to the PT BLM in control animals, exhibiting different intensities in specific PT segments (Oat1: S2>S3, negative S1; Oat3: S1=S2, negative S3). In the NTM-treated rats, the staining intensity for both transporters clearly diminished in the sequence Cd>Hg>cisPt. In addition, heavily damaged cells and loss of BLM invaginations were observed in S1 and S2 of Cd-treated rats, and in S3 of Hg- and cisPt-treated rats. In RT-PCR studies, the expression of mRNA for both transporters was downregulated strongly in Cd- and Hg-treated, and weakly in cisPt-treated rats. To conclude, previously observed inhibition of the renal PAH secretion/transport in NTM-treated animals may result from: a) loss of the PT BLM (transporting membrane surface), and b) loss of Oat1 and Oat3 protein (transporting proteins for PAH) in the PT BLM.

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Abstract

# P2.20 Evelopmental patterns of KI-67, bcl-2 and caspase-3 proteins expression in the human spinal ganglia

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**Aims**: To study the distribution of the Ki-67, bcl-2 and caspase-3 proteins in tissues of human conceptuses in order to elucidate the role of those factors during development of the spinal ganglia. **Materials and Methods:** Tissues of 10 human embryos and fetuses between 5 and 9 gestational weeks were analysed immunohistochemically using paraffin sections (7$\mu$m thick) with permission of the Ethical Committee of the University Hospital of Split. Primary antibodies to Ki-67, bcl-2 and caspase-3 proteins were visualised using DAB reaction product. Statistical methods ANOVA and Mann-Whitney test were used. **Results**: All mitotic cells positive to Ki-67, display brown-stained nuclei. Ki-67 proliferation marker had the strongest expression in the 5th developmental week (58% of positive cells). Dorsal part of spinal ganglia had a significantly higher proliferation rate in 5th and in 8th week of gestation (Mann-Whitney, $p=0.003$ and $p=0.043$ respectively). During the 6th developmental week, a significant drop in the number of mitotic cell was seen in both parts of the ganglion, as well as at the end of 7th week, and at the beginning of the 9th week. Cells positive to caspase-3 are characterized by dark staining of nuclei or nuclear fragmentations. In the 5th to 7th developmental week, rare caspase-3 positive cells form 4 to 6% of ganglion cell population. During the 7th week, significant increase in the number of apoptotic cells appears particularly in the ventral part of ganglia, reaching the level of 12% at the end of the 7th week (Mann-Whitney, $p<0.05$). A small drop in the number of apoptotic cell was observed at the beginning of the 9th week. Bcl-2 positive cells have a brown-stained cytoplasm. During the 6th and 7th developmental week, number of bcl-2 positive cells increases in population of ganglion cells, reaching the level of 28%. Slight decrease of bcl-2 positive cells appeared during the end of the 7th week. Afterwards, their number rapidly increased at the end of the 8 week reaching the level of 40%. During the 8th and 9th week, significant increase in number of apoptotic cells appeared in the ventral part of the ganglion (Mann-Whitney, $p<0.0001$ and $p=0.018$ respectively). **Conclusions**: Proliferation (Ki-67), apoptotic (caspase-3) and anti-apoptotic (bcl-2) factors appear in spinal ganglia simultaneously even at early developmental stages. Their expression changes in a temporally and spatially restricted manner, thus enabling morphogenesis and differentiation of cells of the spinal ganglia. Changes in that pattern might lead to disturbances of ganglia formation and function.

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Abstract

# P2.21a G-protein-coupled – ketoglutarate (GPR99) are α receptors for succinate (GPR91) and differently localized in the human and mouse nephron

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It has been suggested that the G-protein-coupled receptors GPR91 (receptor for succinate) and GPR99 (receptor for α-ketoglutarate), that are localized in the mammalian nephron, may sense local levels of succinate and α-ketoglutarate, respectively, and then function as controlers of blood pressure via affecting the activity of renin-angiotensin system. In the mouse kidney, mRNA for these receptors was highly expressed and localized to the proximal (GPR91) and distal (GPR99) tubules, but the exact localization of receptor proteins along the mammalian nephron is not known. In order to screen localization of these receptors in the human kidney, in this study we used several commercial polyclonal antibodies against the peptide sequences specific for the human GPR91 and GPR99 and tested their efficiency in detecting the respective proteins by Western blotting (WB) of total cell membranes isolated from the tissue homogenates, and by immunofluorescence cytochemistry (IC) in tissue cryosections. In WB studies, the optimal antibodies from both groups labeled a single protein band of ~90 kDa (reducing conditions at 37 °C) that dropped to ~50 kDa at higher heating temperatures (reducing conditions at 65–95 °C). In IC studies, the optimal GPR91-antibody stained: a) the basolateral domain of thick ascending limbs of Henle, which were distinguished from the high affinity $Na^+$-glucose cotransporter (SGLT1)-positive proximal tubule S3 segments, and b) intercalated cells in the inner medullary collecting duct, where it colocalized with the vacuolar $H^+$-ATPase. The GPR99-antibodies stained only the brush-border of cortical proximal tubules. In conclusion, GPR91 and GPR99 proteins exhibit specific localization in the human nephron which is different from that in the mouse nephron, and thus indicate possible different, species-specific function(s) of these receptors.

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Abstract

# P2.22 Protective effect of cAMP on liver damage by xenobiotics

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**Introduction**. Previously we have shown that inflammatory cytokines (IL-1a/ß and IL-6) have hepatoprotective effect if given to mice before administration of acetaminophen (APAP) or D-Galactozamine + LPS and that this effect is partially mediated by $PGE_2$. Since many inflammatory cytokines stimulate the synthesis of cAMP as well as $PGE_2$, we investigated the effect of agonists, antagonists and inhibitors of cAMP degradation on APAP toxicity. **Matherials and Methods.** APAP (300 mg/kg) was administered by gastric lavage to mice which were given i.p., 1-2 hours before or up to 3 hours after a stable agonist (dibutyryl-cAMP – Db-cAMP) or antagonist (2, 3-dideoxyadenosine – DDA) of cAMP or inhibitor of phoshodiesterase IV (Rolipram). The survival of mice was followed for 72 hours and level of serum aminotransferases (AST and ALT) were determined 18-24 hours after administration of APAP. **Results**. Db-cAMP (25 mg/kg) as well as Rolipram (8 mg/kg) significantly increased the survival of mice and reduced AST and ALT serum concentration if given 1-2 hours before or up to 2 hours after AAP administration. On the contrary, DDA (160 $\mu$g/kg) decreased the survival of mice and increased serum aminotransferase concentration if given 2 hours before or ½ hours after APAP, but only effect on aminotransferase level was statistically significant. The treatment of animals with APAP greatly decreased the level of cAMP (20 or more times) in liver in vivo. IL-1ß, which was previously shown to have protective effect on APAP toxicity, if given before APAP, greatly increased the synthesis of cAMP in liver in vivo in comparison to saline control (to approximately 2/3 of level in normal mice), and this effect could be blocked almost completely with DDA, given after IL-1a but before APAP. Similar results with Db-cAMP were obtained in mice intoxicated with D-galactosamine + lipopolysaccharide. Presently, we are investigating signaling ways of this protective effect, using more specific inhibitors of cAMP or its downstream intracellular mediators. Specially, we are investigating the role of NF-$\kappa$B and protein kinase A (PKA) in protective action of cAMP. **Conclusions**. cAMP has a significant protection on liver toxicity induced by xenobiotics.

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Abstract

# P2.23 Tumor associated glycoprotein-72 (Tag-72) orients the immune response *via* CD1a$^+$ dendritic cells

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**Problem**: We evaluate whether Tumor Associated Glycoprotein-72 (TAG-72), which is also physiologically present in decidual glandular cells can affect dendritic cell (DC) functions and orientate cytokine production in T cells. Methods of study: The first trimester human pregnancy decidua was used for immunohistology studies and the isolation of decidual mononuclear cells (DMC) by enzymatic digestion and gradient density centrifugation. The phenotype and cytokine production in decidual CD1a$^+$ DC was assesed by flow cytometry in DMC cultured in the medium only or in the presence of TAG-72 (200 U/mL) for 18 hours. Decidual CD1a$^+$ cells magneticaly separated (purity ~40%) from the adherent DMC fraction were untreated or treated with TAG-72, and co-cultured with CD6 purified cord blood (CB, purity >98%) or decidual T cells (purity ~70%) for 3 or 6 days. Polarization of T cells was measured by analysing interleukin (IL)-4, interferon-gamma (IFN-γ) and CD45RA expression. **Results**: CD1a$^+$ cells are spreaded all over the stroma of decidual tissue and they are found in intimate contact with glandular epithelial cells. TAG-72 decreased maturation markers expression on CD1a$^+$ DC, as well as the production of IL-15 and IFN-γ. Interleukin-10 was enhanced, wheres IL-18 was unaffected in CD1a$^+$ DC pretreated with TAG-72. Decidual CD1a$^+$ cells enhance IL-4, whereas did not affect IFN-γ production in CB T cells after 3 and 6 days co-culture at DC/T cell ratio 1:5. TAG-72 treated DC decreased IFN-γ in CB T cells and sustain IL-4 production in LPS treated CB T cells after 3 days of the co-culture. Concomitant decrease of CD45RA expression on the surface of CB CD3+ cells was noticed. TAG-72 treated CD1a$^+$ cells enhance IL-4 and decreased IFN-γ in decidual T cells. **Conclusions**: TAG-72 inhibits the maturation and induce anti-inflammatory properties of decidual CD1a$^+$ DC, that support Th2 orientation of the immune response. *Acknowledgement:* The experiments are partially financed by the grants of »EMBIC» European FP6 project No. 512040, LSHM-CT-2004-512040, as well as Croatian Ministry of Science, Education and Sports No. 0376 and No. 0377.

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# P2.24 NHE3 is a novel member of the CaMKII binding proteins

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**Introduction**: $Na^+/H^+$ exchanger isoform 3 (NHE3) in the brush border of mammalian small intestine and colon play a major role in intestinal $Na^+$ absorption. We previously showed that $Ca^{2+}$/calmodulin and $Ca^{2+}$/calmodulin dependent protein kinase II (CaMKII) were involved in regulation of the ileal neutral NaCl absorptive process and its component NHE3. We now investigate the relationship between CaMKII and NHE3 at a molecular level using PS120 cell fibroblasts expressing NHE3. **Materials and Methods:** Stably transfected PS120 fibroblasts expressing rabbit NHE3 wild type or its truncations mutants with +/- NHERF2 protein were used. NHE3 truncation mutants, NHE3/585, /605, /640, /660 and /690 (the final number in the name indicates the amino acid number at the truncation site) were constructed and expressed in the NHE-deficient PS120 cells. $Na^+/H^+$ exchange activity was measured by using the pH-sensitive fluorescent dye BCECF and a computerized fluorometer. Cells were incubated with -/+KN-62, a CaMKII inhibitor, during the dye loading and $Na^+/H^+$ exchange activity was determined after addition of $Na^+$ media. Co-immunoprecipitation studies: PS120 cells stably expressing full-length NHE3 or its truncation mutants were co-immunoprecipitated with anti-CaMKII antibody and subjected to immunoblot analysis using anti-VSVG antibody. »Pull-down« assays were used to determine which domain of NHE3 physically binds CaMKII. Four $His_6$-tag fusion proteins bound to Ni-NTA beads were constructed: $His_6$-F1(aa 475-581), -F2 (aa 582-667), -F3 (aa 668-744), and -F4 (aa 745-832) and incubated with the recombinant a-CaMKII. Protein bands were visualized by immunoblotting using anti a-CaMKII antibody. An *in vitro* back-phosphorylation assay was employed to assess CaMKII-mediated phosphorylation of NHE3. **Results**: CaMKII inhibits NHE3 under basal conditions and this inhibition is mediated by NHERF2, since a CaMKII inhibitor, KN-62, stimulated basal NHE3 activity in the presence of NHERF2 only. Back phosphorylation studies showed that CaMKII activity was associated with increased phosphorylation of NHE3 under basal conditions, an effect decreased by KN-62 treatment. *In vivo* co-immunoprecipitation demonstrated that CaMKII binds NHE3 in intact cells under basal $Ca^{2+}$ conditions in a $Ca^{2+}$ independent manner. The domains of NHE3 that bound CaMKII were further defined using *in vivo* co-immunoprecipitation studies of NHE3 truncated mutants. These studies showed that CaMKII binds to the region of NHE3 between aa 585 to 605. *In vitro* »pull down« assays using His-tagged fusion proteins containing different parts of the NHE3 cytoplasmic domain demonstrated that CaMKII bound NHE3 directly and only at the F2 domain (aa 582-667) of the NHE3 C-terminus. The absence of CaMKII activation did not affect its binding to F2 fragment suggesting that *in vivo* CaMKII might constitutively bind to NHE3. **Conclusion**: These results demonstrate that NHE3 is a novel member of the CaMKII binding proteins. CaMKII binds NHE3 under basal condition at aa 585-605 in a $Ca^{2+}$ independent manner and activation of CaMKII was not required for this binding to occur. Furthermore, NHERF2 is essential for CaMKII inhibition of NHE3 under basal conditions, however CaMKII dependent phosphorylation of NHE3 does not require the presence of NHERF2. CaMKII is part of the physiologic NHE3 regulation that occurs in fibroblasts as well as in BB of ileal Na absorptive cells.

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Abstract

# P2.25 Heat shock proteins and metallothionein expression in tissues of marine mussel Mytilus galloprovincialis as sensors of environmental pollution

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**Introduction**: Exposure of cells to wide variety of stressors, such as heat, heavy metals, organic poison, injuries, hypoxia etc alters the tertiary structure of proteins and leads in cells to general stress response, which consists in transient enhancement of the expression of cytoprotective proteins- heat shock proteins (HSP) and metallothioneins (MT), which act as molecular chaperons or as regulators of heavy-metal homeostasis, respectively. Since both families, as evolutionary conserved proteins are distributed also in animals, in this study the expression of HPS 70 and MTs I/II have been evaluated in tissues of marine mussel Mytilus galloprovincialis collected along the coast of the Kvarnerian bay in the zones, which are differently exposed to environmental pollution (industrial, urban, peri-urban and turistic area), as well as in mussels exposed to heat or to CdCl2 and ZnCl2 *in vitro*. **Material and Methods:** The level of HSP 70 and MTs expression were detected in mussel gills and the digestive gland, using Western blot analysis and immunohistochemistry. **Results**: The data have shown that there are significant differences in HSP 70 and MTs expression in mussels collected at zones with different pollution, as well as that they differently react on stressors (heat shock and toxic metals) used *in vitro*. **Conclusion**: The data suggests that HPS and MTs may be useful biomarkers of environmental pollutions.

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Abstract

# P2.26 Metallothioneins as regulators of liver regeneration and fetal organogenesis

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**Introduction**: Metallothioneins (MTs), the intracellular, ubiquitous, low molecular cysteine rich proteins are stress-proteins, which participate in cell reactions on different kinds of injuries, as well as in host defense responses, affecting the functions of several immune cells, TLR signaling, expression of MHC proteins and co-stimulatory molecules on dendritic cells and the production and release of pro-inflammatory cytokines. Owing to this the induction MTs dramatically increases in response to tissue injury, infection, inflammation and neoplastic disease. It is however, of particular interest that both nuclear and cytoplasmic localization of MTs might be transiently expressed also in several growing tissues and during fetal development, implying that they might participate in DNA synthesis-and apoptosis-related processes, in regulation of transition from proliferation to the differentiation stage, as well as in several immune-related processes that initiate and coordinate the normal growth. In an attempt to underline the physiological functions of MTs in this study we analyzed the tissue distribution of MT I+II after partial hepatectomy (pHx), as well as in syngeneic pregnancy, looking for the changes in the liver, at the fetoplacental unit and in several fetal tissues. Material and methods: Experiments were done on C57/BL6 mice, which were subjected to 1/3 pHx or matted with syngeneic partner. Mice were sacrified in early postoperative period (1, 2, 6, 12 and 24 hours) after one-third hepatectomy (pHx), or on 16th day of syngeneic pregnancy. MT I/II expression was determined immunohistochemicaly, using monoclonal anti-MT I+II antibody and DAKO EnVision System. Additionally, in the regenerating liver MT-I mRNA and apoptosis were analyzed by RT-PCR and by TUNEL method, respectively. **Results**: The data have shown that fast liver growth after pHx is followed by marked increases of MT proteins and MT-I mRNA in the regenerating liver, where areas expressing cytoplasmic and nuclear MT immunoreactivity inversely correlated with those containing apoptotic cells. Similar over expression of MT I/II was found in maternal and fetal liver, as well as on the fetoplacental unit, affecting syncytiotrophoblast, cytotrophoblast, stromal and macrophages-like cells within the core of the chorionic vili and some decidual cells. Additionally, a high cytoplasmic and nuclear MT I/II imunoreactivity was noticed in fetal tissues, such as intestine, pancreas, gallbladder, kidneys and skin. **Conclusion**: The data underline the growth-related properties of MTs, implying that as metal-donors or metal-acceptors they alter the functional state of metal-dependent proteins, such as zinc finger domain-containing transcription factors, DNA synthesis enzymes and signal transduction molecules, regulating cell proliferation and apoptosis, facilitating also the flow of vital nutrient materials to the embryos and providing a barrier to entry of toxic materials from the mother at the fetoplacental unit.

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Abstract

# P2.27 Lipopolysaccharide injection suppresses osteoblastogenesis but stimulates osteoclastogenesis from mouse bone marrow cells

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**Introduction**. Lipopolysaccharide (LPS) from gram-negative bacteria may cause chronic inflammation and subsequent bone loss, and has been involved in the pathogenesis of several bacterially induced bone disseases like peridontitis, osteomyelitis and bacterial arthritis. LPS strongly stimulates osteoclast formation and induces production of many local pro-inflammatory factors, such as tumor necrosis factor a, interleukin-1, prostaglandin $E_2$. The aim of our study is to investigate the effects of LPS on bone cell differentiation in the bone marrow microinvironment. **Materials and Methods.** C57BL/6 mice were injected intraperitonealy once a week during 4 weeks in a dose of 5, 10 and 20 $\mu$g LPS/g. After 5 weeks, spleenocytes from these mice were stimulated by mitogen. Concanavalin A (ConA; 10$\mu$g/mL) or LPS (25$\mu$g/mL) for 4 days in culture followd by MTT cell proliferation assay. Bone marrow cells were cultured under conditions stimulating for osteoblastogenic differentiation (ascorbic acid, dexamethasone and ß-glycerolphosphate) or osteoclastogenic differentiation (receptor activator of nuclear factor-κB ligand and macrophage colony-stimulating factor) cultures. Osteoclast-like cells were identified as tartarat-resistant acid phosphatase (TRAP)-positive multinucleated cells. Osteoblast colonies were detected as alkaline phosphatase-positive colony-forming units. **Results**. There was a dose-dependent stimulation in cell proliferation after LPS injection, which increased further upon restimulation *in vitro* by ConA and LPS. LPS-stimulation significantly suppressed osteoblast differentiation from bone marrow cells after (39.3±2.1 of ALP-positive colonies in mice injected with 20 $\mu$g LPS/g mice vs 76.7±10.8 in control mice, p=0.009, t-test). At the same time the number of TRAP-positive osteoclast-like cells was increased in LPS-injected mice compared with the controls (22.6± 6.2 of TRAP-positive osteoclasts in mice injected with 20 $\mu$g LPS/g mice vs 3.2±0.75 in control mice, p= 0.001, t-test). **Conclusion**. Our preliminary results indicate that LPS-injection *in vivo* induced increase in osteoclast differentiation and inhibition in osteoblast differentiation from bone marrow cells *ex vivo*. Our further aim is to investigate intracellular mechanisms by which LPS affects bone cell differentiation and activity.

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Abstract

# P2.28 Comparison of thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) as biomarkers of oxidative stress in rat (*Ratus norvegicus*) and common carp (*Cyprinus carpio*)

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Oxidative stress can be defined as an imbalance between oxidative-reductive processes due to overproduction of free oxygen radicals that cell homeostatic mechanisms can not neutralize, which leads to harmful biochemical and physiological alterations in cells. Two biochemical alterations resulting from oxidative stress are lipid peroxidation and protein carbonyl formation. There are many conditions that can cause increased free radical production and induce oxidative stress, for example toxic substance exposure, smoking, alcohol consumption, as well as exercise at high intensity. The aim of this work was to evaluate the effects of intensive exercise on concentration of thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) concentration as biomarkers of oxidative stress, in rat (*Ratus norvegicus*) and common carp (*Cyprinus carpio*). For that purpose, a total of 20 male rats were randomly divided into two groups. Group A was the control group (n=10) and did not perform exercise and group B was the experimental group (n=10) that was exposed to intensive exercise on treadmill over a two week period (120 min/day at 1.6 km/h for 5 days/wk). One group of rats was sacrificed after first week (5 rats from both groups) and another group (residual rats) was sacrificed at the end of the experiment, liver was collected from each animal for estimation of TBARS and PC concentrations. Fish were also divided into two groups. Group A was the control group (n=14) and did not perform exercise and group B was the experimental group (n=14) that was exposed to intensive exercise (8 h/day over a 6 days) in 150L pool in which a system for water mixing was constructed (water flow was 15 cm/s). One group of fish was sacrificed after three days (7 fish from both groups) and another group (residual fish) was sacrificed at the end of the experiment, liver was collected from each carp for estimation of TBARS and PC concentrations. The results showed that the acute intensive exercise induced significant increase in rat liver TBARS concentrations after one week in the B group, yet after two week period liver TBARS concentrations were lower in this group of rats. The PC levels after first week of experiment were not significantly increased in the B group of rats, but after two week period a significant increase in the PC levels was observed. The resembling results were obtained with the carp experiment. Significant increase of TBARS levels in carp were obtained after three days of exposure to intensive exercise, although these levels already started to decrease after six days. Significant increase in PC level in carp that were exposed to intensive exercise were obtained after six days of exposure. The levels of TBARS after oxidative stress caused by increased free radical production after intensive exercise increased much earlier than the PC concentrations levels, although the PC levels maintained increased much longer than the TBARS levels. That should be taken into account when using these two biomarkers in the environmental biomonitoring.

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Abstract

# P2.29 The relationship of perfusion pressure and reversibile/irreversibile microcirculation changes in sepsis

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Perfusion pressure is crucial for the tissue perfusion, oxygenation and elimination of metabolites in normal tissue. In septic patients it may be altered by several mechanisms. Endothelial lesions and impaired vasoregulation resulting from bacteriemia may produce vasodilatation, hypotension and tissue hypoxia. Endothelial lesions and hypotension decrease blood velocity. These events may favor disseminated intravascular coagulation in septic patients, and thus pronounce perfusion maldistribution. Since hypotension is commonly treated by vasoactive drugs to increase vascular tone toward normal values, more pronounced peripheral tissue ischemia may result. Until now, there are no recommendations on critical mean arterial pressure (MAP) that should be maintained in septic patients, since diversity of physiological parameters should be encountered, i.e. age, body weight, core temperature, overall patients' cardiovascular performance, anemia, and protein status. Several studies evaluated the MAP value necessary for tissue perfusion with controversial results. One human clinical study conducted on 10 medical patients with sepsis (1) has proved MAP value of 65 mmHg as safe in the terms of regional tissue perfusion, oxygen delivery and consumption, diuresis and cardiac performance in one hour period. This study suggests little benefit in increasing MAP over 65 mmHg, but did not confirm how long such hypotension may persist. Together with specific antibiotics, numerous therapeutic procedures like normovolemic haemodilution, use of vasoconstrictors, vasopressin and its analogue terlipressin, high doses of corticosteroids or inhalation of NO are currently used to improve outcome of hypotensive septic patients. Numerous preclinical and clinical studies were undertaken to point biochemical tests and their values requiring prompt intervention to improve outcome. The values of arterial lactate, cortisol response, TNF, interleukine (IL) 6, IL-12p70 and IL-12p40 production, together with submucosal (gastric intramucosal or sublingual) $CO_2$ values may be useful. These may indicate whether microcirculatory impairment is reversible or not, and which therapeutic maneuver should be appropriate.

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Abstract

# P2.30 $Na^+$-glucose co-transporter SGLT2 in rat kidney

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A bulk of D-glucose reabsorption in the mammalian kidney (K) is mediated by the low affinity, high capacity $Na^+$-glucose co-transporter SGLT2. In various transport and hybridization studies, this transporter has been localized in the cells of proximal tubule segments S1 and S2. The transporter exhibits the 1:1 $Na^+$:glucose coupling ratio, and unlike SGLT1, a high affinity $Na^+$-glucose cotransporter localized mainly to the proximal tubule S3 segments, it does not transport D-galactose. cDNA for SGLT2 has been identified first in humans and later in rats and mice. The expression of its mRNA has been detected in most human tissues, the highest levels being present in kidney. A convincing immunolocalization of SGLT2 along the mammalian nephron has not been reported. In this work we generated a novel polyclonal antibody (Ab) specific for peptide sequence in the rat SGTL2 protein, and performed detailed Western blotting (WB) and immunocytochemical (IC) studies of the expression of SGLT2 protein along the nephron in variously-treated adult (3 months) and prepubertal (3 weeks) male (M) and female (F) rats. WB experiments were performed with brush-border (BBM) and total cell membranes isolated from tissue homogenates, whereas the IC studies were performed in tissue cryosections. In addition, the expression of SGLT2 mRNA was determined by RT-PCR using RNA isolated from various tissue zones. In WB of isolated membranes, the Ab labeled a single protein band at ~75 kDa. The protein band and the immunostaining was absent in isolated membranes and tissue cryosections, respectively, from the rat small intestine, indicating a specific localization of SGLT2 in the K. In membranes from the K cortex (C) and outer stripe (OS) of intact adult rats, the density of 75 kDa protein band: a) exhibited strong zonal (C>OS) and gender differences (F>M), and b) increased weakly by castration and remained unaffected by ovariectomy. In castrated rats, the protein band was downregulated by testosterone and upregulated by estradiol treatment. By IC, the Ab stained BBM of S1 and S2 (S1>S2) with gender-dependent manner (F>M). The staining intensity in gonadectomized animals, and in sex hormone-treated castrated M matched the pattern of WB data. In prepubertal rats, the respective protein band in isolated K membranes and the staining intensity in PT were weaker than in adult animals, and gender-independent. The expression of SGLT2 mRNA was found to be high in the C and low in the OS, but gender-independent. Our data indicate that in the rat K: 1) SGLT2 is localized to the BBM of proximal tubule S1 and S2 segments (S1>S2) in the C, 2) at the protein level, SGLT2 expression exhibits zonal (C>OS) and gender differences (F>M) that occur after puberty, and 3) at the mRNA level, the expression of SGLT2 exhibits no gender differences, thus indicating the involvement of posttranslational mechanisms in sex hormone-regulated expression of SGLT2 protein.

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Abstract

# P2.31 The changes of serum enzyme activity as an indicator of injuries in irradiated chickens

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**Introduction**: Many authors have reported that organic lesions and metabolic disorders of many organs, especially the liver, are followed by changes of some enzyme activities in blood plasma of domestic animals and poultry. It is also known that the liver in birds is equally affected by radiation as the intestine, bone-marrow and sex glands, which is different in mammals. In our previous investigation we showed that the activities of some enzymes in blood plasma of chickens are significantly changed after a single parenteral injection of $^{32}$P. In this paper an attempt was made to investigate the influence of gamma ray irradiation of whole body of chickens upon activity of several enzyme (enzymatic profile) in blood plasma. We also wanted to evaluate whether investigation of the enzymatic profile in blood plasma can help in the diagnosis of organic or functional liver damages caused by gamma ray in the chickens. **Materials and Methods:** The experiments were performed on hybrid chickens of heavy Jata breeds of booth sexes. Chickens were irradiated by gamma ray in the dose of 7,23±0.95 Gy. Blood samples were taken from the wing vein on days 1, 3, 5, 7, 9 and 15 after irradiation.The activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), leucine aminopeptidase (LAP), lactate dehydrogenase (LDH) and alpha-hydroxy-butyrate dehydrogenase (HBDH) in blood plasma of irradiated chicken was determined spectrophotometrically by using Boehringer Mannheim GmbH optimized kits. At the end of the experiment all birds were sacrificed and the organs were pathomorphologically and histologically investigated. **Results:** Throughout the experimental period, only ALT and GGT activity did not statistically significantly change. All other enzymes activity showed a decreased tendency during the experiment; statistically significant decreases were recorded as follows: on the 1st day of the experiment – LAP and LDH activity; on the 3rd day – LAP activity; on the 5th day – LDH activity; on the 7th day – AST, LDH and HBDH activity, on the 9th day – LAP and HBDH activity, and on the 15th day – LAP activity. **Conclusion**: The determination of a so-called enzyme profile in blood plasma which include measurement AST, LAP, LDH and HBDH activities may serve as an additional test for functional liver damages in chickens caused by ionizing radiation before the appearance of clinical simptoms.

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Abstract

# P2.32 Antioxsidant status in chicken liver after exposure to low dose gamma radiation

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**Introduction**: Although radiation is a known producer of reactive oxygene species in living systems it has been shown that acute low dose ionizing irradiation induced various antioxidant mechanisms, enzymatic and non-enzymatic, that defend cells from oxidative stress. It is also shown in increased level of antioxsidant substances such as superoxide dismutase, catalase, glutathione peroxidase and glutathione in different organs after low dose irradiation. However, newly studies of low dose radiation in chickens were performed mostly on hatchability, body weight and egg fertility in commercial meat chicken line. Whereas, there are lack of results on antioxsidant status in chicken embryo and chicks hatched from irradiated eggs the aim of this study was to investigate low dose radiation effects upon antioxidant status in chicken liver after low dose gamma irradiation. **Materials and methods:** The eggs of a heavy breeding chickens COBB 500 were irradiated with the dose of 0,3 Gy gamma radiation (source $^{60}$Co) on the 19th day of incubation. Liver samples were taken on 3, 6, 24, 48, 72 and 96 h after irradiation. The thiobarbituric acid reactive substances (TBARS), glutathione levels (GSH) and activity of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were measured spectrophotometrically. **Results**: The GSH levels increased in the liver of chickens hatched from irradiated eggs at 6 hours after irradiation and decreased 48 h after irradiation. Antioxsidant enzyme activities for GSH-Px and SOD significantly decreased in the liver of chickens hatched from irradiated eggs 48 h after exposure to the dose of 0.3 Gy. TBARS levels and CAT activity during the experiment were not statistically different when they compared to controls. **Conclusion**: Obtained results indicated that acute irradiation of chicken eggs on the 19th day of incubation with the dose of 0.3 Gy gamma radiation could have a different effects on antioxidant parameters in embryo liver of chicken, i.e. the enzyme activity of SOD and GSH-Px were decreased, however, catalase and TBARS were not affected by the irradiation. The level of glutathione was firstly increased and after that it decreased. These findings suggest that glutathione is an important antioxidant in the protection of liver cells against ionizing radiation and that this dose does not stimulate antioxidants enzymes in the same tissue.

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Abstract

# P2.33 Hematological and biochemical values of ecologically bred Cres sheep

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**Introduction**: Ecological breeding of sheep should be based on indigenous breeds, which are already well adapted to their environment. This practice eliminates most of the problems usually encountered when imported foreign breeds have to be adjusted to new conditions. Systematic monitoring of health and productivity of the animals helps to detect problems before they develop in earnest and incur economical losses. Hematological and biochemical analyses are accepted methods for such monitoring. In order to apply them to Cres sheep ecologically bred in Croatia, normal physiological values of key hematological and biochemical parameters have been determined in this study. This was necessary because some of these parameters are specific for each breed and are heavily influenced by the conditions in which the animals are kept, particularly the relatively harsh conditions of ecological breeding on Cres. Therefore, values found in general literature are of little value, except as rough guidelines. Specific values for Croatian indigenous breeds in their native environment have not been determined previously. **Materials and Methods:** The test group comprised 30 clinically healthy Cres sheep, aged one to six years. Their food intake was limited to free grazing. As the control group we used 20 clinically healthy Merinolandschaf sheep, kept on a farm situated on Žumberačka gora, at the elevation of 450 m. This group was fed by free grazing and additionally with hay and grain. Blood samples were taken in December. Samples were treated with EDTA and kept at 4°C until analyzed. Beckman Coulter ACT diff Hematology Analyzer instrument was used for most of the hematological measurements; differential blood count was determined by manually counting May-Grünwald-Giemsa stained smears. Biochemical parameters were determined by standard laboratory tests. Statistical significance of differences between blood parameters of Cres and Merinolandschaf sheep was determined by the t-test. **Results**: Difference between erythrocyte, leukocyte and thrombocyte count, hemoglobin concentration, MCV, MCH and neutrophil count in Cres and Merinolandschaf sheep was statistically significant to the level of $p<0.01$. Differences in hematocrit were significant to the level of $p<0.05$, while differences in lymphocyte, monocyte, basophyl and eosinophyl count and MCHC were not significant. Differences in biochemical values – phosphate, urea, creatine level and AST activity were significant to the level of $p<0.01$; calcium, glucose and GGT to $p<0.05$. Magnesium, total protein and albumin levels were not significantly different. **Conclusion**: The obtained results demonstrate specificity of Cres sheep and provide a solid basis for referent physiological values. They also point to the necessity of standardizing parameters of other indigenous Croatian breeds, which could prove beneficial to the development of ecological breeding.

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Abstract

# P2.34 Hematologic and biochemical parameters of ostriches after vaccination against Newcastle disease

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**Introduction**: Newcastle disease is one of the most important diseases of poultry and other avian species. The usual means for controlling the disease is specific immunoprophylaxis. Although chickens are routinely vaccinated against Newcastle disease, vaccination of ostrich is less well understood. To assess the effect of vaccination on the health of ostriches, key biochemical parameters and differential blood count were monitored after vaccination by La Sota strain of the Newcastle disease virus, which is widely used in chickens. **Materials and Methods:** The investigation was performed in 24 adult ostriches weighing approximately 120 kg, hybrids between blue-neck and African black ostriches. The ostriches were divided into four groups, three study groups and a control group. Each group comprised six ostriches divided into two families. In the three study groups, animals were vaccinated by different routes: drinking water, oculo-nasaly or by spraying. Blood samples for haematology tests were collected from the wing vein into test tubes with potassium citrate immediately before the vaccination and on days 7, 14, 21 and 28. In the collected blood, total erythrocyte counts, haemoglobin concentrations, hematocrit values, as well as total and differential leukocyte counts were assessed. **Results**: Erythrocyte count, haemoglobin concentration, hematocrit values and total leukocyte count were not significantly changed in any trial group over the whole investigation period. Only leukocyte differentiation yielded a significant decrease in eosinophil percentage in all groups and a significant monocyte increase in the groups that received the vaccine in drinking water and oculo-nasally. While the lower eosinophil count could be attributed to the stress which the animals experienced as a result of experimental procedures and blood sampling, increased monocyte percentage indicates successful immunological reaction against a pathogen, in this case the virus used as the vaccine. **Conclusion**: The results represent the normal response to vaccination and do not indicate any adverse health effects. Therefore, the vaccine which is already routinely used for chickens can be safely applied in ostrich.

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Abstract

# P2.35 The role of blood meal in the life of hematophagous horse flies (diptera: tabanidae)

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Females of most horse flies species require a blood meal to complete egg development, because many species play an important role in spreading diseases of livestock and other animals. Also, some species are medically important to humans. Mainly, females of horse flies can lay eggs only after taking a blood meal. However, this is not always true. During 1960's the autogeny in horse flies of the genus *Chrysops* was established for the first time. After this report many research works have been performed on development cycles of Nearctic and Palaearctic species of horse flies. Several authors have found that many species occur autogenously in the first gonotrophic cycle throughout the world. In our research on the material collected from 1995 we have tried to determine whether one from the most abundant species *Tabanus bromius* L., 1758 and *Tabanus sudeticus* Zeller, 1842 in Eastern Croatia is autogeneous or anautogeneous. Analysis was performed on the ovarioles in the ovaries of 25 females of the species *T. bromius* and *T. sudeticus*. The follicles of the ovarioles of both species were mostly in stage I and II of the maturation, proving that the blood meal is essential for the egg maturation. Based on these results, we can conclude that the *T. bromius* and *T.sudeticus* are anautogeneosus species in the studied area. The anautogeneous species presumably have some food reserves from the larval stage deposited in terminal oocytes, which do not suffice and mainly require a blood meal for egg maturation. Unfavorable climatic conditions such as low air temperature during spring months (June 1995) also prolong the pupal stage, which causes the consumption of the nutritive substances necessary for the development of the egg follicles. Thus, climatic conditions are the reason for appearance of anautogeneous species of horse flies in the first gonotrophic cycle.

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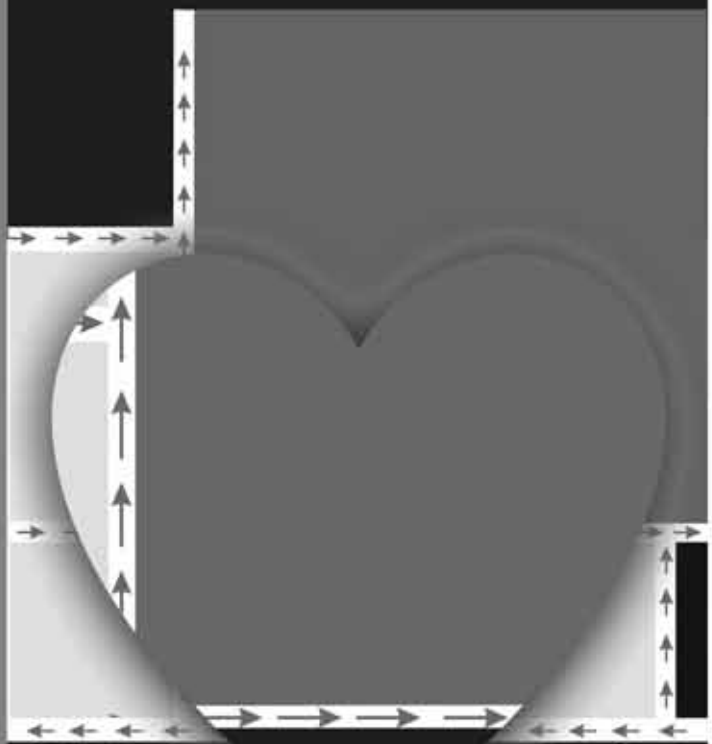

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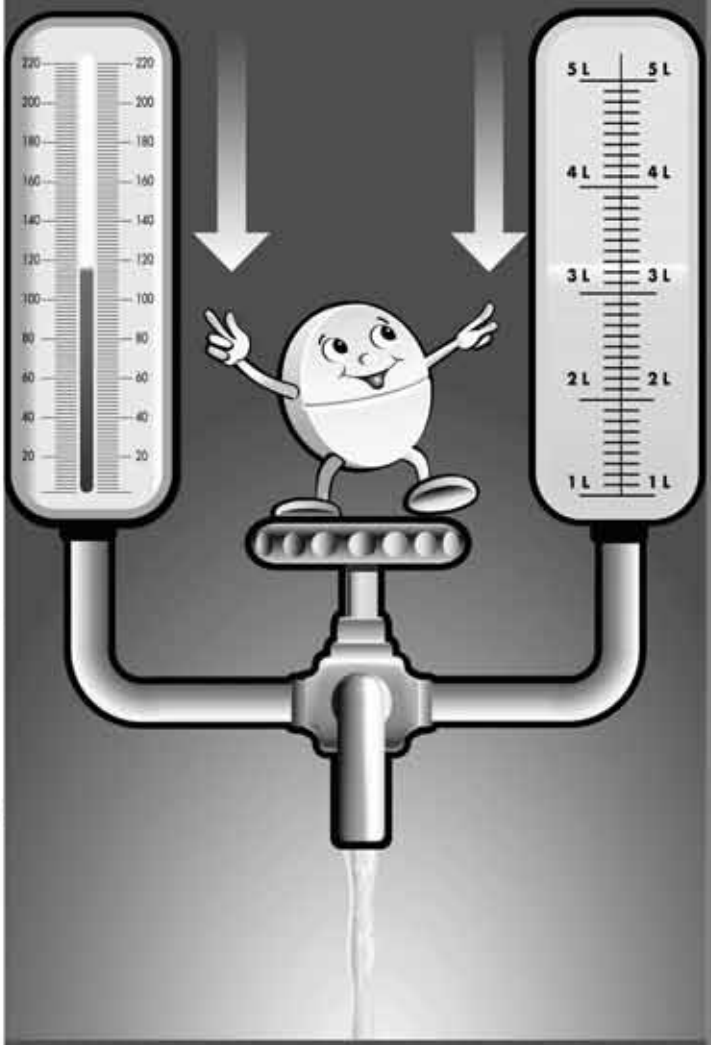


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- NovoMix® 30 osigurava postizanje ciljne vrijednosti HbA1c[1]
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