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## physiology

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“Doua tari, un scop, succes comun!”

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## Bilateral Cooperation of Doctoral Schools Timisoara-Szeged – an European Model for Setting up a Doctoral Programme in the Medical Field

University of Medicine and Pharmacy Victor Babes Timisoara,  
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### CONFERENCE PROGRAMME, 05-06.05.2011

#### THURSDAY, 05.05.2011

<b>14.00-14.15</b>	Opening ceremony
<b>PAPER SESSION I</b>	
<b>14.15-14.35</b>	<b>Prof. Dr. Adrian Neagu:</b> Biological Physics Tools for Investigating Multicellular Systems
<b>14.35-14.55</b>	<b>Prof. Dr. Ferenc Bari: Optical Methods for Studying Cerebro-Cortical Microcirculation</b>
<b>14.55-15.10</b>	PhD Stud. <b>Şerban Comşa:</b> Correlations between the Vascular Endothelial Growth Factor Expression, Microvascular Density in Tumor Tissues and the TNM Staging in Breast Cancer
<b>15.10-15.25</b>	PhD Stud. <b>Dénes Garab:</b> Beneficial Effects of Remote Limb Ischemic Preconditioning against the Microcirculatory Consequences of Partial Hepatic Ischaemia in Rats
<b>15.25-15.40</b>	PhD Stud. <b>Ştefana Feflea:</b> Evaluation of EG-VEGF Antibody Effect on Chick Embryo Chorioallantoic Membrane and Organs
<b>15.40-15.55</b>	PhD Stud. <b>László Juhász:</b> Examination of the Trigger Role of Peroxynitrite in the Antiarrhythmic Effect of Ischaemic Preconditioning and Peroxynitrite Infusion
<b>15.55-16.10</b>	PhD Stud. <b>Adina Duşe:</b> Expression and Possible Significance of Vascular Endothelial Growth Factor in Non Hodgkin Lymphoma
<b>16.10-16.40</b>	Coffee Break
<b>PAPER SESSION II</b>	
<b>16.40-17.00</b>	<b>Prof. Dr. Carmen Bunu Panaitescu:</b> Dendritic Cells Response to <i>in vitro</i> Allergen Challenge and Impact of Corticosteroids on Local Inflammation
<b>17.00-17.15</b>	PhD Stud. <b>Adrian Gruici:</b> Carotid Intima-Media Thickness and the Prediction of Clinical Cardiovascular Events in Patients with Manifest Arterial Disease
<b>17.15-17.30</b>	PhD Stud. <b>Tünde Tóké:</b> Protective Effect of L- $\alpha$ Glycerylphosphorylcholine against Ischaemia-Reperfusion Injury in the Rat Small Intestine
<b>17.30-17.45</b>	PhD Stud. <b>Laura Maruscia:</b> Specific Chemokine-Chemokine Receptors Interactions and Their Role in hMSC Migration
<b>17.45-18.00</b>	PhD Stud. <b>Gabor Kisvári:</b> Acute Administration of Simvastatin Reduces the Severity of Ventricular Arrhythmias in Anesthetized Dogs
<b>18.00-18.15</b>	PhD Stud. <b>Adelina Mavrea:</b> Ventricular Asynchronism - Prognosis Marker in Heart Failure with Preserve Ejection Fraction
<b>19.00</b>	Dinner

## FRIDAY, 06.05.2011

<b>PAPER SESSION III</b>	
<b>9.00-9.20</b>	<b>Prof. Dr. Mihály Boros: Ischemia-Reperfusion Injuries, from Laboratory Studies to Clinical Practice</b>
<b>9.20-9.35</b>	PhD Stud. <b>Simona Bota</b> : The Value of Acoustic Radiation Force Impulse Elastography (ARFI) for the Assessment of Chronic Hepatopathies
<b>9.35-9.50</b>	PhD Stud. <b>Csaba Gombár</b> : Effect of Calcaneo-Stop Procedure on Load Conditions of Paediatric Flexible Flatfoot
<b>9.50-10.05</b>	PhD Stud. <b>Bogdan Bălinișteanu</b> : Prevalence of Ki-67 in Pituitary Adenomas
<b>10.05-10.20</b>	PhD Stud. <b>Bernadett Borda</b> : Functional and Histopathological Changes in Renal Transplant Patients with New-Onset Diabetes and Dyslipidemia
<b>10.20-10.35</b>	PhD Stud. <b>Viorica Bocan</b> : Neuroendocrine Differentiation in Benign Prostate Diseases. An Autopsy Report
<b>10.35-11.05</b>	Coffee Break
<b>PAPER SESSION IV</b>	
<b>11.05-11.25</b>	<b>Assoc. Prof. Dr. Gyula Sáry: Beyond the Orbit - Cortical Mechanisms of Shape and Form Vision</b>
<b>11.25-11.40</b>	PhD Stud. <b>Nóra Szabó</b> : Epidemiology of Central Nervous System Malformations in a Region in Hungary
<b>11.40-11.55</b>	PhD Stud. <b>Diana Naiche</b> : Mesostructure. Types of Bars in Implant Overdenture
<b>11.55-12.10</b>	PhD Stud. <b>Levente Szalardy</b> : Study on the Levels of Tau and $\beta$ -amyloid in CSF of Patients with Multiple Sclerosis
<b>12.10-12.25</b>	PhD Stud. <b>Claudia Corici</b> : The Properties of the Transient Outward, Inward Rectifier and Acetylcholine Sensitive Potassium Currents in Atrial Myocytes from Dogs in Sinus Rhythm and Experimentally Induced Permanent Atrial Fibrillation
<b>12.25-12.40</b>	PhD Stud. <b>Magdolna Kósa</b> : Efficiency of Plasmid Transfection along the Regenerating Rat Soleus Muscle
<b>12.40-14.00</b>	Lunch
<b>PAPER SESSION V</b>	
<b>14.00-14.20</b>	<b>Assoc. Prof. Dr. Cristina Dehelean</b> : New Trends in Toxicological Screening Applied on Experimental Models Correlated to Tumours
<b>14.20-14.35</b>	PhD Stud. <b>Péter Szerémy</b> : Investigation of Transporter Interactions of Antimalarials In Vitro
<b>14.35-14.50</b>	PhD Stud. <b>Tuboly Eszter</b> : The Effects of Exogenous Methane Inhalation on Macro- and Microcirculatory Changes during Intestinal Ischemia-Reperfusion in Rats
<b>14.50-15.05</b>	PhD Stud. <b>Vanya Melinda</b> : Childbearing among Women with Multiple Sclerosis (MS)
<b>15.05-15.20</b>	PhD Stud. <b>Simona Trancotă</b> : Characterization of the Effects of Two Porphyrin Compounds on Rat Heart Mitochondrial Respiration
<b>15.20-15.35</b>	PhD Stud. <b>Bere Zsófia</b> : Multimodal, Live Imaging of Global Forebrain Ischemia- Induced Periinfarct-Depolarisation (PID) in the Rat Cerebral Cortex
<b>15.35-15.50</b>	PhD Stud. <b>Georgel Țăranu</b> : Ischemic Limb Salvage Using Neovascularogenic Therapy – Case Report
<b>15.50-16.05</b>	PhD Stud. <b>Emese Asztalos</b>
<b>16.05-16.15</b>	Concluding Remarks

## 1. PREVALENCE OF KI-67 IN PITUITARY ADENOMAS

**Bogdan Balinisteanu, Raluca Ceausu, Anca Maria Cimpean, Marius Raica**

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**Introduction.** Pituitary adenomas represent the third most common primary intracranial tumors in neurosurgical practice. To understand the biological behaviour of the pituitary adenomas, cell proliferation markers, such as monoclonal antibodies targeted against the Ki-67 antigen, have been applied. The Ki-67 antigen is a protein related to cell proliferation and is expressed in cell nuclei throughout the entire cell cycle. **Aim.** The aim of this study was to correlate the Ki-67 index with hormone phenotype of pituitary adenomas. **Materials and methods.** Our study included 30 cases of pituitary adenomas. Sections from each case were stained with routine haematoxylin and eosin method for histopathologic evaluation. Immunohistochemistry was performed on additional slides in order to detect specific pituitary adenomas. The percentage of Ki-67-positive nuclei (the Ki-67 Label Index) was determined by counting approximately 1000 nuclei of tumor cells at 400-fold magnification. **Results.** Ki-67 immunostaining was detected in 21 cases, with values between 0.2% and 4.8%. The highest values were noticed in 3 prolactin (PRL)-secreting adenomas (range 3.1 – 4.8%). The Ki-67 was negative in 9 cases, the majority of them being non-secretory adenomas (6 of 9). **Discussion.** Pituitary adenomas are heterogeneous in growth rate, invasiveness and recurrence. They frequently invade surrounding structures such as the cavernous sinus, sphenoid sinus, and even the brain. **Conclusions.** The Ki-67 index was significantly higher in adenomas with prolactin secretion.

**Keywords:** Immunohistochemistry, Ki-67, pituitary adenomas

## 2. MULTIMODAL, LIVE IMAGING OF GLOBAL FOREBRAIN ISCHEMIA- INDUCED PERIINFARCT-DEPOLARISATION (PID) IN THE RAT CEREBRAL CORTEX

**Zsófia Bere<sup>ab</sup>, Adrienn Kulmány<sup>ab</sup>, Tihomir P. Obrenovitch<sup>ac</sup>, Ferenc Bari<sup>a</sup>, Eszter Farkas<sup>ab</sup>**

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Propagating electrical inactivation called periinfarct-depolarization (PID) and associated changes in cerebral blood flow (CBF) spontaneously occur in the cortex during cerebral ischemia. These phenomena

may contribute to the extension of brain infarcts. We have set out to characterize PID-related changes in membrane potential and hemodynamic variables in rat cerebral cortex.

In anesthetized, male Sprague-Dawley rats (n=13) a closed cranial window was mounted over the parietal bone and loaded with voltage sensitive (VS) dye. The left femoral artery and vein were cannulated for the monitoring of mean arterial pressure (MAP) and for blood withdrawal. Global forebrain ischemia was induced by bilateral common carotid artery occlusion combined with subsequent hypovolemic hypotension (MAP<40 mmHg). Using selected illuminations we acquired synchronous changes in membrane potential (VS dye method); cerebral blood volume with green (540–550 nm); hemoglobin saturation with red (620–640 nm) illumination, and CBF by laser speckle contrast imaging.

PID occurred at a MAP value of 41.2±3.7 mmHg and at a CBF value of 43.4±4.9%. In most cases the depolarization was not followed by the recovery of membrane potential, and these events were associated with CBF reduction (19±3.6%).

In conclusion, PID appears to generate at the lower limit of the autoregulatory range of CBF, originates at a focus with high vulnerability to ischemia, and propagates similar to that known for cortical spreading depression. PID associated CBF responses most often display inverse neurovascular coupling.

This work was supported by grants from the EGT Norwegian Financial Mechanisms (NFM, NNF 78902), and Hungarian Scientific Research Fund (OTKA, K81266).

**Keywords:** multimodal imaging, global forebrain ischemia, periinfarct-depolarisation, neurovascular coupling

## 3. NEUROENDOCRINE DIFFERENTIATION IN BENIGN PROSTATE DISEASES. AN AUTOPSY REPORT

**Elena Viorica Bocan<sup>1</sup>, Horatiu Ieremia<sup>2</sup>, Dorin Agapie<sup>3</sup>, Ovidiu Mederle<sup>1</sup>, Radu Minciu<sup>4</sup>, Marius Raica<sup>1</sup>**

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Basal cell hyperplasia is occasionally a component of untreated usual, nodular, glandular and stromal hyperplasia. Prostatic intraepithelial neoplasia (PIN) has been identified as precursor lesions to prostatic carcinoma.

The aim of the study was to identify and evaluate neuroendocrine differentiation in autopsy cases of patient that died of other causes than prostate lesions.

The study was performed on a number of 17 cases. The cases have been harvested from the Forensic Medical Institute from patients without prostatic pathology. 3µm sections were performed and then immunohistochemical marker antibodies anti-chromogranin A and PSCA (prostate stem specific antibody) were applied.

After standard hematoxylin-eosin staining was performed, a series of benign prostatic lesions were identified: basal cell hyperplasia, prostatic intraepithelial neoplasia, cystic and lobular degeneration. In some sections, zones of post-mortem autolysis of various sizes

were identified. The presence of neuroendocrine differentiation was proved in both small normal prostate zones and also at the level of benign lesions. PSCA was negative for neuroendocrine cells. Aside of neuroendocrine differentiation, neuroendocrine cell hyperplasia was identified (more than 3 cells per gland) on the same type of lesions. The neuroendocrine differentiation was identified on a number of 10 cases out of the 17 cases. In the same sections we identified hyperplasia of neuroendocrine cells.

PSCA has been shown as negative marker of neuroendocrine cells with positive control in secretory cells. We found neuroendocrine differentiation in cases of patients without prostate pathological conditions, diagnosed post-mortem with benign prostate lesions.

**Keywords:** post mortem diagnosis, benign lesion, chromogranin A, neuroendocrine differentiation

#### 4. FUNCTIONAL AND HISTOPATHOLOGICAL CHANGES IN RENAL TRANSPLANT PATIENTS WITH NEW-ONSET DIABETES AND DYSLIPIDEMIA

**Bernadett Borda, György Lázár**

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The principal risk factors of post-transplantation cardiovascular mortality are hyperglycemia, hypertriglyceridemia, obesity and smoking.

115 patients were included in the study. We assessed the risk factors of diabetes (NODM) and dyslipidemia (NODL), and the effect of these on the function and histopathological changes of the allograft, one year after the transplantation.

When evaluating the risk factors and the initial recipient data, we found a significant difference in age when comparing normal (N) and NODM patients ( $p=0.004$ ), N and NODL patients ( $p=0.002$ ) and N and NODL+NODM patients ( $p=0.0001$ ). The difference in body mass index (BMI) was significant when comparing N and NODM+NODL patients ( $p=0.003$ ). In regard to immunosuppressive therapy, NODM was significantly more frequent in those taking tacrolimus (Tac) ( $p=0.005$ ), whereas those who received cyclosporine (CsA) showed a significantly higher incidence of NODL ( $p=0.001$ ). The triglyceride level was  $3.02\pm 1.51$  mmol/L in those taking CsA and  $2.15\pm 1.57$  mmol/L in those taking Tac, which is a significant difference ( $p=0.004$ ). The difference proved to be significant also when assessing the total cholesterol level: it was  $5.43\pm 1.23$  mmol/L in those receiving CsA-based immunosuppression and  $4.42\pm 1.31$  mmol/L in patients taking Tac ( $p=0.001$ ). In regard to allograft function, one year after the transplantation a significant difference was found between the NODM+NODL group and the N group in serum creatinine level ( $p=0.02$ ), as well as in eGFR ( $p=0.004$ ).

When assessing the morphological changes of the kidney, we found that interstitial fibrosis/tubular atrophy occurred significantly more frequently in all three groups, compared to normal patients. Our own clinical study proves that one year after transplantation the allograft function is already impaired if both medical conditions (NODM and NODL) are present; however, in regard to morphology, a single condition (NODM or NODL) is sufficient to lead to histological changes in the kidney.

**Keywords:** new-onset diabetes mellitus and dyslipidemia

#### 5. THE VALUE OF ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY (ARFI) FOR THE ASSESSMENT OF CHRONIC HEPATOPATHIES

**Simona Bota, I. Sporea, Roxana Şirli, Alina Popescu, Mirela Dănilă**

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**Introduction:** ARFI is a new noninvasive method for the assessment of liver fibrosis.

**Aim:** To assess the value of liver stiffness measurement by means of ARFI as a predictive factor for the severity of fibrosis and for the presence of cirrhosis complications.

**Methods:** We performed ARFI measurements in 523 patients(p), mean age  $59.6\pm 10.2$  years, 210 women and 313 men. In 150p (28.6%) we performed liver biopsy and 373p were diagnosed with liver cirrhosis by clinical ultrasound, endoscopy criteria. In 163 cirrhotic patients we performed also ARFI in spleen. 10 valid ARFI measurements were performed in each patient and a median value was calculated, expressed in meters/second (m/s).

**Results:** We obtained valid liver ARFI measurements in 511p (97.7%). In patients with liver biopsy we obtained a direct, linear correlation between ARFI values ( $r=0.527$ ) and liver fibrosis ( $p<0.0001$ ). The correlation was stronger in patients with HCV vs. HBV hepatitis, but not statistically significant ( $r=0.557$  vs.  $r=0.49$ ,  $p=0.67$ ). The medium ARFI value in cirrhotic patients with significant esophageal varices-EV (at least grade II-150p) was not statistically significant vs. those without or with grade I EV (157p):  $2.8\pm 0.73$  vs.  $2.93\pm 0.72$  m/s ( $p=0.13$ ), also between patients with (69p) or without hepatocellular carcinoma (287p):  $2.76\pm 0.73$  vs.  $2.86\pm 0.72$  m/s ( $p=0.27$ ). ARFI values were statistically higher in patients with ascites (140p) vs. those without ascites (216p):  $2.99\pm 0.71$  vs.  $2.74\pm 0.71$  m/s ( $p=0.003$ ). Spleen ARFI values were statistically higher in patients with significant EV (95p) vs. those without EV or grad I EV (68):  $3.31\pm 0.50$  vs.  $3.09\pm 0.56$ ,  $p=0.01$ . For a cut-off value  $>3.47$  m/s, ARFI in spleen had 43.6%Se, 80.3%Sp, 75.9%PPV, 50%NPV, 58.7% accuracy (AUROC=0.60) for the prediction of significant EV.

**Conclusion:** ARFI is a good method for the assessment of liver fibrosis. In cirrhotic patients can predict the vascular decompensation and ARFI values in the spleen can predict the significant EV.

**Key words:** liver fibrosis, Acoustic Radiation Force Impulse Elastography (ARFI), liver stiffness, spleen stiffness, liver cirrhosis

#### 6. CORRELATIONS BETWEEN THE VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION, MICROVASCULAR DENSITY IN TUMOR TISSUES AND THE TNM STAGING IN BREAST CANCER

**Serban Comsa, Anca Maria Cimpean, Raluca Ceauşu, Cristian Suci, Marius Raica**

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**Background:** The breast cancer is the most frequent malignancy of women all over the world. In spite of its doubtful reliability, the TNM staging system is widely used for the prognosis of this disease. On the other hand, angiogenesis is considered to have an essential role in the evolution of breast cancer and the Vascular Endothelial Growth Factor (VEGF) has proven to be the key regulator of this process. Quantitation of VEGF and the tumor vasculature might play an important role in predicting tumor behavior and patient management. The aim of our study was to evaluate the potential correlation between the VEGF expression, microvessel density (MVD) in the tumor tissues and the TNM staging in breast cancer.

**Method:** We included 34 patients with breast cancer, who had undergone surgical treatment, and we evaluated each case histopathologically and by immunohistochemistry for VEGF and CD31 expression in the tumor tissues. VEGF expression was evaluated by calculating the average percentage of cytoplasmic positive cells from 3 high power fields. MVD was expressed as the average number of the CD31+ microvessels from 3 high power fields.

**Results:** Expression of VEGF was significantly associated with the number of lymph-nodes with metastasis, the number of cells in mitosis, the presence of necrosis and the T-stage (inverse correlation). MVD correlated very well with the histological grading, the number of cells in mitosis, the presence of inflammation and the presence of necrosis.

**Conclusion:** Although their relationship with the TNM staging remains unclear, VEGF expression and MVD prove to be important indicators of the malignant status in breast cancer, confirming the major involvement of angiogenesis in this type of cancer. Both of them are valuable prognostic factors in breast cancer, but the pattern of their relationship needs further analysis of the VEGF receptors in order to be described.

**Keywords:** angiogenesis, breast cancer, microvessel density, TNM staging, VEGF

## 7. THE PROPERTIES OF THE TRANSIENT OUTWARD, INWARD RECTIFIER AND ACETYLCHOLINE SENSITIVE POTASSIUM CURRENTS IN ATRIAL MYOCYTES FROM DOGS IN SINUS RHYTHM AND EXPERIMENTALLY INDUCED PERMANENT ATRIAL FIBRILLATION

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**Background:** The aim of present study is to investigate and compare the properties of repolarizing currents which contribute to AF induced remodeling, i.e. the transient outward ( $I_{to}$ ), inward

rectifier ( $I_{K1}$ ), acetylcholine sensitive potassium currents ( $I_{K,ACH}$ ) in isolated atrial canine myocytes obtained from normal (SR) and tachypaced atrial fibrillating (ATR) dogs by applying *in vitro* (patch clamp) and *in vivo* electrophysiological techniques.

**Results:**  $I_{to}$  current was slightly downregulated in ATR cells when compared with that of recorded in SR.  $I_{K1}$  was larger in the myocytes isolated from ATR dogs, however, this upregulation is not so extensive between -80-20 mV, the voltage range close to an atrial action potential (AP).  $I_{K,ACH}$  was activated by cholinergic agonist carbachol (2  $\mu$ M). In SR, carbachol activated a large current either at outward or inward directions. Selective  $I_{K,ACH}$  blocker, tertiapin (10nM) blocked the carbachol induced current by 57%. In atrial myocytes from ATR dogs we could measure the presence of a constitutively active  $I_{K,ACH}$  which could be blocked by 26% with 10nM tertiapin. However, in atrial myocytes from ATR dogs, carbachol in addition could also activate a significant ligand-dependent and tertiapin sensitive  $I_{K,ACH}$  current. *In vivo* application of tertiapin in ATR dogs even in low concentration (<3 nM) effectively prevented burst induced AF.

**Conclusion:** In contrast to the human, ATR slightly downregulated  $I_{to}$  in dogs. The slower  $I_{to}$  current inactivation may contribute more to atrial repolarization than that of described in ventricular myocytes. The presence of the constitutively activated  $I_{K,ACH}$  in atrial myocytes from ATR dogs shows that electrical remodeling developed in our model. The constitutively active and ligand-dependent  $I_{K,ACH}$  currents together play an important role in dogs by contributing to AF remodeling, since selective  $I_{K,ACH}$  blockade effectively prevented AF.

**Keywords:** atrial fibrillation (AF), tachypaced atrial fibrillating (ATR) dogs, AF induced remodeling

## 8. EXPRESSION AND POSSIBLE SIGNIFICANCE OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN NON HODGKIN LYMPHOMA

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**Background.** Non-Hodgkin's lymphoma (NHL) is a heterogeneous group of lymphoid malignancies, characterized by unpredictable behaviour. The potential significance of angiogenesis in NHL is related to the association between the disease progression and the increased angiogenic activity. VEGF plays a crucial role in both normal and tumor-associated angiogenesis and together with its receptors, it has been shown to represent a promising target for antiangiogenic therapy. Lymphoma cells express VEGFR-1 and VEGFR-2 which promote survival, proliferation and metastasis through autocrine mechanisms. Only few things are known about the exact trigger mechanisms of VEGF expression in hematolymphoid malignancies, but the recent findings have showed

some similar features to those observed in solid tumors.

**The aim** of this study was to investigate the expression of VEGF in malignant non-Hodgkin lymphoma, in terms of its immunohistochemical distribution and clinico-pathological significance.

**Material and methods.** Twenty one specimens of lymph node biopsies were included in this study. For the immunohistochemical procedure we used anti-VEGF antibody clone VG-1 (ready-to-use, 30 minutes incubation time at RT), avidin/biotin/HRP based working system, and 3,3 diaminobenzidine as chromogen. Counterstain was performed by using modified Lille's hematoxylin. We evaluated the distribution in tumour cells, macrophages and in nontumoral cells. VEGF immunoreactivity was estimated as percent of positive cells according to this score: 0 (0% positive cells); 1 (<10%); 2 (10-30%); 3 (>30%).

**Results.** Histopathologic evaluation revealed 18 cases of diffuse type lymphoma and 3 cases of follicular type lymphoma. VEGF was positive in 95,23% of cases and in one case, VEGF was negative in all cell types. We noticed a significant correlation in VEGF expression between tumor cells and macrophages ( $p=0,001$ ). It was also found a significant correlation between tumor cells and nontumoral cells ( $p=0,002$ ).

**Conclusion.** In non-Hodgkin lymphoma the main mechanism of angiogenesis seems to be dependent on the VEGF pathway and its expression particularly by stromal cells.

**Key words:** angiogenesis, lymph node, immunochemistry, VEGF

## 9. EVALUATION OF EG-VEGF ANTIBODY EFFECT ON CHICK EMBRYO CHORIOALLANTOIC MEMBRANE AND ORGANS

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**Introduction.** Endocrine gland derived vascular endothelial growth factor (EG-VEGF), also called prokineticin 1 (PK1) is a highly specific mitogen that regulates proliferation and differentiation of the vascular endothelium in a tissue specific manner. The inhibition of EG-VEGF/ PK receptor pathway could represent a more selective antiangiogenic and anticancer strategy. This study aimed to evaluate the modifications produced by an antibody anti-EG-VEGF on the vascularized chick chorioallantoic membrane (CAM) and in embryonic organs.

**Material and methods.** The in vivo CAM assay was performed *in ovo*, by injecting different dilutions of the antibody EG-VEGF (T-16) in the allantoic vesicle of the embryo, from incubation day 7. Test and control specimens were macroscopically observed, histologically processed and interpreted.

**Results.** Hemorrhagic areas of the capillary plexus were

observed after only one day of administration of the antibody which can be correlated with a very low survival rate. On hematoxylin-eosin staining of the CAM, there was evidence of vascular disruption and erythrocyte extravasations in the chorion. There were signs of CAM epithelium vacuolization while the high density stroma showed a tendency for blood vessel dilatation and bundle-like organization of medium and large vessels. The organs of the treated specimens were also investigated, indicating massive hemorrhagic areas and blood stasis in brain and lungs. These signs of vascular impairment could be possibly explained by the role of the EG-VEGF in the vascular and tissue development of embryonic membranes, eventually blocked by the specific inhibition of EG-VEGF; nevertheless different signaling pathways seem to be involved.

**Conclusion.** An antibody targeting the endogenous EG-VEGF might represent a possible anticancer strategy involving pathways of impairing angiogenesis, mesenchymal and epithelial structure in endocrine - derived embryonic tissues.

**Keywords:** EG-VEGF, angiogenesis, chick chorioallantoic membrane

## 10. BENEFICIAL EFFECTS OF REMOTE LIMB ISCHEMIC PRECONDITIONING AGAINST THE MICROCIRCULATORY CONSEQUENCES OF PARTIAL HEPATIC ISCHAEMIA IN RATS

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**Objective:** We investigated whether the harmful microcirculatory consequences of liver ischemia can be modulated by a remote ischemic preconditioning protocol which can easily be performed and potentially accessible in the clinical practice

**Methods:** Partial hepatic ischemia (I/R) was elicited in male Sprague-Dawley rats by occluding the portal vessels of the left liver lobe for 60 min. The microcirculatory changes induced by I/R were examined by two methods; leukocyte-endothelial interactions were determined by fluorescence intravital videomicroscopy, while tissue perfusion and oxygen saturation changes by the O<sub>2</sub>C ("oxygen-to-see") technique. The reperfusion-caused alterations were compared in I/R and sham-operated animals and in rats that were also subjected to limb ischemic preconditioning (IPC, 2 x 10 min I/R) prior to hepatic I/R (n=5-8). Hepatic myeloperoxidase (MPO) enzyme activities and serum AST, ALT, LDH levels were measured.

**Results:** I/R caused a significant, 4-fold increase in the number of rolling leukocytes and a 3-fold increase of adherent leukocytes in the central venules of the liver as compared to the values of sham-operated animals (rolling:  $64 \pm 12$  1/mm/s; sticking:  $61 \pm 7$  1/mm<sup>2</sup>). The blood flow and tissue oxygen saturation were

decreased by approx. 20% (from control values of ~230 AU and ~46%, respectively), while MPO activity increased approx. 4-fold in comparison with those in the sham-operated animals. All of the above parameters were ameliorated significantly by limb IPC.

**Conclusions:** Remote IPC exerts marked protection against the detrimental microcirculatory consequences of hepatic I/R. Preconditioning applied on the extremities may represent a potential, clinically applicable approach to ameliorate the postischemic microcirculatory failure. (Supported by OTKA K75161, TAMOP-4.2.2-08/1-2008-0013, TAMOP 4.2.1/B-09/1/KONV-2010-0005 grants)

**Keywords:** microcirculation, partial hepatic ischemia, limb ischemic preconditioning, myeloperoxidase

## 11. EFFECT OF CALCNEO-STOP PROCEDURE ON LOAD CONDITIONS OF PAEDIATRIC FLEXIBLE FLATFOOT

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**Background:** Flatfoot is a frequent postural deformity in childhood and adolescence. Operative methods may be considered in severe cases, after ineffective conservative treatment, where the calcaneus is in remarkably valgus alignment and pain is associated. Aim of this study is to evaluate the pedographic outcome of percutaneous lateral arthroereisis with use of a screw through sinus tarsi into the talus.

**Materials and methods:** 25 patients (44 feet) underwent calcaneo-stop surgery at our department between 1<sup>st</sup> June 2008 and 31<sup>st</sup> January 2010. Mean age was 10 years (7-14, SD 2.04). Sole pressures were recorded with the EMED pedographic analyser before surgery. Of these, 17 cases (28 feet) were available for follow-up. The mean follow-up time was 9.7 (7-19, SD 4.98) months.

**Results:** Area, relative contact time and pressure-time integral (load amount) values increased after surgery on lateral heel, midfoot and metatarsal sections. On medial areas a decrease of mentioned parameters was observed. The most remarkable differences were measured at midfoot region. Here the contact area value medially decreased from pre op 19.69 (2-28, SD 6.61) cm<sup>2</sup> to post op 7.31 (1-22, SD 5.6) cm<sup>2</sup>, pressure-time integral increased laterally from 3.99 (0.88-7.31, SD 1.48) Ns/cm<sup>2</sup> to 8.44 (2.3-19.88, SD 3.56) Ns/cm<sup>2</sup> after surgery. Total contact area of the sole decreased post op from 120.00 (82-176, SD 27.16) cm<sup>2</sup> to 107.23 (73-149, SD 20.856) cm<sup>2</sup>. These changes are significant (p<0.001).

**Conclusion:** Calcaneo-stop procedure seems to provide an excellent correction for calcaneovalgus deformity at severe flexible paediatric flatfoot. Pedographic analysis of sole after operation showed a load shift from medial to lateral, predominantly on the midfoot and hindfoot, giving a load map close to normal. Further investigation are required to evaluate the long term outcome of this method, included the conditions following implant removal.

**Keywords:** Paediatric flatfoot; Calcaneovalgus; Calcaneo-stop; Arthroereisis

## 12. CAROTID INTIMA-MEDIA THICKNESS AND THE PREDICTION OF CLINICAL CARDIOVASCULAR EVENTS IN PATIENTS WITH MANIFEST ARTERIAL DISEASE

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**Introduction** - Carotid intima-media thickness (IMT) is an independent predictor of cardiovascular events in the general population and an important marker regarding the evaluation of subclinical vascular damage. Its use relies on its ability to predict future clinical cardiovascular end points.

**Aims** - Currently, little is known about the relationship between IMT and new cardiovascular events in patients with manifest arterial disease. We investigated whether IMT is associated with cardiovascular risk in patients who already have vascular disease or atherosclerotic risk factors.

**Materials and Methods** - The study was performed between 2008-2011 and included 283 patients with manifest arterial disease or atherosclerotic risk factors, aged >50 years, mean age 63±5 years, 68% men and 32% females, registered in family medicine offices in Timiș County. IMT was measured at baseline in both carotid arteries by high resolution ultrasound as agreed in Mannheim consensus. Increased IMT (>0.9 mm) or presence of carotid atherosclerotic plaques are evidences of subclinical vascular damage. A value of IMT, measured by echo-Doppler carotid, greater than 0.9 mm shows vascular lesions earlier than ankle-brachial index.

**Results** - Major vascular events were vascular death, ischaemic coronary events, or stroke. Adjusted for age and sex, an increase in carotid IMT of 1 SD (~0.32 mm) was associated with an increased risk of any vascular event (Hazard Ratio-HR 1.17; 95% CI 1.04-1.30). Increasing IMT was most strongly related to ischaemic stroke incidence (HR 1.37; 95% CI 1.16-1.58), than to myocardial infarction incidence (HR 1.21; 95% CI, 1.04 to 1.38). Results were similar in patients without large carotid plaques (IMT<2 mm at all measurements sites).

**Conclusions** - Carotid IMT is a strong predictor of future cardiovascular events. IMT is associated with the occurrence of new vascular events, mostly for ischaemic stroke, in patients with manifest arterial disease or atherosclerotic risk factors.

**Keywords:** Intima-media thickness, cardiovascular events, atherosclerotic risk factors, stroke.

## 13. EXAMINATION OF THE TRIGGER ROLE OF PEROXYNITRITE IN THE ANTIARRHYTHMIC EFFECT OF ISCHAEMIC PRECONDITIONING AND PEROXYNITRITE INFUSION

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**Background and purpose:** The present study examines whether peroxynitrite (PN) generated during the preconditioning (PC) procedure, or administered in brief intracoronary infusions plays a trigger role in the antiarrhythmic effects of PC and PN in anaesthetised dogs. **Experimental approach:** The PN scavenger urate (UA;  $0.2 \text{ mg kg}^{-1} \text{ min}^{-1}$ ) was administered in PC (UA+PC;  $n=8$ ) and in PN treated (UA+PN;  $n=8$ ) dogs or in control animals (UAC;  $n=9$ ) over 30 min, just prior to a 25 min occlusion of the left anterior descending (LAD) coronary artery. Severities of ischaemia (ST-segment elevation, inhomogeneity of electrical activation) and ventricular arrhythmias (VPBs, VT, VF), plasma nitrite/nitrate ( $\text{NO}_x$ ) levels as well as superoxide and myocardial nitrotyrosine (NT) productions were determined. **Key results:** Both the PC procedure ( $n=10$ ) and the infusion of PN ( $n=10$ ) resulted in elevations in NT formation, and compared to the controls (C;  $n=14$ ) markedly reduced the number of VPBs ( $276 \pm 85$  vs  $24 \pm 10$  and  $55 \pm 19$ ), increased survival (S: 0% vs 66% and 50%), preserved  $\text{NO}_x$  formation and attenuated superoxide and NT productions that resulted from a prolonged ischaemia and reperfusion insult. Although UA inhibited NT formation in both groups the protective effects were only abolished in PN (VPBs:  $226 \pm 121$ ; S: 25%) but not in PC dogs (VPBs:  $12 \pm 6$ ; S: 62%). Interestingly, urate itself was also antiarrhythmic (VPBs:  $50 \pm 14$ ; S: 55%). **Conclusions and Implications:** Low concentration of PN can induce an antiarrhythmic effect but PN generated during the PC procedure is not necessary for the PC-induced antiarrhythmic protection.

**Keywords:** Preconditioning; peroxynitrite; urate; ischaemia/reperfusion; arrhythmias

#### 14. ACUTE ADMINISTRATION OF SIMVASTATIN REDUCES THE SEVERITY OF VENTRICULAR ARRHYTHMIAS IN ANESTHETIZED DOGS

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During the past decade statins were found to have a pleiotropic effect beyond their lipid-lowering ability. These effects, especially that occur following acute drug administration and may contribute to the cardioprotection, are still poorly understood. Our aim was to investigate the effect of a single bolus injection of simvastatin ( $0.1 \text{ mg/kg}$ ) on the ischaemia and reperfusion-induced ventricular arrhythmias in chloralose-urethane-anaesthetized dogs. In one group of dogs ( $n=7$ ) activated simvastatin, in the other group ( $n=8$ ) the solvent of the compound were given just prior to a 25 min occlusion of the left anterior descending coronary artery. The severity of ischaemia (degree of inhomogeneity of electrical activation, epicardial ST-segment) and of arrhythmias, as well as the plasma nitrate/nitrite ( $\text{NO}_x$ ) levels were assessed during the occlusion. Compared to the control group, simvastatin decreased the total numbers of VPBs ( $206 \pm 44$  vs.  $139 \pm 50$ ) and episodes of

VT ( $5.8 \pm 1.9$  vs.  $0.5 \pm 0.3$ ), the incidences of VT (70% vs. 38%) and VF (40% vs. 0%) during the occlusion. Survival from the combined ischaemia/reperfusion was also higher (38%) in the simvastatin treated group than in the controls (0%). Whereas in the control group the plasma level of NO metabolites in the coronary sinus blood was markedly reduced, in the simvastatin treated animals  $\text{NO}_x$  levels were significantly increased particularly during the second phase of the occlusion. We conclude that acute simvastatin treatment may provide protection against the ischaemia and reperfusion-induced severe ventricular arrhythmias perhaps through the elevation of NO availability during ischaemia. This work is supported by the Hungarian Scientific Research Foundation (OTKA, project No. K75281).

**Keywords:** simvastatin, ischaemia, ventricular arrhythmias, NO

#### 15. EFFICIENCY OF PLASMID TRANSFECTION ALONG THE REGENERATING RAT SOLEUS MUSCLE

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Regeneration plays an important role in skeletal muscle diseases and injuries. Experimental regeneration can be induced in different ways; we use direct intramuscular injection of snake venom (notexin) to achieve a complete necrosis and subsequent regeneration in the rat soleus muscle. The regenerating soleus can be successfully transfected by naked plasmid injection. However this procedure results in a low efficiency transfection which has not been characterized along the entire length of the muscle.

The regenerating soleus muscles were transfected with plasmids encoding green or red fluorescent proteins. After one week the muscles were dissected, frozen and cut into 6 approximately equal segments before frozen sectioning. Transversal sections were studied with fluorescent microscope for transfected fibres in each segment. The neuromuscular endplates were also stained in each muscle segment for determination of fibre length. We found transfections mostly in the central segments, near to the site of intramuscular injection. The transfected fibres were usually organized in groups, next to each other on cross sections. The neuromuscular endplates were also found mostly in the central segments. Considering that each fibre has one endplate, this concentration suggests that most fibres run from proximal to distal ends along the entire muscle. From these results we conclude that the transfection efficiency is even further reduced along the fibres in the regenerating muscle. This is not due to reduced fibre length, since most fibres extend from proximal to distal ends of the muscle, but rather caused by the limited migration of the transfected plasmids and expressed proteins along the fibres.

**Keywords:** muscle regeneration, transfection distribution, fibre length

## 16. SPECIFIC CHEMOKINE-CHEMOKINE RECEPTORS INTERACTIONS AND THEIR ROLE IN HMSC MIGRATION

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**Background and aims:** Human mesenchymal stem cells (hMSCs) are non-hematopoietic stromal cells that can differentiate into different tissues. They are rare in the bone marrow (0.001-0.01% of nucleated cells). Minimal criteria have been established to define hMSCs. They have leukocyte-like functions, expressing various chemokine receptors, integrins, and adhesion molecules, and displaying migratory capacity towards ligands released from injury sites. The aims are to isolate, characterize, differentiate hMSCs, to determine their migratory signals, and the *in vitro* & *in vivo* migration of hMSCs to specific chemokines.

**Material and methods:** hMSCs were isolated from human bone marrow using the density gradient method. After 2 weeks expansion, they were characterized using flow cytometry. The hMSCs were then cultured in adipogenic, chondrogenic, and osteogenic self-made media for 3 weeks, and stained with specific stains for adipose tissue (Oil Red O), bone (Alizarin Red), and cartilage (Alcian Blue). The surface and intracellular expression of 7 pro-inflammatory chemokines and 13 chemokine receptors were analyzed on undifferentiated hMSCs.

**Results:** hMSCs have been successfully isolated from 8 bone marrow samples, obtaining an average of  $110 \times 10^6$  bone marrow cells after isolation. They have been expanded and kept in culture for up to 6 passages. They fulfilled the minimal criteria for hMSCs (expression of CD105, CD73, and CD90, lack of CD11a, CD34, and CD45 expression). The hMSCs were successfully differentiated into three lineages using self-made media. There was no chemokine receptor surface expression, due to trypsinization. The chemokine receptor intracellular expression was donor-dependent, with the constant expression of CCR3, CCR4, and CXCR3. The chemokine intracellular expression was also heterogenic, only CCL2 and CXCL12 being constantly expressed.

**Conclusion:** The expression of chemokines and chemokine receptors in healthy donors is highly heterogenic. There are specific interactions between chemokines and chemokine receptors during inflammation, and these specific interactions influence hMSC migration.

**Keywords:** hMSC, chemokines, chemokine receptors, migration, inflammation

## 17. VENTRICULAR ASYNCHRONISM - PROGNOSIS MARKER IN HEART FAILURE WITH PRESERVE EJECTION FRACTION

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It is now recognized that at least 40% of patients with heart failure have normal ejection fraction (FE). If left ventricular (LV) systolic function is normal, it may be difficult to make a conclusive determination of the relative role of diastolic heart failure compared with other concomitant conditions.

I propose to investigate the association between ventricular asynchrony, LV diastolic dysfunction and other biomarkers of prognosis in diastolic heart failure

The objectives are to identify new prognostic markers that correlate with clinical and cardiac functional exploration, to assess the progression of heart failure, prognostic stratification of heart failure and identifying patients at high risk of cardiovascular events.

**Inclusion criteria:** clinical symptoms of heart failure NYHA II, III; sinus rhythm; FE > 45%.

**Exclusion criteria:** atrial fibrillation, relevant valve disorders, hypertrophic cardiomyopathy and severe lung disease.

For echocardiography diagnosis of diastolic heart failure the following are determined: mitral Doppler profile, the profile of pulmonary venous flow Doppler, early diastolic flow propagation velocity and myocardial performance index (GMI).

Ventricular asynchronism is assessed by the ratio of left ventricular filling time and cardiac cycle, determining the interventricular mechanical delay (IVMD), Septal – posterior wall motion delay (SPWMD), aortic preejectional time. Using tissue Doppler the differences between peak systolic velocities of opposite walls will be measured.

All patients will be physically and echocardiography examined, 6-minute-walk-testing will be assessed and determination of serum prognostic markers of heart failure (serum pro-BNP, serum troponin, serum D-dimmer) will be performed.

After one year we will reevaluate the parameters used for the study and we will compare patients' evolution with and without ventricular asynchrony and the presence of prognosis biomarkers.

With multimarker strategy I intend to identify the combinations of biomarkers and echocardiography parameters that are optimal at various stages during the evolution of diastolic heart failure, ranging from their use for screening, diagnosis, determining prognosis, and guiding management.

**Keywords:** diastolic dysfunction, heart failure, asynchronism, marker prognosis

## 18. MESOSTRUCTURE. TYPES OF BARS IN IMPLANT OVERDENTURE

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Prosthetic rehabilitation of totally edentulous patients, especially on the mandibular jaw, with complete dentures is no longer the first treatment method choice. After the Consensus held in 2002, at McGill University, Canada, it was decided that the standard of care for this category of patients is represented by the overdenture supported on 2 interforaminal implants. In time, this treatment concept became viable and other treatment solutions have emerged. The best known classification of prosthetic implant options is that one proposed by C.E. Misch. The number of implants inserted varies for each patient, depending on its financial availability and anatomical limitations. The connection between the implant and overdenture is done through different connection systems: balls, bar / rider, magnets and telescopic systems. In case of using implant bar overdentures, we can distinguish three levels at this type of prosthetic restorations: infrastructure, mesostructure, and superstructure. There are several types of bars used for implant overdentures, and the purpose of this presentation is to expose them: **cemented and screwed bars, short and long bars, intraorally welded prefabricated bars, metallic (titanium) milled bars, zirconium milled bars.**

**Keywords:** mesostructure, zirconium, overdenture

## 19. EPIDEMIOLOGY OF CENTRAL NERVOUS SYSTEM MALFORMATIONS IN A REGION IN HUNGARY

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**Background:** Few data are available on the epidemiology of central nervous system (CNS) malformations despite their major aetiological role in mental and neurological handicap and epilepsy in childhood.

**Aim of the study:** To describe the population-based epidemiological characteristics and spectrum of various malformations of the CNS in South-Eastern Hungary and compile

a registry.

**Methods:** A retrospective survey of patients born with CNS malformations in the region between July 1, 1992 and June 30, 2006 was performed. The malformations were classified into 22 subgroups and the livebirth prevalence per 10 000 births was calculated.

**Results and Conclusions:** CNS malformations were found in 214 children among 185 486 livebirths, which correspond to a livebirth prevalence of 11.54 per 10 000. Neural tube defects (spina bifida, anencephaly, encephalocele) and corpus callosum anomalies were the most common malformations with livebirth prevalence figures of 2.21 and 2.05 per 10 000 births.

The livebirth prevalence of holoprosencephaly, septo-optic dysplasia, cavum septum pellucidum, microcephaly, microlissencephaly, lissencephaly-agyria/pachygyria and subcortical band heterotopia spectrum, neuronal heterotopia, polymicrogyria, schizencephaly, focal cortical dysplasia, Joubert syndrome, unilateral cerebellar hypoplasia, Dandy-Walker malformation, cerebellar vermis hypoplasia, cerebellar aplasia/hypoplasia, Chiari I malformation, pontocerebellar hypoplasia and arachnoid cysts was 0.49, 0.11, 0.59, 1.62, 0.11, 0.27, 0.16, 0.43, 0.54, 0.05, 0.05, 0.11, 0.32, 0.49, 0.38, 0.11, 0.16, and 1.29 per 10 000 livebirths, respectively. Somatic anomalies in association with the CNS dysgenesis were observed in 39.2% of the patients. Seizures appeared and epilepsy was diagnosed in 35.9%. Developmental delay, intellectual disability or abnormal neurological signs occurred in more than half of the patients.

Our registry of CNS malformations provides a database for further studies on the genetic and environmental aetiology of CNS dysgenesis.

**Keywords:** epidemiology, central nervous system malformations, livebirth prevalence

## 20. STUDY ON THE LEVELS OF TAU AND $\beta$ -AMYLOID IN CSF OF PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a progressive central nervous system (CNS) disorder characterized inflammation, demyelination, axonal damage and neurodegeneration. The course of the disease can vary in a wide range and finding a proper way to predict disease progression is a matter of great significance.

Our objective was to examine if the cerebrospinal fluid (CSF) levels of proteins previously proved to be relevant in common neurological diseases could predict the progression and future clinical manifestation of MS.

We evaluated the levels of h-Tau, p-Tau and  $\beta$ -amyloid in human CSF samples collected from patients presenting with different clinical forms of MS by commercially available ELISA kits. The lumbar punctures were performed from 1999 to 2007 and patients underwent regular clinical follow-up and were classified as clinically isolated syndrome (CIS), relapse remitting form (RR)

and primary progressive MS (PP).

We did not find any difference in the examined protein levels between MS and control groups. No significant difference could be observed between groups with different progression tendencies; however, there was an observable decrease in p-Tau concentration in the primary progressive group, which did not reach the level of significance.

The examined proteins seem to be inappropriate as biomarkers in predicting disease progression in MS. More focus should be devoted to the examination of primary progressive MS with an increased number of patients involved.

**Keywords:** sclerosis multiplex, demyelination, biomarker, ELISA

## 21. INVESTIGATION OF TRANSPORTER INTERACTIONS OF ANTIMALARIALS IN VITRO

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Options to control spread of malaria are increasingly limited due to emergence of parasites resistant to widely used antimalarials, therefore, discovery of novel antimalarials appears crucial as ever. However, animal experiments are too expensive and laborious for the pharmacokinetic characterization of large number of compounds. The fate of administered drugs may largely depend on their interactions with transporter proteins, which are present in all major pharmacologically relevant barriers. Furthermore, transporters are key determinants of antimalarial drug resistance of plasmodiums as well. The aim of this study was to examine whether the high-throughput (HTS) cell and membrane-based transporter assays can be applied to characterize the transporter interactions of candidate antimalarials.

Reference antimalarials, such as artemisinin, chloroquine, mefloquine, quinine, etc. have been tested for their interaction with the ABC-transporters MDR1, MRP1 and BCRP using the Solvo PredEasy ATPase kits and the interaction with the SLC family members OCT1 and OCT2 uptake transporters in cell-based assay. Measured IC<sub>50</sub> and EC<sub>50</sub> values were correlated with the clinical observations on the tested antimalarials.

In case of Amodiaquine, Hydroxychloroquine, Primaquine, Pyrimethamine, Proguanil, Artemisinin, Artesunate, Atovaquone, Clindamycin, Halofantrine, our data are the first proof for transporter interaction of these clinically important drugs. Artemisinin is a substrate for MDR1, chloroquine is inhibitor of the MDR1 and substrate for the MRP1 and BCRP, mefloquine is substrate for the MDR1 but at higher concentrations is a not specific inhibitor of all the transporters and quinine is substrate for the MDR1. These results corresponded exactly to the clinical data on the antimalarials tested.

We conclude that the membrane- and cell-based HTS in vitro

assays can be applied to facilitate the ADME characterization of candidate antimalarials.

**Keywords:** antimalarial, transporter interaction, ADME, *in vitro* assays

## 22. ISCHEMIC LIMB SALVAGE USING NEOANGIOGENIC THERAPY – CASE REPORT

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The present paper describes the case of a 50-year old patient, heavy smoker, diagnosed at the age of 48 with peripheral occlusive arterial disease (occlusion of the left superficial femoral artery), when a revascularization procedure was attempted (not possible because of extensive thrombosis in the popliteal artery and poor distal arterial bed – highly suggestive for thromboangiitis obliterans). In the next period the patient developed a tissue loss in the calf; skin graft was performed (in some other surgical department) but with no result. Considering the current symptoms (rest pain), the peripheral angiographic aspect and the trophic lesions in the calf, in September 2008 the first gene therapy procedure was performed, followed by repeated administration at one month. The patient was monitored for the next 18 month and the clinical course was good, with relief of rest pain and secondary healing of trophic lesions.

**Keywords:** neoangiogenic therapy, VEGF, HGF, critical limb ischemia

## 23. PROTECTIVE EFFECT OF L- $\alpha$ GLYCERYLPHOSPHORYLCHOLINE AGAINST ISCHAEMIA-REPERFUSION INJURY IN THE RAT SMALL INTESTINE

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**Introduction:** Our previous studies showed that phosphatidylcholine (PC) inhibits the production of reactive oxygen species (ROS) both *in vitro* and *in vivo*, and displays anti-inflammatory features during ischaemia-reperfusion (IR) conditions. Our present aims were to examine whether the anti-inflammatory effects of PC are linked to the fatty acids of the molecule or the headgroup, therefore we studied the effects of L- $\alpha$  glycerylphosphorylcholine (GPC), the deacetylated polar derivative of PC, in a rat model of IR.

**Methods:** Anesthetized rats were randomized to control

(n=8), mesenteric IR (n=8), IR with GPC pre-treatment (n=8) or IR with GPC post-treatment (n=8) groups. We monitored the macrohaemodynamics (mean arterial pressure, arteria mesenterica superior flow, mesenteric vascular resistance (MVR) and microhaemodynamic parameters (serosal blood flow, with intravital OPS videomicroscopy) and biochemical markers (tissue superoxide ( $O_2^{\cdot-}$ ), xanthine oxidoreductase (XOR) activity, and liver ATP-contents, after 45 min ischemia and 180 min reperfusion period.

**Results:** The IR insult increased MVR and tissue  $O_2^{\cdot-}$  production, and reduced liver ATP content after reoxygenation. Nevertheless, GPC pre-treatment reduced the elevation of MVR and decreased the tissue  $O_2^{\cdot-}$  production, while post-treatment diminished both XOR activity and ATP depletion, and normalized the intestinal microcirculatory dysfunction.

**Conclusion:** Exogenous GPC has anti-inflammatory effect and decreases IR-induced ROS production. The similar efficacy of PC could be linked to a reaction involving the polar part of the molecule.

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**Keywords:** Mesenteric vascular resistance (MVR), superoxide, xanthine oxidoreductase, ATP

## 24. CHARACTERIZATION OF THE EFFECTS OF TWO PORPHYRIN COMPOUNDS ON RAT HEART MITOCHONDRIAL RESPIRATION

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Mitochondria play a central role in health and disease, and compounds such as metallo-porphyrins, able to enter mitochondria, are nowadays intensively investigated. The aim of the present study was to assess the effects of two porphyrins: Zn(II)-5,10,15,20-tetrakis(4-pyridyl)porphyrin (ZnTPyP) and 5,10,15,20-tetrakis(4-allyloxyphenyl) porphyrin (TAPP) on respiratory function of rat heart mitochondria isolated by means of differential centrifugation technique. Oxygen consumption of freshly isolated mitochondria (0.5 mg/mL, final concentration of mitochondrial proteins) was measured using a Clark-type respirometer at 37°C in a sucrose buffer (pH 7.2) in the presence of NAD-linked (glutamate/malate) and FAD-linked (succinate) substrates, respectively. Basal (state 2) and ADP-stimulated (state 3) respiratory rates were recorded and expressed as nanoatoms oxygen/min/mg protein and respiratory control index (RCI) was calculated. Three concentrations (3, 5 and 10  $\mu$ M, respectively) of each porphyrin were studied. In the presence of complex I substrates (glutamate/malate), ZnTPyP (5  $\mu$ M final concentration) elicited a significant increase of state 2 and state 3 respiratory rates ( $70.5 \pm 2.2$  and  $468 \pm 22.6$ ,  $p < 0.0001$  vs. Control) but had no effect on complex II dependent

respiratory rates. However, in the presence of TAPP (10  $\mu$ M final concentration) oxidation of glutamate and malate was diminished: state 3 respiratory rate was decreased by 29% and RCI by 31%. TAPP (10  $\mu$ M) also decreased basal respiration by 16% in the case of succinate-supported respiration. In normal isolated rat heart mitochondria Zn(II)-5,10,15,20-tetrakis(4-pyridyl) porphyrin and 5,10,15,20-tetrakis(4-allyloxyphenyl) porphyrin elicited different effects on complex I and complex II supported respiration, an observation that clearly warrants further investigation view their potential application in pathological conditions associated with ischaemia/reperfusion injury.

**Keywords:** mitochondria, metallo-porphyrins, rat, respiration

## 25. THE EFFECTS OF EXOGENOUS METHANE INHALATION ON MACRO-AND MICROCIRCULATORY CHANGES DURING INTESTINAL ISCHEMIA-REPERFUSION IN RATS

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**Introduction:** The small intestinal mucosa is particularly sensitive to ischemia-reperfusion (I/R) injuries. I/R initiates a complex biochemical cascade reaction in which the generation of reactive oxygen species (ROS) and the micro-and macrocirculatory perfusion deficiencies will lead to tissue damage and barrier function failure. Mammalian methanogenesis is considered an exclusive indicator of carbohydrate fermentation of the anaerobic **gastrointestinal** flora, but in our previous studies we have shown preventive effects of methane on hypoxia-induced inflammatory processes (Ghyczy et al., FASEB J 2003; Cell Physiol Biochem 2008). Based on this background, we hypothesized that the damaged intestinal microperfusion during I/R could be improved by exogenous methane inhalation.

**Methods:** Male Sprague-Dawley rats were randomized to control (n=7), I/R without methane (I/R, n=7) and I/R with 2.5% methane added to 21% oxygen (n=7) groups. The superior mesenteric artery (SMA) was occluded for 45 min, which was followed by 120 min reperfusion. Methane treatment was started during the last 10 min of ischemia and lasted for 5 min during reperfusion. Systemic and mesenteric haemodynamics were monitored, the microcirculation of the serosa of the terminal ileum was observed by intravital videomicroscopy (IVM) using orthogonal spectral polarisation imaging technique (A/R Cytoscan). The tissue ROS generation was detected by a chemiluminescence assay, the myeloperoxidase (MPO) activity was assessed by fluorimetric analysis.

**Results:** The significant microcirculatory dysfunction (as evidenced by IVM) after I/R was remarkably improved by methane treatment. Moreover, a significantly decreased superoxide production and MPO activity were detected in the treated animals, in contrast with the control group.

**Discussion:** Exogenous methane improves the perfusion deficiency of the mucosa after I/R, and this effect may be linked to ROS scavenging or inhibition of leukocyte activation in the reperfused intestinal tissues.

**Keywords:** intestinal ischemia, reactive oxygen species, microcirculation, leukocyte activation

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## 26. CHILDBEARING AMONG WOMEN WITH MULTIPLE SCLEROSIS (MS)

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**Introduction:** Pregnancy management issues an extra challenge to MS patients and their physicians. There are few epidemiological studies reporting databases on this subject.

**Objectives:** To report the results between 1998 and 2010 from the University of Szeged, Department of Obstetrics and Gynecology, Department of Neurology database on multiple sclerosis and pregnancy. **Patients**

**and methods:** A retrospective analysis of pregnancy data from MS patients, who became pregnant at any time of their disease, compared to international research was carried out. **Results:** In 65 MS mothers, the average age at pregnancy was 35.87 ( $\pm 7.9$ ). EDSS at start pregnancy was 1.38( $\pm 1.4$ ) and relapse rate before antepartum period was 1.37( $\pm 1.3$ ). The average number of pregnancies was 2.14 (SD $\pm 1.3$ ), the average fetal weight: 3106.1 $\pm 630$  g. Exposure to MS drugs at any time during pregnancy was high (67%) In our study the overall spontaneous abortion rate was significantly higher in MS patients, as the age-matched healthy control ( $p = 0.0006$ ). Vaginal delivery incidence was relatively high (67.9%) Incidence of Cesarean section was 35.4%, and 33.8% was the incidence of interruption. The prevalence of pregnancy complications was relatively low with 12.3 % premature births outcomes. Metrorrhagia was seen in 26. 1% and myomas were seen in 13.8%.

**Conclusion:** Our results confirm previous findings lower relapse rate during pregnancy and add to present literature informing on data related to immunomodulatory treatment, spontaneous abortion, changes of BMI and obstetrical complications.

**Keywords:** immunomodulatory treatment during pregnancy, spontaneous abortion, obstetrical complications

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