Title of the project: Innovation of laboratory classes in embryology

Grant Agency: Ministry of Education ***Project Number:*** 364/F3a

Principal Investigator: D. Čížková

Co-investigators: D. Šubrtová

Starting date: 1.1.2008 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 138

Summary of 2008 results

Title of the presentation: Innovation of laboratory classes in embryology

Authors: Dana Čížková, Danuše Šubrtová, Damian Šimčík

Charles Univ. Prague, Fac. Med. Hr. Králové: Dept. Histol. Embryol.

The main goal of the project was to increase quality of laboratory classes in embryology, firstly to change the passive form of teaching to the active one. For innovation of these laboratory classes, newly prepared histological sections of human fetal lung, intestine, liver, pancreas, kidney, suprarenal gland, testis and epididymis were processed for routine hematoxylin eosin staining and inserted in sets (boxes) of slides used by students during the laboratory classes. A new programme and a protocol of innovated laboratory classes in embryology were prepared as electronic files (MS PowerPoint, Word) at students disposal. New histological sections of other embryonic and fetal human and mouse tissues and organs were processed for routine staining and peroxidase immunohistochemistry and then inserted in sets of slides used by teachers. From embryological point of view interesting markers such as intermediate filaments nestin, desmin and vimentin were immunohistochemically detected. Moreover, semithin sections of mouse embryonic tissues and organs of two developmental stages were prepared using LR White resin that enables hematoxylin eosin stainig. Majority of new histological sections were photographed to complete a database of digitalized images. Last but not least, three-dimensional plastic models of human development were bought: a set of 13 models of human embryo development from fertilization to the first week, a model of a 4-week-old human embryo and a model of the uterus with an embryo in the second month of development. Programmes and protocols of laboratory classes in embryology and several digitalized images are available at web sites: http://www.lfhk.cuni.cz/histologie/.
Supported by Grant: FRVŠ No. 364/2008/F3/a.

Address for correspondence: Dana Čížková, Dept. of Histology and Embryology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Intravitreal application of triamcinolone acetonide in macular edema of differential etiology

Grant Agency: Charles University ***Project Number:*** 24807/2007C

Principal Investigator: J. Dusová

Co-investigators: J. Dusová

Starting date: 1.1.2007 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 356

Summary of 2008 results

Title of the presentation: Triamcinolon in treating macular edema of varying etiology

Authors: J. Dusová, D. Hejcmanová
Dept. of Ophthalmology, Faculty of Medicine and Faculty Hospital Hradec Králové

One year follow-up to evaluate the efficacy of a 4mg injection of triamcinolone in patients with macular edema (ME) of varying etiology. This prospective study included 54 eyes. Of these, 30 eyes had diabetic macular edema, 16 eyes had ME after vein occlusion and 8 eyes had cystoid macular edema (CME) after cataract extraction. Before injection and at the 1, 3, 6, 9 and 12 months all patients had undergone biomicroscopic examination, best-corrected visual acuity (BCVA) and macular thickness (MT) estimation using optical coherent tomography. Patients with a history of glaucoma and patients with tangential vitreous traction on the macula were excluded from the study.BCVA mean in group I improved from 0,19 to 0,23 after 12 months. Improvement of vision was significant in the 1st, 3rd and 6th month ($p<0,0005$). Vision improvement was not significant at the 12th month. Macular thickness decreased from 518µm to 374µm after 12 months. Improvement in MT was significant in all follow-up months ($p<0,0005$). BCVA mean in group II was improved from 0,19 to 0,39 after 12 months. Improvement of vision was significant in all follow-up months (1month $p=0,001$, 12 month $p=0,003$). Macular thickness decreased from 476µm to 309µm. Improvement in MT was significant in all follow-up months (1 month $p<0,0005$, 12 month $p=0,007$). BCVA mean in group III improved from 0,22 to 0,75. Improvement of vision was significant in all follow-up months (1month $p=0,012$, 12 month $p=0,011$). Macular thickness decreased from 536µm to 244µm after 12 months. Improvement in MT was significant in all follow-up months (1 month $p=0,012$, 12 month $p=0,012$). Conclusion: The best results were observed in group with pseudophakic CME.

Supported by the Charles University Grant Agency, No 7248/2007

Address for correspondence: J.Dusová, Dept. of Ophthalnology, Charles University in Praque, Faculty of Medicine and Faculty Hospital in Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Study of factors in a tissue microenvironment that influence the process of skeletal muscle reparation

Grant Agency: Czech Republic ***Project Number:*** 304/08/0329

Principal Investigator: S. Filip

Co-investigators: J. Mokrý, D. Čížková, M. Řezáčová, S. Mičuda, J. Vávrová, Z. Řeháková-Šinkorová, A. Tichý, L. Zárybnická

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 4020

Summary of 2008 results

Title of the presentation: The homing of hematopoietic stem cells after transplantation in lethally irradiated mice

Authors: S. Filip (1), J. Mokrý (2), D. Čížková (2), J. Vávrová (3), S. Mičuda (4), M. Řezáčová (5)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Oncology and Radiotherapy (1), Dept. of Histology and Embryology (2), Dept. of Pharmacology (4), Dept. of Biochemistry (5); Fac. Milit. Health Sci., Univ. Defence, Hr. Králové (3)

We describe the homing of hematopoietic stem cells (HSCs) to non-hematopoietic tissues in lethally irradiated (9 Gy) mice transplanted intravenously with lin- / CD117+ bone marrow cells from ROSA-26 mice. On day 8 post irradiation, we demonstrated recovery of hematopoiesis as the the number of CD117+ / B220- cells in the bone marrow reached the level of non-irradiated controls. The numbers of CFU-GM in spleen of irradiated transplanted mice were well above the levels found in non-irradiated group as early as day 8 after transplant. In contrast, bone marrow CFU-GM numbers did not reach levels found in non-irradiated controls even 33 days after transplant. Regeneration of cells in peripheral blood was significantly slower. Transplanted lacZ+ cells were detected histochemically and their location in the thymus, liver, stomach and ileum was followed during 33 days. We found massive presence of donor (lacZ+) cells in the thymic cortex. In the stroma of intestinal villi, positivity to β-galactosidase was observed on day 33. By day 33 individual lacZ+ cells were dispersed in relatively great numbers throughout the liver parenchyme. Transplantation of sorted lin- / CD117+ effectively resulted in hematopoietic recovery ensuring survival of lethally irradiated animals. Hematopoietic stem cell transplantation led not only to recovery of hematopoietic and lymphoid tissues but also facilitated recovery of the small intestinal mucosa, which was significantly damaged by ionizing radiation. Our results show that cells derived from donor bone marrow were found in small intestine of lethally irradiated recipients and they participated in lymphopoiesis.

Supported by the Grant Agency of the Czech Republic 304/08/0329.

Address for correspondence: J. Mokrý, Dept. of Histology and Embryology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Quantitative dopaminergic receptors expression in clinically non-functioning pituitary tumours

Grant Agency: Charles University ***Project Number:*** 79008/2008C

Principal Investigator: F. Gabalec

Co-investigators: J. Čáp, M. Beránek

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 223

Summary of 2008 results

Title of the presentation: Quantitative dopaminergic receptors expression in clinically non-functioning pituitary tumours by real-time PCR

Authors: Filip Gabalec (1), Jan Čáp (1), Martin Beránek (2)

Fac. Med., Charles Univ. and University Hospital, 2nd Deparment of Internal Medicine, Hradec Králové (1), Fac. Med., Charles Univ., and University Hospital, Department of Clinical Biochemistry , Hradec Králové(2)

Dopamine agonists are treatment of choice in human prolactinomas. Dopamine D2 receptors are present on most pituitary cells and also in other types of pituitary adenomas - growth hormone secreting tumors and according to some recent studies in clinically non-functioning adenomas too. However, in non-functioning adenomas the roles of dopamine agonists treatment remain uncertain and result of this treatment in clinical series are conflicting. The aim of this project is quantitative detection of dopamine receptors expression on cells of pituitary adenomas indicated for surgical treatment, using quantitative PCR method to measure receptor mRNA. The receptors expression will be correlated with clinical and radiological characteristics and with hormone expression, detected by immunocytochemistry. Our goal is to learn, which patients would have profit from dopamine agonists treatment in the case of tumor recurrence after surgery.

Actually we are in the phase of collecting pituitary tumors from two clinics of neurosurgery – from Hradec Králové and from Central Military Hospital in Prague. Until now, we have received 29 specimens. Pituitary tissue mRNA isolation from stabilization solution – RNAlater by Trizol reagents was successfully performed. The isolation of D2 receptor mRNA in sufficient amount to enable its cloning and designing a calibration curve is pro progress.

Project is supported by the Charles University Grant Agency, No 79008.

Address for correspondence: Filip Gabalec, University Hospital, 2nd Department of Internal Medicine – Clinical Hematology, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Physiological modelling of nitric oxide bioavailability and effects in the airways

Grant Agency: Ministry of Education ***Project Number:*** 1P05C066

Principal Investigator: J. Chládek

Co-investigators: Z. Havlínová, J. Martínková, M. Hroch

Starting date: 1.1.2004 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 2800

Summary of 2008 results

Title of the presentation: Methodological issues related to exhaled nitric oxide measurement in children

Authors: Jiřina Chládková (1), Zuzana Havlínová (2), Irena Krčmová (3), Tomáš Chyba (1) Jaroslav Chládek (2,4)

Faculty of Medicine, Charles University, and Teaching Hospital, Hradec Kralove: (1) Department of Pediatrics, (2) Department of Pharmacology, (3) Department of Clinical Immunology and Allergology, (4) Department of Medical Biochemistry

In a prospective clinical study with 82 children and adolescents (44 males) with corticosteroid-treated allergic rhinitis and/or asthma, several techniques for measurement of fractional exhaled nitric oxide (FENO) were developed and compared. In agreement with the published guidelines, different techniques and evaluation methods were used in younger (4.9-11.8 yr, N=36) and older children (12.2-18.7 yr, N=46), respectively. Regarding the single-breath chemiluminiscence measurement at the expiratory flow rate of 50 mL/s performed with the Ecomedics analyzer in children younger than 12 yr, the guideline-derived plateau FENO concentrations evaluated at variable time intervals of exhalation were 34% higher than the concentrations from the fixed time interval of 2-4 s used by the software of the analyzer. Contrary to that, the FENO concentrations of older children calculated in the fixed interval of 7-10 s represent the plateau values. In both groups of children, the mean difference between the FENO concentrations from Ecomedics and the results of electrochemical monitor NIOX MINO was less than 21%. In individual patients however, a higher than 100% difference can not be excluded. Geometric mean (range) FENO values obtained using the tidal breathing technique achieved 5.1 (1.0 - 32) ppb in older group, and 3.0 (0.3 - 30) ppb in younger group, respectively. Compared to single exhalation at 50 mL/s, the concentrations were 3 to 4-fold lower but the results of both techniques were highly correlated ($r=0.84$, $p<0.0001$ in younger, and $r=0.90$, $p<0.0001$ in older children, respectively).

Address for correspondence: J. Chládek, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Šimkova 870, 500 38 Hradec Kralove, Czech Republic.

Title of the project: Influence of extracellular matrix on gene expression in liver myofibroblasts

Grant Agency: Charles University ***Project Number:*** 86/2006 C

Principal Investigator: A. Jiroutová

Co-investigators: J. Kanta, L. Majdiaková, M. Hajzlerová

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 723

Summary of 2008 results

Title of the presentation: *Expression of matrix metalloproteinases in liver myofibroblasts.*

Authors: A. Jiroutová, J. Kanta, L. Majdiaková, M. Hajzlerová
Fac. Med., Charles Univ., Hradec Králové, Dept. of Medical Biochemistry

Hepatic stellate cells (HSC) and liver myofibroblasts (MFB) are the cells responsible for the synthesis of most connective tissue in fibrotic liver. In liver tissue, they are surrounded by extracellular matrix (ECM) which can affect their behavior. In the present study we investigated the influence of 3D extracellular matrix environment – collagen type I and fibrin gel on gene expression in MFB. In the 3rd year of our grant we focused on the activation of matrix metalloproteinases (MMPs). MMPs are secreted and membrane anchored enzymes which are able to degrade all components of extracellular matrix (ECM) and play important role in the development of liver cirrhosis.

MFB were isolated by 4fold passaging HSC fraction obtained by centrifugation on an Optiprep gradient. They were then transferred on fibrin or collagen type I gels and overlayed with another layer of the protein. Previously, total cellular RNA was isolated and analyzed by cDNA arrays, and the results were validated by real-time RT-PCR. This year we aimed to confirm the upregulation of MMPs at protein level. We used immunocytochemical analysis for this purpose. The cells embedded in gels were fixed with cold acetone and then stained for MMP 2, 9 and 13 using polyclonal antibodies.

Using immunofluorescence in MFB in collagen gels we observed strong positivity for MMP 2, 9, 13, positivity for MMP 2 in fibrin gels and no positivity in cells on plastic.The activation of MMPs leads to breakdown of gels.

MFB produced gelatinase A and collagenase 3, which are able to cleave collagenous and fibrin matrix. We suggest that MFB could contribute to liver tissue remodelling in vivo.

This work was supported by The Charles University Grant Agency, No 86/2006/C

Address for correspondence: A. Jiroutova, Department of Medical Biochemistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic.

Title of the project: Antiproliferative effects of zinc in colon cancer cell lines

Grant Agency: Charles University ***Project Number:*** 132808/2008C

Principal Investigator: S. John

Co-investigators: E. Rudolf

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 246

Summary of 2008 results

Title of the presentation: Antiproliferative effects of zinc in colon cancer cell lines

Authors: S. John, L. Klvačová, M. Červinka, E. Rudolf

Zinc is a bioelement involved in many aspects of cellular physiology. Pertubations in zinc metabolism due to changes in its external or internal concentrations may seriously affect cellular growth, proliferation and signaling, ultimately leading to cell cycle arrest and cell death. Colon cancer is among the leading types of cancers in western populations, and in particular in the Czech Republic. Several studies have shown the involvement of zinc in colorectal carcinogenesis. Thus the aim of this study was to investigate a potential chemopreventive role of externally supplemented zinc on growth and proliferation of colon cancer cell lines representing different stages of this malignancy: HCT-116, HT-29 and SW620. The results suggest that that there are differences in sensitivity of colon cancer cells to zinc and that zinc inhibits cell growth and proliferation possibly via several specific and nonspecific mechanisms including cytoskeleton and cell cycle checkpoints.

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Title of the project: Examination of differentiation potential of dental pulp stem cells

Grant Agency: Czech Republic ***Project Number:*** 304/07/P307

Principal Investigator: J. Karbanová

Co-investigators:

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1167

Summary of 2008 results

Title of the presentation: Endothelial differentiation of human dental pulp stem cells isolated from impacted third molars

Authors: J. Karbanová (1), T. Soukup (1), J. Suchánek (2), J. Mokrý (1)
(1)Dept. of Histology and Embryology, Fac. Med. Hr. Králové, Charles Univ. in Prague, (2) Dental Clinic, Univ. Hospital in Hr. Králové, Czech Republic

Previously we isolated stem cells from dental pulp of extracted third molars using innovative culture method of serum-low-content medium supplemented with growth factors PDGF-BB and EGF. These cells exhibit an immature phenotype, i. e. they express many stem cell associated markers like nanog, nucleostemin, nestin, CXCR4, β1-integrin, Bcrp1 and MDR1 and share antigenic properties of neural and mesenchymal stem cells (nestin, nucleostemin, STRO-1). In the present study we evaluated differentiation potential of dental pulp stem cells (DPSCs) towards endothelial cells. DPSCs were cultured in serum-free medium supplemented with vascular endothelial growth factor (VEGF) and optionally with bFGF growth factor and ITS supplement. Their phenotype was evaluated immunocytochemically after 7 and 16 days of differentiation. When placed to the serum-free differentiation medium cells stopped their proliferation and changed their morphology. Originally flattened large cells become elongated spindle or triangular shaped and tent to form chains. After the 7 days cells were found to express endothelial markers vWF, CD31, CD34, endoglin, VCAM1, VEGF-R1, -R2, c-kit and nestin. They also bound lectin GSA-IB4. After 16 days the expression of CD31, CD34 and endoglin was downregulated. The addition of ITS and bFGF to medium was found beneficial as it increased survival of differentiated cells and enhanced their differentiation. If the cells were replated after 7 days of culture in differentiation medium to 10% FCS and VEGF containing medium, cells maintained endothelial phenotype and continued again in proliferation. Our results demonstrate that under suitable in vitro conditions DPSCs can be induced to differentiate in cells with antigenic properties characteristic for endothelial cells. Moreover these cells are further expandable in serum-supplemented medium. The ability of DPSCs to generate vascular cells can be beneficial for future use in transplantation stem cell therapy.

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Title of the project: The influences of standard preoperative starvation and thirst on general patients conditions.

Grant Agency: Ministry of Health ***Project Number:*** NR/8037-5

Principal Investigator: M. Kaška

Co-investigators: T. Grosmanová, E. Havel, D. Bareš, P. Bareš, M. Brtko, R. Hyšpler, V. Tošnerová, Z. Petrová, B. Schusterová, M. Sluka, L. Pyszková

Starting date: 1.1.2004 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 3383

Summary of 2008 results

Title of the presentation: Preoperative drinking – positive influence on a physiological functions and safety for elective surgical patients.

Authors: Milan Kaška (1), Taťána Grosmanová (2), Eduard Havel (1), Radomír Hyšpler (1), Zbyňka Petrová (2), Miroslav Brtko (1), Bronislava Schusterová (2), Lucie Pyszková (2), Vlasta Tošnerová (1), Martin Sluka (2)

1 - Medical Faculty and Teaching Hospital, Hradec Králové, 2 - Teaching Hospital, Olomouc

Methods.The group of 241 patients in this bicentric, randomised, prospective, and blinded clinical trial was divided into three groups according to their preparation for operation: A – patients fasting from midnight; B – patients supported by 10% glucose, magnesium and potassium; and C – patients supported preoperatively by oral consumption of a specifically-composed solution [“potion”]. The study was performed on surgical patients with malignant or benign disease of the large bowel, with the constraints of no peri-operative or postoperative complication, no significant co-morbidity (ASA≤II –American Society of Anaesthesiologists classification of physical status), no diabetes mellitus, no peri-operative blood transfusion etc. Biochemical parameters, muscle power, stomach condition, psychosomatic parameters, and heart function were evaluated.

Results. The peri-operative conditions of patients in group C were better than those in group B and very substantially better than those in group A. The best results in psychosomatic status, in some biochemical, heart and muscle parameters, and in stomach physiology were found in group C. Significant differences were in the serum concentrations of insulin and QUICK index. In other evaluated biochemical markers the best results were found in group C but without statistical significance. The best psychosomatic outcome was achieved significantly in group C with good statistical significance. The heart functions were best in group C during the peri-operative period.

Conclusions. Preoperative fasting does not confer any benefit on either patients or medical staff. In contrast, consumption of an appropriate potion composed of water, minerals and high molecular weight sugar confers protection to patients against surgical trauma.

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Title of the project: Examination of function and structure of cilia of nasal respiratory epithelium stable chronic obstructive pulmonary disease patients (CILIARY STUDY).

Grant Agency: Ministry of Health ***Project Number:*** NR/8407-4

Principal Investigator: V. Koblizek

Co-investigators: F. Salajka, D. Pohnetalova, M. Tomsova, K. Dedic, J. Chladek, H. Vanicek

Starting date: 1.1.2005 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3835

Summary of 2008 results

Title of the presentation:Impairment of function and structure of nasal ciliary epithelium in stable chronic obstructive pulmonary disease(COPD)patients(CILIARY STUDY).

Authors: V.Koblizek[1], F.Salajka[1],M.Tomsova[2],D.Pohnetalova[2],H.Vanicek[3],P.Papousek[4], E.Cermakova[5], J.Chladek[6] Department of Pneumology[1], The Fingerland Department of Pathology[2], Department of Pediatrics[3], Department of Pathological Physiology[4], Department of Medical Biophysics[5], Department of Pharmacology[6]

Objective:To decribe function and structure of cilia of respiratory(nasal)epithelium in subjects suffering fromCOPDand to compare them with healthy adults(HA) and adults patiens with cystic fibrosis (CF).***Study Design***:Observational descriptive cross–sectional study.***Methods***:98 stable COPD exsmok. (79male,mean 65,1y.) were randomly select from file of ourCOPDpts.COPDpts and both control groups(involve 15adultCF pts-6male,26,3y. and 39 HA non-smok.,free of allergy and ENT path.-22male,63,2y.) were evaluated about ciliary tests:saccharine test,digital high speed videomicroscopy and electronmicroscopy of samples of nasal mucosa.COPDpts were also undergone to complex of lab.exam and pulm.function. tests for verification of proven COPDdiagnosis,for the purpose of determination of COPD staging,for exclusion of still unrecognized alpha1-antitrypsin deficiency,moderate or severe imunodeficiency and less expressed adult forms of CF.***Results:*** Saccharin test [sec.] and index of ciliary dyskinesia [0-3] significantly rose from HA, to COPD and CF pts: 756, 1188, 1578 sec. and 0,82, 1,77, 2,18.Ciliary beat frequency [Hz] in COPD was significantly reduced 6,13 against HA 7,21 and CF 7,21. The presence of ciliary akinesia was more frequent in COPDpts in comparison to HA. Electron microscopy revealed an icnrease in the percent of subjects exhibiting microtubular disarrangement among severe and very severe stages of COPD. ***Conclusion:*** We detected the presence of secondary ciliary dyskinesia in patients with COPD.

Project was supported by the Ministry of Health Grant Agency, *NR8407-4/2005*

Address for correspondence: V.Koblizek, Dept.of Pneumology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Simkova 870, 500 38 Hradec Kralove, Czech Republic.

Title of the project: The study of the potential therapeutic importance of pravastatin in the treatment of the liver injury caused by acute and chronic cholestasis and biliary cirrhosis in rats

Grant Agency: Charles University ***Project Number:*** 122408/2008C

Principal Investigator: G. Kolouchová

Co-investigators: S. Mičuda, E. Brčáková, F. Štaud

Starting date: 14.4.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 240

Summary of 2008 results

Title of the presentation: The study of the potential therapeutic importance of pravastatin in the treatment of the liver injury caused by chronic cholestasis in rats

Authors: G. Kolouchova (1), S. Micuda (1), E. Brcakova (1), F. Staud (3), J. Cermanova (1), P. Hirsova (1), L. Fuksa (1), J. Mokry (2)
(1) Dept. of Pharmacology, Charles Univ., Fac. Med., Hr. Kralove, (2) Dept. of Histology, Charles Univ., Fac. Med., Hr. Kralove, (3) Dept. of Pharmacology; Charles Univ., Fac. of Pharmacy, Hr. Kralove

The presented project is focused on the evaluation of potential therapeutic usage of pravastatin in the treatment of cholestatic liver injury. It concern both detailed characterization of the drug impact on the morphology of cholestatic changes in livers and kidneys and the expression and function of enzymatic and transport mechanism involved in the elimination of anions such as bile acids and bilirubin which are cumulating during cholestasis. Simultaneously, the paracellular permeability of hepatocytes is evaluated in vivo by melibiose/rhamnose permeability test. During the first year, the in vivo experiments (induction of chronic cholestasis by bile duct ligation for 7 days and pravastatin pretreatment – 5 mg/kg and 1 mg/kg) were performed. Melibiose/rhamnose biliary permeability was measured by HPLC analysis of the bile samples. Respective samples of bile, urine, and plasma were further examined for bile acids, hepatic enzymes, HDL, LDL, TAG, kreatinin, bilirubin, glucose, and proteins. The samples of the liver were used for the determination of glutathione and malondialdehyde. Furthermore, the samples of the liver were evaluated histologically. Up to now results indicate, that pravastatin can not moderate the liver injury during extrahepatic cholestasis in rats.

The study was supported by Charles University Grant Agency, No. 122408.

Address for correspondence: G. Kolouchova, Dept. of Pharmacology, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Clinical dental implantology – educational video presentations

Grant Agency: Ministry of Education ***Project Number:*** 622/F3 d

Principal Investigator: D. Kopecká

Co-investigators: A. Šimůnek, T. Brázda

Starting date: 1.1.2008 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 100

Summary of 2008 results

Title of the presentation: Clinical dental implantology – educational video presentations

Authors: Dana Kopecká, Antonín Šimůnek, Tomáš Brázda

Fac. Med., Charles Univ., Hr. Králové: Dept. of Dentistry

The Centre for Dental Implantology of the Department of Dentistry at the Medical Faculty in Hradec Králové is the largest fully specialized workplace dealing with dental implantology in the Czech Republic. In addition to clinical implantological care it offers pre- and postgraduate training. The aim of the project is innovation and improvement of education in dental implantology with help of video.

The video presentations target the theoretical education of students of dentistry and students of general medicine. They are also integral parts of courses organized by the Department of Dentistry and other organizations in the Czech Republic and abroad.

In terms of the project, 6 videos were produced: (1) Bone splitting, (2) Mental bone graft, (3) Fixed bridge in the edentulous maxilla, (4) Immediate loading, (5) Single tooth replacement, (6) One-stage sinus lift – close to 60 minutes in length. They record step-by-step reconstruction of dentition from the surgical to the restorative view. Different surgical and restorative procedures are used. Some of these are unique and students and dentists are not able to see them elsewhere. Such films are rare in Czech dental implantology.

Address for correspondence: Dana Kopecká, Faculty Hospital, Dept. of Dentistry, 500 05 Hradec Králové

Title of the project: The clinical significance of angiogenic markers in patients with renal cell carcinom

Grant Agency: Ministry of Health ***Project Number:*** NR/8914-4

Principal Investigator: O. Kopecký

Co-investigators: Š. Lukešová, V. Vroblová, D. Vokurková, C. Andrýs, P. Morávek, M. Podhola, H. Šafránek

Starting date: 1.1.2006 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 4600

Summary of 2008 results

Title of the presentation: The Correlation of Soluble Angiogenic Factors and Clinical Stage of Renal Cell Carcinoma

Authors: O. Kopecký (1), Š. Lukešová (1), D. Hlávková (2), V. Vroblová (2), C. Andrýs (2), P. Morávek (3), E. Čermáková (4)

Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Králové: Second Department of Internal Medicine (1), Institute of Clinical Immunology and Allergology (2), Department of Urology (3), Computer Technology Center (4)

The aim of the study: To determine the selected serum angiogenic factors in 54 patients with newly diagnosed renal cell carcinoma (RCC) and to correlate them with the clinical stage of the disease.

Results: In case of GROα, a significant difference was found between patients with and without progression period (p = 0.006), between surviving and dead patients (p = 0,038), between the1st and the 4th grade of HPG (p = 0.05), between the 2nd and the 4th grade of HPG (p = 0.0043) and between the 3rd and the 4th grade of HPG (p = 0,044). A statistically significant difference in serum concentration of IL-8 was found between patients with and without progression (p = 0,007), but no differences were found between the dead and surviving patients and between the various grades of HPG. A statistically significant differences in serum concentration of IL-6 were found between patients with and without progression (p = 0.0006) between dead and surviving patients (p = 0.0042), between the1st and the 4th grade of HPG (p = 0.05) and between the 2nd and the 4th grade of HPG (p = 0.0041). A statistically significant difference in serum levels of VEGF was found between patients with and without progression (p = 0.0026), between dead and surviving patients (p = 0,021). A statistically significant difference in serum levels of bFGF was found between dead and surviving patients (p = 0,024).

This study was supported by the grant from the Internal Grant Agency of the Czech Republic Ministry of Health: NR/8914-4.

Address for correspondence: O. Kopecký, Dept. of Haematology, University Hospital in Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Chronic disorders induced by deregulated reactivity of immune system; their pathogenesis, diagnosis and treatment

Grant Agency: Ministry of Education ***Project Number:*** 0021620812

Principal Investigator: J. Bartunkova

Co-investigators: J. Krejsek and co-workers

Starting date: 1.1.2005 ***Duration (years):*** 6

Total funds allocated for project - Kč (thousands): 1600

Summary of 2008 results

Title of the presentation:

Authors:

Krejsek J (1), Kunes P (2), Kolackova M (1), Kudlova M (1), Lonsky V (2), Mandak J (2), Andrys C (1): The expression of Toll-like receptor 2 and 4 on innate immunity cells is modulated by cardiac surgical operation. Scand J Clin Lab Invest, 2008; 68(8): 749-758. IF=1,235

Kolackova M (1), Kudlova M (1), Kunes P (2), Lonsky V (2), Mandak J (2), Andrys C (1), Jankovicova K (1), Krejsek J (1): Early expression of FcgammaRI (CD64) on monocytes of cardiac surgical patients and higher density of monocyte anti-inflammatory scavenger CD163 receptor in “on-pump” patients. Med Inflam, 2008; doi:10.1155/2008/235461. IF=1,162

Kunes P (2), Krejsek J (1), Brtko M (2), Mandak J (2), Kolackova M (1), Trojackova Kudlova M (1), Andrys C (1): Neutrophil apoptosis by Fas/FasL: harmful or advantageous in cardiac surgery? Thorac Cardiovasc Surgeon, 2008. IF=0,741 (in press)

Kunes P (2), Mandak J (2), Kolackova M (1), Krejsek J (1): Mystery of pentraxin-3 not yet resolved: still a long way to its prime in surgery. Nephrol Dial Transplant, 2008. IF=3,167 (in press)

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(2) Department of Cardiac Surgery

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Title of the project: New variants of visual evoked potentials for diagnostics of functional disorders of CNS

Grant Agency: Ministry of Health ***Project Number:*** NR/8421-4

Principal Investigator: M. Kuba

Co-investigators: Z. Kubová, J. Kremláček, F. Vít, J. Langrová, J. Szanyi

Starting date: 1.1.2005 ***Duration (years):*** 4

Total funds allocated for project - CZK (thousands): 1228

Summary of 2008 results

Title of the presentation: Recommendation for implementation of motion-related visual evoked potentials (VEPs) into clinical neurophysiological examinations

Authors: M. Kuba, J. Kremláček, Z. Kubová, J. Szanyi, J. Langrová, F. Vít
Dept. of Pathophysiology, Charles University - Faculty of Medicine in Hradec Králové, Czech Republic; http://www.lfhk.cuni.cz/ELF

After completion of testing of motion-onset VEPs methods a set of pilot studies was accomplished (see http://www.lfhk.cuni.cz/ELF/elf_publications.html) to verify facilities of the extended VEP examination for diagnostics of CNS disorders.
Since the use of visual moving stimuli enables an early recognition of the magnocellular system and/or the dorsal stream disorders of the visual pathway, introduction of the new method into the set of standard examinations in neuro-ophthalmology was suggested.
Our Electrophysiological lab provides the needed software and assistance for new implementations.

Supported by Ministry of Health of the Czech Republic (Grant NR8421-4/2005).

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Title of the project: Pathophysiology of neuro-psychiatric disorders and its clinical applications

Grant Agency: Ministry of Education ***Project Number:*** 0021620816–4a

Principal Investigator: M. Kuba

Co-investigators: Z. Kubová, J. Kremláček, F. Vít, J. Langrová, J. Szanyi

Starting date: 1.1.2005 ***Duration (years):*** 7

Total funds allocated for project - CZK (thousands): 4500

Summary of 2008 results

Title of the presentation: Visual mismatch negativity examination for cognitive deficit detection

Authors: M. Kuba, J. Kremláček, J. Szanyi, Z. Kubová, J. Langrová, F. Vít
Dept. of Pathophysiology, Charles University - Faculty of Medicine in Hradec Králové, Czech Republic.

As a part of a complex testing of visual information processing, the visual modality of mismatch negativity examination (using motion-direction detection paradigm) has been introduced in our Electrophysiological lab (http://www.lfhk.cuni.cz/elf). Together with evaluation of P300 parameters in event related evoked potentials, it might contribute to the objective assessment of a cognitive deficit in some neuro-psychiatric disorders.
In co-operation with Psychiatric Clinic of our Faculty Hospital, we verified sensitivity of various types of visual evoked potentials, including visual mismatch negativity,
for detection of the suspected cognitive deficit in the group of methamphetamine-dependent individuals and in the group of patients with schizophrenia (1,2,3).
In both applications the obtained results indicate that the visual mismatch negativity could be used for quantitative analysis of changes in visual cognitive functions.

References:

1. Kremláček J, Hosák L, Kuba M, Libiger J, Cizek J. (2008).Visual information processing in recently abstaining methamphetamine-dependent individuals: Evoked Potentials study. Documenta Ophthalmologica, 117, 245-55.
2. Hosák L, Kremláček J, Kuba M, Libiger J, Cizek J. (2008).Mismatch negativity in methamphetamine dependence: A pilot study. Acta Neurobiol Exp, 68, 97-102.
3. Urban A, Kremláček J, Masopust J, Libiger J. (2008). Visual mismatch negativity among patients with schizophrenia. Schizophrenia Research, 102, 320-328.

Supported by Ministry of Education of the Czech Republic (VZ 0021620816).

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Title of the project: Is the liver affected by non-alcoholic liver disease (NAFLD) more sensitive to toxic injury?

Grant Agency: Czech Republic ***Project Number:*** 305/08/P184

Principal Investigator: O. Kučera

Co-investigators:

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1419

Summary of 2008 results

Title of the presentation: Model of Non-Alcoholic Fatty Liver Disease in Rats for Toxicological Studies

Authors: O. Kučera (1), P. Křiváková (1), T. Roušar (1), T. Garnol (1), R. Bolehovská (1), M. Hroch (2), Y. Mazurová (3), H. Lotková (1), Z. Červinková (1)
Charles University in Prague, Faculty of Medicine in Hradec Králové: Department of Physiology(1), Department of Pharmacology(2), Department of Histology and Embryology(3)

Non-alcoholic fatty liver disease (NAFLD) is a common condition associated with metabolic syndrome and represents a wide spectrum of liver affections ranging from benign hepatic steatosis, to non-alcoholic steatohepatitis (NASH) that may progress to cirrhosis and even to end-stage liver disease.
The aim of this project was to introduce and describe a suitable nutritional model of NAFLD on rats for comparison of toxic effects of model hepatotoxins on intact liver and liver affected by NAFLD.
Wistar and Sprague-Dawley male rats were fed ad libitum a standard pelleted diet (ST-1, 10% of energy from fat); medium-fat gelled diet (MFGD, 35% of energy from fat) and high-fat gelled diet (HFGD, 71% of energy from fat) for 3 and 6 weeks. Then serum ALT, AST, glycaemia, total bilirubin, levels of triacylglycerols (TAG) and cholesterol were measured. Respiration of isolated liver mitochondria was assessed using high-resolution respirometry. Expression of UCP-2 (uncoupling protein 2) cDNA in liver samples was determined by real-time PCR (ABI Prism 7900HT Instrument). Malondialdehyde (MDA) content and reduced glutathione (GSH) in the liver and tissue cytokines (IL-6 and TGFbeta1) were measured and histopathological samples were prepared (H+E, Sudan III).
Feeding with HFGD for 6 weeks in both strains (less in MFGD and for 3 weeks) induced periportal small-droplet steatosis with very mild focal inflammation without necrosis in comparison with control group (ST-1). There were no significant differences among groups in serum biochemical parameters except for lower concentration of TAG in MFGD and especially in HFGD against ST-1 after 3 and 6 weeks. There was a significant increase in expression of UCP-2 and tissue IL-6 (but not TGFbeta1) in groups fed by diets with higher fat content in comparison with ST-1.
This work was supported by Grants GAČR 305/08/P184 and MSM 0021620820.

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Title of the project: Pathophysiology of neuropsychiatric disorders and its application in clinical practice

Grant Agency: Ministry of Education ***Project Number:*** 0021620816-4c

Principal Investigator: R. Rokyta

Co-investigators: J. Libiger

Starting date: 1.1.2005 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 827

Summary of 2008 results

Title of the presentation: Diagnostic and therapeutic issues in early schizophrenia

Authors: Libiger J, Urban A , Hons L, Masopust J, Tůma I, Hosák L

Department of Psychiatry, Charles University Medical School and Faculty Hospital Hradec Králové, Hradec Králové, CZ- 500 05, Czech Republic

The research support funds served to focus on two major issues : the role of visually evoked "mismatch negativity" in detecting and eventually classifying subtypes of schizophrenia and the significance of excitatory amino acids (glycine, serine and D- serine) plasma levels for the symptomatic profile of patients with schizophrenia. We confirmed that there is a deficit in generation of the an ERP phenomenon "mismatch negativity"(MMN) to aberrant visual motion stimuli, that corresponds with a similar phenomenon evoked by aberrant auditory stimuli. This is a new finding that rises questions as to the cognitive nature of the MMN phenomenon. We also collected data on excitatory amino acids (EAA) in plasma of patients with schizophrenia and their relatioship to clinical symptomatology monitored by the PANSS instrument. We found a significant negative correlation of the negative symptoms score and glycine serum levels. However, we did not detect the hypothesized association of low D-serine levels and negative symptoms and also our data do not support the putative association of low D- serine levels with schizophrenia among patients in comparison to healthy individuals. The third main focus of the research effort within the framework of the research programme was the identification of thromboembolic risks associated with an effect of antipsychotic medication and work on guidelines to avoid it, as well as monitoring the metabolic adverse effects after antipsychotics active on multiple receptors (MARTAs) .

References:

Urban A., Kremláček J., Masopust J., Libiger J.: Visual mismatch negativity among patients with schizophrenia. Schizophrenia Research 102 (2008) 320-328.

Hons J., Žirko R., Ulrychová M.,et al.: D-serine serum levels in patients with schizophrenia: Relation to psychopathology , Neuroendocrinology Letters, 2008;29:485-492

Address for correspondence: prof. Jan Libiger, Psychiatric Clinic and Dept. of Psychiatry, Charles University Medical School and Faculty Hospital, Hradec Králové, CZ-500 05 Czech Republic

Title of the project: Quality of vision in premature children

Grant Agency: Charles University ***Project Number:*** 36007/2007C

Principal Investigator: H. Adámková

Co-investigators: D. Liláková, D. Hejcmanová

Starting date: 1.1.2007 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 414

Summary of 2008 results

Title of the presentation: Quality of vision in premature children

Authors: D. Liláková (1), D. Hejcmanová (1), H. Adámková (2)

Fac. Med., Charles University and University Hospital Hradec Králové:Dept of Ophthalmology (1), Department of ophthalmology, Hospital Pardubice (2)

The aim of this study is to document the quality of visual functions of children born prematurely with a weight bellow 1500 g (visual acuity, refraction errors, spherical equivalent, contrast sensitivity, axial length, binocularity and strabismus). The best corrected visual acuity was lower than in the control group. We also describe worse results of contrast sensitivity in premature children than in the control group. The biggest difference in comparison with the control group was found on the level of 4.0 c/deg. Statistically significant difference was found out in measurement of axial length. Mean axial length was 22.31 mm and 23.18 mm in control group. Refractive errors were more common in group of premature children. Only 48.05% of premature children did not wear glasses, in a group of full term children almost 95% of patients did not wear glasses. Strabismus was found in 19.99% of premature children. General healthy problems were detected in 26.32% of premature children. More serious visual problems were higher in children with neurological disorders. Visual functions of premature children are worse than those of full term children. We detected higher frequency of rerefractive error. The most common is hypermetropia. Myopia is more frequent in children with neurological disorders and in children after cryotherapy of ROP. Also strabismus is more common in the group of premature children, exotropia was often detected. Contrast sensitivity is statistically significant lower even in premature children, who did not suffer from the retinopathy of prematurity.

The project was supported by the Charles University Grant Agency, No 7360/2007/C

Address for correspondence: D. Liláková, Dept. of ophthalmology, University Hospital, Hradec Králové, 500 05

Title of the project: The use of miniinvasive extracorporeal circuit (mini-CPB) in cardiac surgery

Grant Agency: Ministry of Health ***Project Number:*** NR/9090-4

Principal Investigator: V. Lonský

Co-investigators: J. Manďák, J. Harrer, M. Bartoš, P. Kuneš, J. Kubíček, M.Volt, P.Valentová

Starting date: 1.1.2006 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3916

Summary of 2008 results

Title of the presentation: THE IMPACT OF THE CARDIAC SURGERY ON LEUKOCYTE EXPRESSION OF FCΓ RECEPTOR (CD64) AND THE SCAVENGER RECEPTOR (CD163)

Authors:V.Lonský*, J.Krejsek, M.Kudlova, M.Kolackova, J.Manďák, J.Kubíček, M.Volt, M.Bartoš J.Harrer, P.Valentová

BACKGROUND: The cardiac surgical intervention is accompanied by the activation of innate immunity arm. This feature is associated with both pro-inflammatory and anti-inflammatory changes. The increase in the expression of a high affinity receptor for Fc fragment of immunoglobulins – CD64 and the scavenger receptor for haemoglobin-haptoglobin complexes – CD163 have been recently identified to be involved in the immunopatogenesis of sepsis and post-infection recovery phase. The changes of these cell surface receptors were studied during and after cardiac surgery.

METHODS: 54 patients who underwent isolated CABG were divided into three groups (each of 17 patients) according to the different surgical approach (OFF PUMP, routine CPB, mini CPB). Peripheral venous blood samples were collected before and after surgery and at the 1st, 3rd and 7th postoperative day.The receptors were detected by double fluorescence staining using flow cytometry.

RESULTS: The earliest changes were observed in the decreased expression of anti-inflammatory molecule CD163 on monocytes after finishing of CPB. The expression of CD163 was decreased on monocytes and granulocytes after finishing surgery in all 3 groups. The highest level of both receptors CD163 and CD64 on monocytes was found at the 1st postoperative day in all 3 groups whereas the subsequently activated granulocytes expressed the highest level of the receptors at the 3rd postoperative day. There were no differences between all groups except more significantly decreased expression of CD163 on monocytes after finishing surgery in on-pump patients group.

CONCLUSIONS: The results indicate that the activation of immunity due to surgical intervention is self regulating process which is balanced with changes in expression of pro- and anti-inflammatory parameters. The main impact of different surgical approaches was found when conventional extracorporeal circuit was used.

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Title of the project: Optimalization of the therapy of multiple myeloma patients in Czech republic

Grant Agency: Ministry of Health ***Project Number:*** NR/9225-3

Principal Investigator: I. Špička

Co-investigators: V. Maisnar, J. Straub, M. Krejčí, J. Bačovský, E. Gregora

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 6085

Summary of 2008 results

Title of the presentation: The Registry of Monoclonal Gammopathies - RMG

Authors: V. Maisnar (1), J. Straub (2), M. Krejčí (3), J. Bačovský (4), E. Gregora (5), I. Špička (2)

2nd Dept. of Medicine - Clin. Haematology, Faculty Hospital Hradec Králové (1), 1st Dept. of Medicine, General Faculty Hospital Prague (2), Hemato-oncological Dept., University Hospital Brno (3), 3rd Dept. of Medicine, Faculty Hospital Olomouc (4), Dept. of Clinical Haematology, Univ. Hospital Prague (5)

The purpose of the project is the prospective data analysis of multiple myeloma patients in Czech republic, including incidence of disease, therapeutical modalities used, the results of treatment and the most frequent adverse events of the therapy. The principal aims are the analysis of the factors which could influence the long-term results of both the first-line treatment and therapy of relapsed/refractory disease, evaluation of the effect of consolidation chemotherapy vs. standard maintenance therapy and the economic aspects of the different therapeutical protocols. Further, the benefit of standard and new prognostic factors for the myeloablative regimens and new drugs (thalidomide, bortezomibe) protocols will be analyzed to propose the risk-adapted and cost-effective therapeutical approach for patients with different prognosis. The analysis of the benefit of early diagnosis, profylaxy of the most frequent complications of both the disease and therapy will be performed as well. The long-term, prospective analysis of the data of patients with single cancer could be started due to the close cooperation of the principal hematological centers and the working group of these centers in Czech republic. The Registry of Monoclonal Gammopathies = RMG was started from 1. 5. 2007. Currently 1090 patients with monoclonal gammopathies are registered: 544 patients with multiple myeloma and 546 patients with monoclonal gammopathies of undetermined significance (64 patients with MM and 110 with MGUS in Hradec Králové).

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Title of the project: Monitoring of biochemical changes in skeletal muscle during coronary surgery and during postoperative care using interstitial microdialysis

Grant Agency: Ministry of Health ***Project Number:*** NR/8944-3

Principal Investigator: J. Manďák

Co-investigators: V. Lonský, P. Živný, V. Palička, M. Pojar, J. Kubíček, D. Kakrdová

Starting date: 1.1.2006 ***Duration (years)*** 3

Total funds allocated for project - Kč (thousands): 2122

Summary of 2008 results

Title of the presentation: Biochemical changes in skeletal muscle during cardiac surgery with or without cardiopulmonary bypass - a microdialysis study

Authors: J. Manďák, V. Lonský, P. Živný, V. Palička, M. Pojar, J. Kubíček, D. Kakrdová

Serious complication of cardiac surgery using cardiopulmonary bypass (CPB) could be a hypoperfusion of peripheral tissues. The aim of this study was to monitor and to compare the metabolism changes in the skeletal muscle during the cardiac operations in CPB and operations without CPB by means of interstitial microdialysis. Surgical revascularization (CABG) was performed in 40 patients. 20 patients (group On-pump) were operated using CPB, 20 patients (group Off- pump) without CPB. Interstitial microdialysis was performed by special probes inserted into the patient´s deltoid muscle. Microdialysis measurements were performed at 30 minutes intervals. Glucose, lactate, pyruvate and glycerol were measured in samples using. Dynamic changes of interstitial concentrations of the measured analytes were found in the both groups of patients during the operation. There was no significant difference in dialysate concentrations of glucose and lactate between the groups. Significant difference was detected in pyruvate and glycerol concentrations, lactate/pyruvate ratio and lactate/glucose ratio between On-pump vs. Off-pump patients. Pyruvate concentrations were higher in Off-pump group and the values of the concentrations of glycerol were lower in Off-pump group. The lactate/pyruvate ratio and the lactate/glucose ratio indicating the aerobic and anaerobic metabolism status were lower in Off-pump group.

The dynamic changes in the interstitial concentrations of the glucose, glycerol, pyruvate and lactate were found in the both groups of patients (On-pump and Off-pump). These results showed significantly higher aerobic metabolic activity of the peripheral tissue of patients in Off-pump group vs. On-pump group during the course of cardiac revascularization surgery. Results suggest that extracorporeal circulation, cardiopulmonary bypass compromises peripheral tissue (skeletal muscle) energy metabolism.

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Title of the project: Quality of life in children and adolescents

Grant Agency: Czech Republic ***Project Number:*** 406/06/0035

Principal Investigator: J. Mareš

Co-investigators: M. Rybářová, D. Skorunka, L. Hadaš, L. Hodačová, J. Marešová; S. Ježek, B. Koukola, E. Ondřejová; T. Svatoš; M. Dostálek

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1086

Summary of 2008 results

Title of the presentation: Quality of life in children and adolescents

Authors: J.Mareš (1), M. Rybařová (1), D. Skorunka (1), L. Hadaš (1), L. Hodačová (1), J. Marešová (2)

Fac.Med. , Charles Univer., Hradec Král.: Dept. Social Medicine (1), Dept. Pediatrics (2)

Review studies: Six theoretical frameworks for studying QL including its definition and assessment were reviewed. Currrent approaches in research of health-related quality of life (HRQL) were outlined. Several aspects related to childhood and adolescence were depicted including 35 generic methods and qualitative approaches used with children and/or adolescents. **Empirical studies** were carried out in 6 different population groups: 1. 2242 healthy children and adolescents under the age of 15; 2.1701 healthy adolescents in the age of 16-19; 3. 211 ill children (50 children with oncological disease, 55 children with severe scoliosis, 50 children with vision impairment, 61 children with epilepsy); 4. 41 parents of children with vision impairment; 5.138 students of Faculty of Education; 6. 240 students of Faculty of Medicine. **Methods:** internationally accepted questionnaire such as SeiQoL, QLQ, PedsQL 4.0, ComQol-S5, BSFC, HRQoLCE, various qualitative methods including the computer analysis of interviews. Open questionnaire aimed at child´s preconception of QL term and content analysis of childrens´statements. **Results:** Thorough exploration of childrens´ideas of excellent, normal and bad QL in two age groups: 8-11 and 12-15. Variations in these were also influenced by age and ethnicity. Children´s perceived quality of life is more intense than adult assume. **Outcome:** 6 peer reviewed collection of papers, 2 succesfully defended doctoral thesis, 2 articles in a journal with IF (the third is in press), 8 articles in peer reviewed journals, 60 texts in peer reviewed collections of papers, translated and standardised methods, developed 3 original methods, 15 oral presentations abroad and 48 at national conferences. Contract with Grada Publishing Prague for a monography.

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Title of the project: Pharmacokinetic dosage optimization of gentamicin in neonates

Grant Agency: Ministry of Health ***Project Number:*** 1A/8671

Principal Investigator: J. Martínková

Co-investigators: P. Pokorná

Starting date: 10.10.2005 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 548

Summary of 2008 results

Title of the presentation: Therapeutic drug monitoring (TDM) of gentamicin in neonates critically ill during the first week of life.

Authors: Jiřina Martínková (1), Pavla Pokorná (2), Jiří Záhora (3), Jaroslav Chládek (1), Václav Vobruba (2)

Fac. Med., Charles Univ., Hr. Králové, Dept. of Pharmacology (1), Dept of Med. Biophysics (3), General Teach.Hospital, Prague: Dept. of Pediatrics and Adolesc.Medicine(2)

The standard dosage regimen of gentamicin (GE) used to treat critically ill neonates during the first week of life is traditionally based on birth weight and gestational age. After the first dose, peak plasma GE concentrations (Cpeak) within the range of 7-8 mg/l are recommended for rapid bacteridical effects. In this study, individual values of pharmacokinetic (PK) parameters were estimated after the first standard dose by compartmental modelling according to the maximum a posteriori Bayesian fitting method, using the MWPHARM 3.15 computer program and four plasma concentrations assayed by immunochemistry (TDx Abbott). The concentration-time profile was predicted in each child and the dosage regimen was individualized in order to reach the target therapeutic range of 6-10 mg/L for Cpeak, and < 2 mg/L for Ctrough at the steady state. The individual regimen differed from the standard one in 46 out of 60 neonates (77%). The rate of dosing had to be decreased in 45 neonates while in other two a higher rate was needed. TDM at the steady-state showed that in 50% of neonates, the PK-guided dosing resulted in the Cpeak below the target range. Overprediction of the true steady-state Cpeak was associated with a low volume of distribution and/or low renal clearance of GE after the first dose. Several covariates such as fluid accumulation, cardiovascular failure, asphyxia, hyperdynamic circulation, renal function, and others may undergo dramatic changes during the first week of life and strongly influence PK of GE. Therefore, individual dosing guided by the PK parameters after the first dose and predictions of the steady-state concentrations are prone to errors. Improvement in efficacy and safety of GE therapy of critically ill neonates during the first postnatal week requires active TDM based on population kinetic models which include time-dependent changes of main covariates.

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Title of the project: Changes in pharmacokinetics and pharmacodynamics of drugs in the presence of impaired liver function

Grant Agency: Ministry of Education ***Project Number:*** 1P05OC062

Principal Investigator: J. Martínková

Co-investigators: S. Mičuda

Starting date: 1.1.2005 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 2800

Summary of 2008 results

Title of presentation: Monitoring of changes in drug pharmacokinetics and dynamics due to impaired hepatic function

Authors: E. Brčáková, S. Mičuda, L. Fuksa, J. Cermanová, G. Kolouchová, P. Hiršová, J. Chládek, M. Hroch, J. Martínková, F. Štaud

Dpt.of Pharmacology, Charles University in Prague, Faculty of Medicine in Hradec Králové

The goal of the phase was to evaluate the impact of two models of cholestasis on the pharmacokinetics of methotrexate (MTX) in rats. MTX is an acidic drug used for cytostatic, antiinflammatory and imunosuppressive action. Its efficacy and safety is dependent on plasma concentration which in turn is determined by elimination (described by total clearance - CLtot) effectuated by both renal clearance (CLren- including glomerular filtration, active tubular secretion, and passive reabsorption) and hepatic clearance (CLhep consisted of biliary clearance CLbil and metabolic transformation to 7-OHMTX). The extrahepatic cholestasis (ECHO) was induced by bile duct obstruction for one and seven days (acute and chronic phase). Intrahepatic cholestasis (ICHO) was obtained 18 hr after i.p. administration of lipopolysaccharide (endotoxin isolated from Salmonella Typhimurium, 4 mg/kg). ICHO is considered a symptome of septic syndrome - the response to an inflammatory agent. MTX CLbil dropped significantly to 12% and 5% of control value in rats during both acute and chronic phase of ECHO, respectively, while CLtot was reduced only to 56% due to compensatory increase of CLren (250%). In contrast, ICHO caused decrease of both MTX CLbil and CLren to 42% and 55%, respectively, due to multiorgan failure presented by the septic model (glomerular hypoperfusion). The outcome was complemented by examination of specific transporter expression at both hepatic (canalicular and basolateral biomembranes) and renal level. This study was supported by a grant from the Ministry of Education of the Czech Republic No.1P05OC062 (COST B25.002)

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Title of the project: Risk factors of atherosclerosis in cancer patients

Grant Agency: Ministry of Health ***Project Number:*** NR/9096-4

Principal Investigator: B. Melichar

Co-investigators: J. Doležal, R. Hyšpler, Z. Zadák, P. Eliáš, D. Solichová, L. Ungermann, D. Vokurková, J. Dvořák, J. Šťásek, K. Melicharová, J. Vižďa

Starting date: 1.1.2006 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 14 381

Summary of 2008 results

Title of the presentation: Atherosclerosis and cancer: a coincidence or a causal link?

Authors: Melichar S, Melicharová S.
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Cancer and the complications of atherosclerosis represent two leading causes of death in the industrialized nations. A causal link in the pathogenesis of these two disorders that would exist in addition to the shared risk factors (e.g. age or smoking) is a matter of dispute. Inflammatory response, both local and systemic, plays an important role in the progression of both atherosclerosis and cancer. Systemic inflammatory response resulting from chronic infections, reflected in increased serum concentrations of C-reactive protein, has been postulated to play an important role in the progression of atherosclerosis. Advanced/metastatic cancer is also commonly associated with systemic inflammatory response and elevation of serum C-reactive protein, but poor prognosis of patients with advanced/metastatic cancer might have prevented the clinical manifestation of accelerated atherosclerosis in this group of patients. The progress in the therapy of some tumors in the past decades may lead to unmasking of the effect of systemic inflammatory response associated with advanced/metastatic cancer on the progression of atherosclerosis. Moreover, the toxicity of anticancer therapy may result in the progression of atherosclerosis. The clinical data so far indicate increased incidence of cardiovascular disorders in survivors of childhood cancers or germ-cell tumors that is associated with a history of chemotherapy. The data on more common adult tumors is scanty. More pronounced adverse effects on the progression of atherosclerosis could be expected from the use of drugs targeting the vascular endothelial growth factor or its receptors, but these drugs were introduced only recently and are now used almost exclusively in patients with incurable metastatic tumors. In conclusion, we are just beginning to appreciate the extent to which advanced/metastatic cancer and/or the anticancer therapy causes progression of atherosclerosis. With improved therapy of more common tumors, a causal link between cancer and atherosclerosis mediated by inflammatory response may become obvious.

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Title of the project: Physiologically based prediction of pharmacokinetics prior to in vivo studies: Focus on hepatic and renal elimination

Grant Agency: Ministry of Education ***Project Number:*** 1P05OC061

Principal Investigator: S. Mičuda

Co-investigators: J. Martínková, J. Cermanová, L. Fuksa, E. Brčáková, J. Chládek

Starting date: 1.1.2005 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 2800

Summary of 2008 results

Title of the presentation: Release of methotrexate from bone cement and its harmful influence on stem cells

Authors: Stanislav Micuda (1), Tomas Soukup (2), Milos Hroch (3), Egon Prochazka (3), Leos Fuksa (1), Eva Brcakova (1), Jolana Cermanova (1), Gabriela Kolouchova (1), Petra Hirsová (1), Karel Urban (2), Jaroslav Mokry (2)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Histology (2), Dept. of Orthopedic Surgery (3)

Methotrexate (MTX) released from bone cement showed useful local effect in the animal models of bone tumors. Nevertheless, impaired wound healing was observed in the areas surrounding the MTX-loaded implant. Therefore, we hypothesized that MTX released from bone cement would have harmful effects on human mesenchymal stem cells as the basic component of bone and tissue reparatory processes. The cells were cultured with acrylic bone cement containing two concentrations of MTX (1 mg, and 10 mg per g of powdered monomer) for 14 days. Moreover, elution kinetics of MTX was evaluated from implants prepared either with liquid or powdered MTX. Using the original liquid formulation with the highest concentration of MTX available, the incorporation of MTX to cement was limited to concentration of 10 mg per g of polymer powder. During the 28 day incubation, the cement compounded with liquid MTX showed higher elution rate of the drug. The half-lifes for elution varied between 8.1 days and 12.4 days. However, during evaluated period of 28 days, only 1.1%-4.2% of MTX was released from pellet. The highest rate of elution was observed during the first 14 days. During this period, MTX released from pellets produced significant decrease in proliferation of mesenchymal stem cells as a consequence of blockade of their cell cycle in S/G2 phase. These finding indicate impairment of stem cell function in marginal areas surrounding the MTX-loaded cement and may help to explain problems with regeneration of tissues in these locations.

This study was supported by a grant from the Ministry of Education of the Czech Republic No. 1P05OC061

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Title of the project: Isolation, cultivation, differentiation and phenotypic characterization of dental pulp stem cells

Grant Agency: Ministry of Health ***Project Number:*** NR/9182-3

Principal Investigator: J. Mokrý

Co-investigators: R. Ivančaková, J. Karbanová, J. Suchánek, T. Soukup

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2330

Summary of 2008 results

Title of the presentation: Human dental pulp stem cells

Authors: T. Soukup (1), J. Suchánek (2), J. Mokrý (1), R. Ivančaková (2), R. Pytlík (3), L. Kučerová (4)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Histology and Embryology (1), Dept. of Dentistry (2), Dept. of Clinical Genetics; 1st Fac. Med., Charles Univ., Prague: 1st Dept. of Internal Medicine (3)

Our aim was to isolate stem cells from the dental pulp (DPSCs) and characterize their basic biological properties and phenotype to evaluate influence of media composition and effect of ionizing radiation on DPSCs population. In the second year of the study, we isolated 16 DPSC lines derived from the third molars and 2 lines derived from the exfoliated incisors. The dental pulp was yielded under the sterile conditions and exposed to enzymatic treatment prior seeding in alpha-MEM medium supplemented with growth factors EGF and PDGF, either 2%FCS or 10% FCS, and ITS. We were able to cultivate DPSCs in all tested cultivation media over 40 population doublings. Our results showed that DPSCs cultivated in medium supplemented with ITS had shorter average population doubling time (24.5 hours) compared to DPSCs cultivated in 2% FCS (55.4 hours) or 10% FCS (42.6 hours). In the course of the long-term cultivation, DPSCs did not show any signs of degeneration or spontaneous differentiation and cell viability reached 96%. Our phenotypical analysis presented same results for all tested cultivation media - high positivity for CD29, CD44, CD90 and HLA I, and negativity for CD34, CD45, CD71 and HLA II.

Effect of ionizing radiation was studied after reaching 50% culture confluence. Cells were irradiated using 60Co irradiator with dose 1 Gy/minute in the distance 1 m from the source. We have studied doses 2, 6 and 20 Gy. Our results proved that DPSCs react to high doses of ionizing radiation (6 and 20 Gy) by cell cycle arrest (G2 phase), changes in karyotype and premature senescence (detected as beta-galactosidase activity), but not by apoptosis. Viability following irradiation was not affected.

Supported by the project of the Ministry of Health, Czech Republic NR 9182-3/07.

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Title of the project: Changes of matrix metalloproteinases and their inhibitors on the model of chronic cardiotoxicity of anthracyclines

Grant Agency: Charles University ***Project Number:*** 53107/2007C

Principal Investigator: O. Popelová (A. Potáčová)

Co-investigators: M. Adamcová, M. Štěrba

Starting date: 1.1.2007 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 491

Summary of 2008 results

Title of the presentation: Role of matrix metalloproteinases during the development of chronic anthracycline cardiotoxicity in rabbits.

Authors: O. Popelová (1), A. Potáčová (2), M. Štěrba (1), V. Geršl (1), M. Adamcová (2)
Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Physiology (2)

The development of heart failure is a chronic process which is associated with myocardial remodeling. Matrix metalloproteinases (MMPs) are supposed to significantly contribute to this process. The present study was performed using an accepted model of chronic anthracycline cardiotoxicity in rabbits (daunorubicin, DAU, 50 mg/m^2, i.v., once weekly). Animals were sacrified 24 hours or 7 days after different cumulative dose of DAU (0-500 mg/m^2). Invasive examination of the left ventricular (LV) contractility revealed progressive and significant impairment of the left systolic function, starting with the 8th week. At the same time interval, plasma concentractions of troponin I and T (biomarkers of cardiac injury) were found to be significantly increased. Furthermore, markedly elevated levels of malondialdehyde (marker of lipoperoxidation) were determined in the LV myocardium already after the 1st administration of DAU and the peak concentrations were associated with the 5th dose of DAU (150 mg/m^2). In order to measure the activity of MMPs, LV homogenates were analyzed using MMP Gelatinase Activity Assay kit (Chemicon). During the time-course of the study, the gelatinase activity progressively decreased with increasing cumulative dose of DAU. In addition, MMPs activities were assayed by SDS-PAGE (zymography) using gelatine as degrading substrate under denaturing (SDS), non-reducing conditions. Using this approach a predominant activity of MMP-2 (72 kDa, expressed ubiquitously) was detected, whereas no activity of inducible MMP-9 was identified through-out of the study. The outcomes of both methods showed the similar trend, albeit they are different in principle of MMP activity measurement. Unlike in the case of Chemicon assay, in zymography MMP enzymes are physically separated from their natural inhibitors. Although other data are currently analyzed, the present results strongly suggest that cardiac remodeling associated with anthracycline cardiotoxicity is not based on the ROS-induced increase in activity of MMPs with gelatinase activity.

Project was supported by the Charles University Grant Agency No. 53107/2007/C.

Address for correspondence:
O. Popelová, Department of Pharmacology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Šimkova 870, 500 38 Hradec Králové, Czech Republic.

Title of the project: Utilisation of OCT VISANTE in angle examination in patients with secondary glaucoma after pars plana vitrectomy

Grant Agency: Charles University ***Project Number:*** 66107/2007C

Principal Investigator: L. Procházková

Co-investigators: P. Rozsíval, M.Závorková

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 538

Summary of 2008 results

Title of the presentation: Changes in the anterior chamber angle in diabetic patients with secondary neovascular glaucoma using OCT Visante

Authors: L. Procházková, M. Závorková
Masaryk hospital, Ústí nad Labem, Dept. of Ophthalmology

Purpose: To document the changes in the anterior chamber angle in diabetic patients with secondary neovascular glaucoma (SNVG) using OCT Visante.
Methods: OCT Visante is an imaging technology that enables anterior segment evaluation including corneal thickness and anterior chamber angle anatomy. We examined the anterior segment of the eye in diabetic patients with SNVG using OCT Visante and, subsequently, we assessed the findings – the corneal thickness, the anterior chamber depth and the potential angle adhesion. We compared the findings with the other eye of the patient.
Results: The thickness of the cornea was bigger in the eyes with SNVG than in the other eyes. The depth of the anterior chamber firstly depends on whether the patient is phakic, pseudophakic or aphakic, and secondly on the presence of adhesions in a chamber angle. In the eyes with SNVG the anterior chamber was in all cases shallower than in the other eyes. One of the patients from the group, who has SNVG on both eyes, showed the following: in the right eye, where the anterior chamber was shallower, the clinical finding was worse, the atrophy of the iris was bigger and there were more adhesions in the angle. The anterior chamber angle is in the eyes with SNVG narrower or closed than in the other eyes.
***Conclusion*:** Instead of the description of an angle, the angle can be measured and documented in all sections. OCT Visante is very helpful in diagnostics and in monitoring the changes of the angle in diabetic patients with SNVG. OCT Visante with SNVG is not crucial for the diagnosis. However, it is applicable for a documentation of changes and for taking measures of the thickness of the cornea as well as the depth of the anterior chamber which is directly dependent on the amount of adhesions in an angle.
Supported by the Charles University Grant Agency, No 66107/2007 C

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Title of the project: Protein biomarkers in patients with hypertophic cardiomyopathy

Grant Agency: Ministry of Health ***Project Number:*** NR/9253-3

Principal Investigator: R. Pudil

Co-investigators: J. Stulík, J. Lenčo, M. Hubálek

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2389

Summary of 2008 results

Title of the presentation: Biomarkers in patients with hypertophic cardiomyopathy

Authors: R.Pudil (1), M.Tichý (2), M.Ulrychová (2), J. Stulík (3), J.Lenčo (3)

(1) Charles University in Prague, Faculty of Medicine Hradec Kralove, 1st Dept.of Medicine, Hradec Kralove, Czech Republic

(2) Charles University in Prague, Faculty of Medicine Hradec Kralove, Depat.of Clinical Biochemistry and Diagnostics, Hradec Kralove, Czech Republic

(3) University of Defence Brno, Faculty of Military Health Sciences Hradec Kralove, Czech Republic

The project is aimed at searching for new potential protein biomarkers of hypertrophic cardiomyopathy (HC) in human plasma. For this purpose, we are taking advantage of modern proteomic technologies in order to compare plasma proteomes between patients who suffer from HC and healthy donors.

During the first year, we collected and successfully processed samples from 22 patients (mean of age 58.1 ± 13.5, 15 males, 7 females). In concordance with these statistical parameters a cohort of healthy donors was designed. Totally, 23 of 30 proposed samples were obtained.

During the second year, we performed analyses on new plasma markers of structural changes (glycogen phosphorylase BB, heart fatty acid binding protein, myoglobin, troponin I) and functional changes (N-terminal Pro brain natriuretic peptide) in patients with hypertrophic cardiomypathy.

As far as proteomics is concerned, we used single-dimensional iTRAQ LC-MALDI proteomic workflow, which was specifically optimized for plasma proteome analysis in the first year of the project. Using this approach, we successfully identified and relatively quantified 71 unique plasma proteins with false discovery rate < 1%. Of them, four proteins displayed significantly different relative concentration in plasma in patient with HC. Unfortunately, these proteins belong to the most abundant plasma proteins, which have been previously shown to be not able to fulfill the criteria desired for clinical biomarker. Therefore, in order to exclude proteins with poor diagnostic potential we further introduced immunoaffinity depletion of 14 most abundant plasma proteins. The efficiency of this technology was successfully tested using both SDS electrophoresis and single-dimensional LC-MALDI workflow. The analysis of the depleted plasma samples using two-dimensional LC-MALDI is currently under way. The employment of isoelectric focusing of peptides should allow a significantly deeper insight into the plasma proteome.

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Title of the project: Invasive approach for myocardial salvage and regeneration

Grant Agency: Ministry of Education ***Project Number:*** 0021620817

Principal Investigator: R. Pudil

Co-investigators: J. Ceral, J. Šťásek

Starting date: 1.1.2005 ***Duration (years):*** 7

Total funds allocated for project - Kč (thousands): 212 663

Summary of 2008 results

Title of the presentation: Use of the biochip microarray system in detection of myocardial injury caused by radiofrequency catheter ablation

Authors: R.Pudil (1) P. Pařízek (1) M. Tichý (2,3), L. Haman (1,3), L. Horáková (1), M. Ulrychová (2), J. Vojáček (1), V. Palička (2)

(1)First Department of Medicine, Charles University Prague, Faculty of Medicine, Hradec Kralove, Czech Republic

(2) Institute of Clinical Biochemistry and Diagnostics, Charles University Prague, Faculty of Medicine, Hradec Kralove, Czech Republic

(3) University of Defense Brno, Faculty of Military Health Sciences, Hradec Kralove, Czech Republic

In a prospective study, we measured plasma markers of myocardial damage induced by radiofrequency catheter ablation (RFA) with the protein biochip microarray system.

A total of 32 consecutive patients undergoing RFA for atrioventricular nodal re-entry tachycardia (AVNRT), right atrial flutter (AFL) and atrial fibrillation (AF) were included in the study. Cardiac troponin I (cTnI), creatine kinase isoenzyme (CK-MB), heart-type fatty acid binding protein (hFABP) and glycogen phosphorylase BB (GPBB) were measured using biochip array technology at baseline and 24 h after the procedure.

Values for all markers increased 24 h after RFA (cTnI: 0.92 ± 0.49 μ/L vs. 0.330.06 μ/L, p-0.001; CK-MB: 3.79 ± 2.04 μ/L vs. 1.85 ± 0.55 μ/L, p-0.001; hFABP: 2.82 ± 0.95 μ/L vs. 2.00 ± 0.95 μ/L, p-0.001; GPBB: 9.07± 5.83μ/L vs. 4.70±2.50 μ/L, p-0.001). The correlations between plasma marker levels and RFA time were cTnI: rs0.63, p-0.01; CKMB: rs0.75, p-0.01; hFABP: rs0.55, p-0.05, GPBB: rs0.51, p-0.05; the correlation between RFA time and number of RF applications was significant (rs0.81, p-0.001). Patients with RFA due to AF or flutter had elevated cTnI, CK-MB and hFABP levels compared to patients with AVNRT (cTnI: 1.14 ± 0.49 μ/L vs. 0.59 ± 0.25μ/L, p-0.05; CK-MB: 4.46 ± 2.07 μ/L vs. 2.81 ±1.54 μ/L, p-0.05; hFABP: 3.21± 0.98 μ/L vs. 2.25 ± 0.54 μ/L, p-0.01).

Myocardial injury induced by RFA can be detected by cTnI, CK-MB, hFABP and GPBB.

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Title of the project: Rheopheresis as the method of systemic therapy in age-related macular degeneration

Grant Agency: Ministry of Health ***Project Number:*** NR/9118-3

Principal Investigator: E. Rencová

Co-investigators: M. Bláha, J. Studnička, M. Blažek, D. Solichová, V. Bláha

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2653

Summary of 2008 results

Title of the presentation: Hemorheopheresis could block progression of dry form age-related macular degeneration (AMD)

Authors: E. Rencová (1), M. Bláha (2), J. Studnička (1), M. Blažek (2), V. Bláha (3), H. Langrová (1), J. Malý (2),J. Kvasnička (l), D. Solichová (3)

Fac. Med. Charles Univ., Hradec Králové: Dept. of Ophthalmology (1),2nd Dept. of Internal Medicine-Haematology(2), Dept. of Gerontology and Metabolism (3).

Text: Rheopheresis (cascade plasma filtration) is a method that can improve rheological parameters (i.e. blood and plasma viscosity and erythrocyte aggregation) and so could lead to metabolic and even visual acuity improvement in patients suffering from age-related macular degeneration (AMD).We investigated 16 patients and 16 controls with dry form of AMD (with soft drusen- inclusive confluent and reticular drusen and retinal pigment epithelium detachment- where an imminent danger of conversion to the angiogenic form of AMD exists). Each of 16 patients received a series of 8 haemorheopheresis (double filtration of 1,5 plasma volume) during 10 weeks. Plasma was obtained by the Cobe- Spectra separator and run through the 2nd stage (Evaflux 4A filters, Kuraray). The patients were followed-up using ETDRS charts, optical coherence tomography, fluorescein angiography, electroretinography. We reached for objectivisation of improvement of retinal pigment epithelium detachment (RPED) by digital Reconstruct programme. After the procedures, there was a substantial reduction in rheologically active substances (lipoproteins, ∝2-macroglobulin, fibrinogen, thrombomodulin), and also reduction of plasma and blood viscosity were observed. Visual acuity improved, reattachement of detached retinal pigment epithelium occurred as well as soft drusen absorption.

Conclusion: Repetitive pulses in plasma proteins elimination with rheopheresis seem to be capable of changing the activity of promoters of natural course in the dry form of AMD, its development and progression, improving ocular function and visual acuity. The therapy has shown to be effective and safe in our group of patients.

Literatura: Bláha, M., Rencová, E., Bláha, V. et al.: Significance of changes in microcirculatione during hemorheopheretic therapy of age related macular degeneration- our experience. Vnitř. Lék. 52,2006,11:1102

Project was supported by IGA MH CR NR/9118-3

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Title of the project: Using of spectrofluorimetric methods in detection of oxidative stress during toxic liver injury

Grant Agency: Charles University ***Project Number:*** 90/2006C

Principal Investigator: T. Roušar

Co-investigators: Z. Červinková, R. Endlicher, O. Kučera, P. Křiváková, J. Ondráková

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 755

Summary of 2008 results

Title of the presentation: Estimation of oxidative stress in acetaminophen treated rat hepatocytes in culture

Authors: T. Roušar (1), O. Kučera (1), H. Lotková (1), P. Křiváková (1) & Z. Červinková (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1)

Our project was focused on the optimization of fluorimetric methods and consequently on their use in estimation of oxidative stress in cultured rat hepatocytes treated with acetaminophen. We introduced the spectrofluorimetric glutathione assay which become comparable to a reference HPLC/FL method after optimization of reaction and detection parameters. We gained high specificity, sensitivity (5 μM GSH; 0.5 μM GSSG), linearity (up to 200 μM GSH) and precision (CV% << 5% in all the standards and the samples). As well, the method of intracellular reactive oxygen species (ROS) production was introduced in cultured cells. Another aim of our project was to investigate the role of oxidative stress in acetaminophen liver injury. Acetaminophen (APAP; paracetamol) is one of the mostly used antipyretics and analgesics. After overdose, the toxic effect will appear as the centrilobular liver necrosis that may lead to the acute liver failure. We proved that ROS production was enhanced in cultured rat hepatocytes in dependence on APAP dose (1-20 mM) and the time of incubation (1-24 h). In addition, the inhibition effect of APAP on the glutathione reductase (GR) activity was found. At 3 h of treatment, the GR activity was inhibited to 88%, 78% and 58% of control activity in 1, 5 and 20 mM APAP, respectively. This finding has not been published anywhere yet. Thus, we have been trying to find the cause of the GR inhibition. It seems that the inhibition may be caused by a metabolite of APAP metabolism as our preliminary results showed, that is why we will continue in the study of the APAP toxicity.

Literature: H. Jaeschke et al.: Tox. Sci. 89(1), 31-41, 2006.

Project was supported by the Charles University Grant Agency, No. 90/2006.

Address for correspondence: T. Roušar, Dept. of Physiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Influence of inositol hexaphosphate, inositol and sodium selenite on proliferation and apoptosis colorectal carcinoma cells

Grant Agency: Czech Republic ***Project Number:*** 301/06/P047

Principal Investigator: L. Schröterová

Co-investigators:

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 764

Summary of 2008 results

Title of the presentation: Chemopreventive effects of inositol hexaphosphate, inositol and selenium compounds on colorectal carcinoma cells

Authors: L. Schröterová (1), P. Hašková (2), A. Voráčová (2), E. Rudolf (1), M. Červinka (1)

Text:

1Charles University in Prague, Faculty of Medicine in Hradec Kralove, Hradec Kralove, Czech Republic

2Charles University in Prague, Faculty of Pharmacy, Hradec Kralove, Czech Republic

Incidence of colorectal carcinoma is increasing in the Czech Republic and the disease does not respond well to cytostatic treatment. Therefore it is necessary to broaden the spectrum of chemoprevention and therapeutic possibilities. In our project we try to characterize the effect of IP6, Ins and selenium compounds as a potential adjuvant in cancer treatment. We chose three colorectal cancer cell lines HT29, SW480, SW620 with different malignant potential. Cells were treated for 24, 48 and 72 hours. Proliferation was measured as BrdU incorporation, total protein amount using Brilliant Blue staining and colorimetric WST-1, XTT and MTT assays. Cytotoxicity was assessed by neutral red test. Induction of apoptosis was measured by caspase-3 activity fluorescence assay. Changes in cell morphology were studied by phase-contrast microscopy. The changes in expression of E-cadherin (epithelial cell junction protein) in human colon cancer cell line SW 620 after treatment with inositol hexaphosphate (IP6) was determined by means of indirect immunofluorescence.

IP6 increases expression of epithelial marker E-Cadherin in concentration and time dependent manner. Myo-inositol enhanced the proapoptotic effect in all cell lines. All selenium compounds decrease proliferation of tested cell lines. The most potent anti-proliferative and pro-apoptotic compound is Se-(Methyl)selenocysteine and the most considerable decrease in cell proliferation was observe in the SW 620 cell line on that compound.

This study demonstrates ability of chosen selenium compound and phytic acid to reduce the proliferation rate of tested cell lines and to induce the proapoptotic effect.

This work was supported by the Grant Agency of the Czech Republic (grant 301/06/P047)

Address for correspondence: L. Schröterová, Dept. of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Study of wound healing on animal model of type II diabetes mellitus - Zucker Diabetic Fatty rat.

Grant Agency: Charles University ***Project Number:*** 40207/2007 C

Principal Investigator: R. Slavkovský

Co-investigators: J. Kanta, M. Hajzlerová, R. Köhlerová, L. Sobotka

Starting date: 20.4.2007 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 518

Summary of 2008 results

Title of the presentation:
Study of cutaneous wound healing on animal model of type II diabetes mellitus - Zucker Diabetic Fatty (ZDF) rat.

Authors: R.Slavkovský (1,2), J.Kanta(2), M.Hajzlerová(2), R.Köhlerová(2), L.Sobotka(2,3) (1) Laboratory of Wound Healing, CPN, Dolní Dobrouč, (2) Dep.of Medical Biochemistry, Faculty of Medicine in Hradec Kralové, Charles University in Prague, (3) Dep.of Gerontology and Metabolism, Faculty Hospital in Hradec Kralové, Charles University in Prague

Introduction: Impaired wound healing is a frequent complication of type II diabetes mellitus (DM2) and significantly decreases the quality of life. The present project is focused on extending knowledge of skin wound repair in ZDF rats suffering from obesity and DM2 due to mutation in leptin receptor.
Methods: Four groups of experimental rats were created: healthy animals (HA) and obese diabetic (DA) of both sexes. Circular full-thickness wounds were excised on the back of rats. The wounds were periodically photographed and measured. In the first part of experiment the wounds were not bandaged, in the second part the wounds were covered by gauze bandage. The wound tissue was removed after 5 or 10 days for histological and RNA expression analysis.
Results: Diabetic females had higher level of obesity compared to diabetic males; diabetic males had higher hyperglycemia than diabetic females. Plasma levels of insulin, leptin and PAI-1 were elevated in DA. Significant reduction in wound healing in both DA groups was observed. Wounds in DA were covered by a larger crust, accompanied by higher inflammation, contained more adipose tissue. Diabetic wounds repaired extensively by reepitelization, whereas in HA they repaired mainly by contraction. Wound tissue in DA showed increased expression of interleukin 6 (IL6), myeloperoxidase(MPO) and colagenase 3. Imunohistochemistry showed that MPO and IL6 positivity was mainly localized on the top of the DA wound. Bandaged wounds started to contract faster than non-bandaged wounds in all groups.
Conclusion: The wound repair in ZDF animal is impaired and inflammatory response is aberrant in DA due to obesity and hyperglycemia. This model seems suitable for the study of impaired diabetic wound repair and for the testing of active substances.

Project was supported by the grants of Charles Univ. no. 7402/07/C and CPN no. D11-14,52.

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Title of the project: Photosensitizers in Dentistry

Grant Agency: Ministry of Education ***Project Number:*** 2B06104

Principal Investigator: M. Karásková (co-ordinator), R. Slezák

Co-investigators: J. Černý, R. Kořínková, R. Landsmanová, J. Rakušan, V. Buchta, M. Főrstl, R. Ivančaková., D. Kopecká, O. Krs, L. Ryšková, D. Slížová, A. Šimůnek

Starting date: 1.1.2006 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 30500

Summary of 2008 results

Title of the presentation: **Photosensitizers in Dentistry 2008 updated.**

Authors: R. Slezák(1), M. Karásková (2), L. Ryšková (3), O. Krs (4), V. Buchta (3), D. Slížová (4), J. Rakušan (2), R. Ivančaková (1), V. Králová (5), D., Kopecká (1), J. Černý (2)
Faculty of Medicine, Charles Univ., Hradec Králové: Dept. of Dentistry (1), Dept. of Clin. Microbiol. (3), Dept. of Anatomy (4), Dept. of Biology and Genetics (5), Výzkumný ústav organických syntéz a. s., Pardubice (2)

In 2008 the research followed all planned directions. New 5 types of phthalocyanine photosensitizers (FCs) were synthetized and selected for further research (3rd serie of FCs). Their ability to product singlet oxygen was studied using two modified chemical methods. Totally 7 carriers were performed for the fixation of FCs (e. g., silver, chitosan, zeolite, and titanium oxide nanoparticles). The technology for FCs fixation to nanoparticles was worked out. According to the fixation effectiveness certain combinations of FCs with carriers mentioned above were recommended. FCs revealing *in vitro* antibacterial activity was tested for their suspected cytotoxicity using Hep2 cell cultures and special device simulating FCs laser initiation. Additionally, non-tumorous human gingival cells were obtained and isolated, cultivated and prepared for the testing of FCs toxicity. Retention of nanoparticles in aquous suspensions with 3 different concentrations of titanium oxide, silica oxide and zinc oxide on the surface of the dental hard tissue samples (enamel, dentine, cementum) was evaluated using scanning electron microscopy. Procedure preventing nanoparticles aggregation and optimal concentrations of suspensions should be developed. Additionally, samples of various dental materials and gingival tissue could be tested for the retention of nanoparticles. *In vitro* testing of antibacterial activity of FCs using *S. aureus* strain 5887, *E. coli* strain 5276 and *C. albicans* with or without laser initiation was finished. Testing of antibacterial activity of non-toxic FCs fixed to nanoparticles and sensitivity of more specific oral bacterias to FCs will follow.

The project has been supported by the Czech Ministry of Education programme NPV II No 2B06104.

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Title of the project: Smoking and oral health

Grant Agency: Ministry of Health ***Project Number:*** NR/8781-3

Principal Investigator: J. Šmejkalová

Co-investigators: R. Slezák, L. Hodačová, Z. Fiala

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1339

Summary of 2008 results

Title of the presentation: Influence of smoking and other variables on oral health

Authors: Jindra Šmejkalová (1), Radovan Slezák (2), Eva Čermáková (3), Lenka Hodačová (4), Zdeněk Fiala (1), Vimal Jacob (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Hygiene and Preventive Medicine (1), Dept. of Dentistry (2), Computer Technology Centre (3), Dept. of Social Medicine (4)

A cross-sectional population-based study concerning the influence of smoking on oral health has been conducted on a representative sample of 1684 respondents within the age span of 30 to 69 years. The study consisted of two parts - self reported questionnaire inquiry and clinical examination of oral health status including teeth (DMF), periodontal (CPITN) and mucosal findings. The assessment of a dental status has proven that smokers have a significantly higher DMF index when compared with non-smokers (NS) (17.2 vs. 16), which had been caused mainly by a higher number of decayed and missing teeth. Significant difference in periodontitis occurrence and oral mucosal lesions score between smokers and NS was confirmed as well. The smokers revealed a significantly higher prevalence of CPI 3 or CPI 4 findings (shallow or deep pockets), while NS had a higher prevalence of CPI 0 (healthy periodontium). By a multivariable logistic regression we compared the possible influence of chosen external risk factors (smoking included) on the mean number of decayed teeth and on periodontitis of TN III grade. Our results showed that risk of decayed teeth occurrence is being statistically enhanced mainly by eating after evening tooth brushing (OR=1.68), low participation on preventive check-ups (OR=1.77) and smoking (OR=1.23). The risk for periodontitis of TN III grade was statistically increased with age (OR=2.63), lower tooth brushing frequency (OR=2.18), and lower participation in dental prevention (OR=1.8). Smoking increased the risk as well (OR=1.33), but the result was not significant (p=0.065). Conclusion: In our study we proved that smoking habit reveals negative influence on oral health; nevertheless some other factors of live style play important role, too. Project was supported by the Ministry of Health Grant Agency No. NR 8781-3/06.

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Title of the project: Neuropsychiatric Aspects of Neurodegenerative Diseases

Grant Agency: Ministry of Education ***Project Number:*** 0021620849

Principal Investigator: E. Růžička

Co-investigators: S. Nevšímalová, P. Smolík, J. Bušková, D. Kemlink, E. Havrdová, J. Roth, R. Jech, H. Kovářů, Z. Fišar, R. Jirák, K. Kupka, T. Zima, O. Slanař, J. Pláteník, A. Baxová, Z. Seidl, J. Vymětal, M. Hrdlička, J. Vymazal, I. Štětkářová, D. Urgošík

Starting date: 1.1.2007 ***Duration (years):*** 7

Total funds allocated for project - Kč (thousands): 19030

Summary of 2008 results

Title of the presentation: Monitoring and analysis of the spectrum of sleep and mental disorders in patients with neurodegenerative disorders

Authors: P. Smolik

Coincidence of dementia syndrome and sleep disorders in neurodegenerative disorders had been proofed well in many scientific publications. Our study should contribute to the knowledge of the mutual influence of both psychopathology and sleep disorders and, pharmacological interventions in patients with dementia syndrome, first of all in those with dementia of Alzheimer type or mixed Alzheimer and vascular dementia.

Two basic steps have been provided during the first phase of the study:

I. Preparation of the selection of patients and collecting of dates.

1. Overall census of coincidental ICSD-2 sleep disorders and ICS-10 mental and neurodegenerative disorders in the psychiatric segment of the Center for sleep and biorhythms disorders was provided and the collection of dates has been started.
2. Adeqate conditions for the logitudinal study of coincidence of the development of psychopathology, sleep disorders and chronobiological abnormities in patients with the dementia syndrom has been prepared.
3. The design of the study of effectiveness of different pharmacological interventions in patients with demetia in relation to the course of diesase, to defigned sleep disorders an chronobiological abnormities has been prepared.

II. Preparation of the methodology.

1. Actigraphy has been introduced in the out-patients department and screening instruments has been chosen.
2. Systematic screening of patients with dementia syndrome has been started.
3. Systematic screening and evaluation of pharmacological interventions after consultations with statistician has been started.

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Title of the project: Phenotypic analysis of dental pulp and periodontal stem cells

Grant Agency: Charles University ***Project Number:*** 102908/2008C

Principal Investigator: J. Suchánek

Co-investigators: R. Ivančaková, T. Soukup, J. Mokrý, B. Víšek

Starting date: 1.1.2008 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 251

Summary of 2008 results

Title of the presentation: Dental pulp stem cells and their characterization

Authors: Suchánek Jakub, Soukup Tomáš, Víšek Benjamín, Ivančaková Romana, Mokrý Jaroslav

Aims: Our aims were to isolate dental pulp stem cells, to cultivate them in different media and to investigate their basic biological properties and phenotype.
Methods: 16 lines of dental pulp stem cells (DPSCs) were isolated from impacted third molar. After enzymatic dissociation of dental pulp, DPSCs were cultivated in modified cultivation media for mesenchymal adult progenitor cells containing 2% or 10% fetal calf serum (FCS), or in modified 2% FCS cultivation media supplemented ITS. Cell viability and other biological properties were examined periodically every passage using Vi-Cell analyzer and Z2-Counter. DNA analysis and phenotyping were done using flow cytometry.
Results: We were able to cultivate DPSCs in all tested cultivation media over 40 population doublings. Our results showed that DPSCs cultivated in medium supplemented with ITS had shorter average population doubling time (24.5, 15.55 – 35.12 hours) compared to DPSCs cultivated in 2% FCS (55.43, 21.57 – 187.14 hours) or 10% FCS (42.56, 11.86 – 101.3 hours). Cell diameter was not affected and varied from 15 to 16μm. DPSCs viability in 9th passage was over 90 %. Our phenotypical analysis presented high positivity for CD29, CD44, CD90 and HLA I, and negativity for CD34, CD45, CD71 and HLA II. DPSCs lines cultivated in all media did not shown any signs of degeneration or spontaneous differentiation during expansion process.
Conclusions: We proved that ITS supplement in the cultivation media highly increase proliferation activity of DPSCs. Other DPSCs biological properties and phenotype was not affected.
Work was supported by grant project GA UK 102908-3029/2008

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Title of the project: Difference in the behavior of the freshly isolated and cryo-preserved ovarian cancer cells and its clinical significance

Grant Agency: Ministry of Health ***Project Number:*** NR/8768-3

Principal Investigator: J. Tošner

Co-investigators: I.Sedláková, M.Červinka, J.Špaček ,M. Kudela, O.Kopecký, M.Tomšová, M.Hajdúch, R. Pilka, P Měřička.

Starting date: 1.3.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1215

Summary of 2008 results

Title of the presentation: Difference in the behavior of the freshly isolated and cryo-preserved ovarian cancer cells and its clinical significance

Authors: J. Tošner (1), I. Sedláková (2), M. Kudela (3), M.Červinka(1), O.Kopecký (2), P.Měřička (2), M.Tomšová (2), J. Špaček (1), M.Hajdúch (3), R. Pilka (3),
(1)Charles University in Prague, Faculty of Medicine Hradec Králové
(2)University Hospital Hradec Králové
(3)University Hospital Olomouc

One of the major goals of oncology is to predict the response of patients with cancer to chemotherapeutic agents by employing laboratory methods called tumour chemosensitivity assays. Ninety-three MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) chemosensitivity essays were performed in 22 patients with advanced epithelial carcinoma of ovary. Results of MTT assay using freshly isolated tumours cells were compared to results obtained from defrost samples. In four categories scale absolute agreement of results was observed in 53 cases from 93 (56%). In 36 cases the results with defrost cells were different within one category of chemosensitivity.
Five chemosensitive samples turned to chemoresistant after defrosting. In fifteen cases defrost samples were less sensitive, in 20 samples the sensitivity to chemotherapeutical drugs was higher after thawing.
Similar MTT assay results obtained from fresh and defrost tumor tissue could be of essential importance for chemoresistance testing in practice as frozen samples can be transported to specialized laboratory from remote hospitals. Only five tests from 93 (5.37%) presented different values in the sence of chemosensitivty/chemoresistence. That promising observation encourage us to workout more precise laboratory technique which will confirm our hypothesis that results from fresh and defrost tumour tissue are analogical.

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Title of the project: Determination and significance of resistance to antiplatelet therapy

Grant Agency: Ministry of Health ***Project Number:*** NR/9174-3

Principal Investigator: J. Vojáček

Co-investigators: J. Malý, R. Pudil, P. Dulíček, I. Fátorová, H. Ševčíková, R. Ševčík

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2682

Summary of 2008 results

Title of the presentation: Resistance to acetylsalicylic acid (ASA) and clopidogrel in patients with coronary artery disease

Authors: Hana Ševčíková (1), Jan Vojáček (1), Radek Pudil (1), Jaroslav Malý (2), Petr Dulíček (2), Ilona Fátorová (2), Róbert Ševčík (1)
Fac. Med., Charles Univ., Univ. Hosp. Hr.Králové: Dept. Int. Med. I (1), Dept. Int. Med. II (2)

Benefit of ASA and clopidogrel is well established in patients with coronary artery disease. Persistent platelet reactivity despite antiplatelet therapy (so called laboratory resistance) might explain the recurrence of cardiovascular events despite therapy in some patients.There is no clear and accepted definition for aspirin or clopidogrel resistance and none of the method used for monitoring platelet functions is specific and sensitive enough. The aim of our study was to determine clinical outcomes of laboratory established ASA and clopidogrel resistance in patients with coronary artery disease (group A - patients with acute coronary syndromes - ACS, group B - patients with stable coronary artery disease - SCAD) and to compare two platelet function assays (light transmittance aggregometry with cationic propyl gallate and adenosindiphosphate as platelet inductors and MULTIPLATE - Multiple Platelet Function Analyser). Inclusion criteria were defined and blood samples were taken in described study scheme (D0 entry blood sample, D1 second blood sample between 3.-7.day, M1 third blood sample after 1 month, M6 fourth sample after 6 months, M12 the last blood sample after one year). Platelet reactivity was measured in 63 patients with coronary artery disease. Baseline study population characteristics: 11 females, 52 males, 40 patients with ACS, 23 with SCAD, 40 hypertonics, 12 diabetics. All patients were treated with ASA (either loading dose 500mg or maintanance dose 100mg), 52 patients were on dual antiplatelet therapy (clopidogrel in loading or maintanance dose) . MULTIPLATE has been used in 220 patients with coronary artery disease after coronary stenting and in 33 patients as control group. Till January 2009 results were collected and prepared for statistical analysis. Clinical follow up including antiplatelet status is running now.

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Title of the project: Visual mismatch negativity in patients with schizophrenia

Grant Agency: Charles University ***Project Number:*** 24207/2007C

Principal Investigator: A. Urban

Co-investigators: J. Kremláček, J. Libiger, J. Masopust

Starting date: 1.1.2007 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 249

Summary of 2008 results

Title of the presentation: Visual mismatch negativity among patients with schizophrenia

Authors: Aleš Urban (1), Jan Kremláček (2), Jiří Masopust (1), Jan Libiger (1)
Department of Psychiatry, Faculty of Medicine in Hradec Králové, Charles University in Prague and Faculty Hospital Hradec Králové, Czech Republic (1)
Institute of Pathological Physiology, Faculty of Medicine in Hradec Králové, Charles University in Prague, Czech Republic (2)

Event related potentials (ERPs) provide an insight into sensory and cognitive processes in health and disease. Studies of an ERP negative amplitude deflection elicited by a change in a series of auditory stimuli is known as mismatch negativity (MMN). The generation of MMN is impaired in schizophrenia. Its deficit is associated with lower everyday functioning and may be also interpreted as the marker of progression in schizophrenia.
MMN elicited by visual stimuli (vMMN) was described by several research teams, but it has not been investigated in schizophrenia as yet. Using a motion-direction paradigm, we elicited visual MMN in patients with schizophrenia and schizoaffective disorder. The vMMN was computed as differences in areas under curve of visual ERPs to standard and deviant motion direction stimuli recorded from midline derivations at the interval of 100-200 msec. They were compared between groups of patients with schizophrenia and healthy controls. The significantly smaller vMMN indicated an impaired generation of mismatch negativity in patients with schizophrenia. In secondary analyses there was an association of vMMN impairment among patients with higher dose of medication, lower level of functioning and the presence of deficit syndrome. This impairment appears analogous to the impairment of MMN in the auditory domain and is probably related to early visual information processing. Its relationship to cognitive functioning of patients with schizophrenia deserves further attention.
Project was supported by the Charles University Grant Agency, No 24207.

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Title of the project: Cytotoxicity of Dental Materials

Grant Agency: Charles University ***Project Number:*** 81508/2008C

Principal Investigator: L. Vavřičková

Co-investigators: T. Dostálová, J. Ulrichová

Starting date: 14. 4. 2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 232

Summary of 2008 results

Title of the presentation: Cytotoxicity of Dental Materials

Authors: L. Vavřičková, T. Dostálová, J. Ulrichová

Aim:

The problems of dental alloys in the mouth cavity are still widely discussed despite a great development of non-metallic materials. Our aim was to verify the cytotoxic influence of selected dental alloys and ceramic materials on the cell culture.

Experimental method used:

We selected most used chrome-nickel dental alloys, dental alloy based on titanium (86 %) and noble alloy containing 77 % of gold and different types of ceramic materials. The test monitored the cytotoxic influence of the selected dental material solid sample on the cell line of mouse fibroblasts NIH 3T3 in the cell culture. The test of direct contact and the extract test were monitored for testing of the cytotoxicity in vitro.

Results:

Our results show, that all tested dental materials are considered non-toxic in the direct contact test – with zero cytotoxicity level and Zone Index/Lysis Index 0/0. In the extract test, the level of cytotoxicity is also zero, only % of the lifetime differs with 100% extract and when thinning 1 to 1 and 1 to 3. Overall from this point of view the Wiron 99 (Bego, Germany) and the alumina ceramic (Procera®, Nobel Biocare™, Sweden) appear the worst.

Conclusion:

All tested dental materials can be considered non-toxic. There can only be found slight differences in the extract test with different thinning. When thining 1 to 1 the Wirolloy (Bego, Germany) and Heraenium NA (Heraeus Kulzer, Germany) alloy lifetime is still 100%. Therefore we can say that these two alloys and with this thinning do not indicate even the slightest scent of cytotoxicity which however slightly contradicts with the data gained from literature where the chrome-nickel alloys are considered cytotoxic.

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Title of the project: Importance and time course of plasma metalloproteinases, solubile ligand CD40 and tissue factor in acute coronary syndrom

Grant Agency: Ministry of Health ***Project Number:*** NR/9176-3

Principal Investigator: J. Vojáček

Co-investigators: J. Bis, C. Andrys, V. Palička, J. Dušek

Starting date: 1.1.2007 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 600

Summary of 2008 results

Title of the presentation: Importance and time course of plasma metalloproteinases, solubile ligand CD40 and tissue factor in acute coronary syndrom

Authors: J. Bis (1), J.Vojáček (1), C. Andrys (2), J. Dusek (1), V. Palicka (3), V. Pecka (4), V. Dytrychova(4)Fac. Med., Charles Univ., Univ. Hosp. Hr.Králové: 1st Dept. of Int. Medicine(1), Dept. of Imunology (2), Dept. of Clin Biochemistry (3), 2nd Dept. of Int. Medicine (4).

Circulating metalloproteinases are not only a marker of ACS, but play an important role in the processes leading to multiple plaques ruptures. We hypothetize that level of circulating matrix metalloproteinases would correlate to the plasma level of the tissue factor thus suggesting the role of circulating matrix metalloproteinases in multiple coronary plaques rupture. We measured plasmatic levels of solubile ligand CD40 (slCD40), extracelular matrix metalloproteinase 2, 3 and 9 (MMP-2, MMP-3 and MMP-9), inhibitor of tissue metalloproteinases (TIMP-2), tissue factor (TF) and high sensitive C-reactive protein (hsCRP). Method: Group A - 28 patients (age 62,6, 19 men) with acute coronary syndrom and Group B - 24 patients (age 62,3, 21 men) with stable coronary heart disease. We compare levels in coronary sinus, periferal blood and left main coronary artery, in a group A also 24 hours and 7th day after coronarography. Results: Plasma level of tissue factor and MMP-9 was significantly higher in group A then B (239,0±99,3 pg/ml vs 164,3±114,2 pg/ml; p=0.016 and 815,5+451,8 mg/l vs 504,8+245,7 mg/l; p=0,0038). Also transcoronary gradient of slCD40 was significantly higher in a group A than group B. There were no difference between the MMP-2, MMP-3, MMP-9 a TIMP-2 levels. Significant correlation was foud between the TF and hsCRP, TF and slCD40, MMP-9 and hsCRP. Transcoronary production of TF has a linear correlation with MMP-9 level. Conclusion: Levels of TF and MMPs are elevated in group of acute coronary syndrom also in systemic circulation. We found transcoronary elevation of scCD40 marker.

Project was supported by the Internal Grant Agency Ministry of Health, Czech Republic, No NR/9176-3

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Title of the project: Disturbances of synthesis of cholesterol and its depletion - clinical consequences and opportunities of terapeutical influence in intensive care

Grant Agency: Ministry of Health ***Project Number:*** NR/8921-3

Principal Investigator: P.Vyroubal

Co-investigators: Z.Zadák

Starting date: 1.1.2006 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1871

Summary of 2008 results

Title of the presentation: Disturbances of synthesis of cholesterol and its depletion in cardiosurgical patients

Authors: P.Vyroubal (1), J.Samek (2), R.Hyšpler (1), A.Tichá (1), Z..Zadak (1)
(1)Dept. of Gerontology and metabolism, Faculty Hospital, Hradec Králové, (2) Dept.of Cardiosurgery, Faculty Hospital, Hradec Králové.

The aim of the study was to elucidate the role and importance of hypocholesterolemia after cardiosurgical operation in patients with coronary heart disease (CHD).
Methods: Twenty two patients with CHD were recruited to the study. All patients underwent miniinvasive cardiosurgical operation. The blood samples were taken one day prior surgery, the first, fourth and eighth post-operative day. We performed the determination of sterols, their precursors and IL-6 in the blood serum. ACTH stimulation test was performed of the fourth post-operative day. The oxidative burst of granulocytes was evaluated without and with the stimulation by phorbol-myristate acetate. Data were evaluated by software SigmaStat 3.1 and presented as mean+-SD or median (interquartile range).
Results: There was a significant decline of cholesterol level from day –1 (3.559+-1.067) to day +1 (2.765+-0.798, $p< 0.001$) with full recovery at day 8 (4.169+-0.687). Despite a cholesterol level decline, the concentration of lathosterol (a de novo cholesterol synthesis marker) significantly rose from day –1 to day 8: from 2.746 (1.840;3.275) to 5.377 (4.110; 7.565; $p< 0,05$). There was a significantly negative correlation between IL-6 level and total cholesterol and significantly positive correlation between lathosterol/cholesterol ratio (a de novo cholesterol synthesis marker) and IL-6 and cortisol level after the ACTH stimulation test. There was a significant breakdown of bactericidal function of granulocytes along with decline of cholesterol level.
Conclusion: The cholesterol synthesis rate is positively influenced by IL-6 despite its negative effect on total cholesterol levels, caused probably by the supression of apolipoprotein synthesis. The average body cholesterol synthesis rate had a positive effect on cortisol production by the adrenals.
Project was supported by the IGA Ministry of Health ČR, NR 8921-3
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Title of the project: Research and development of the new isolation technique of conventional and unheard-of amaranth grain components for industrial utilization and nutritional products fortification

Grant Agency: Ministry of Commerce ***Project Number:*** FI-IM5/098

Principal Investigator: Z. Zadák

Co-investigators: A. Tichá, R. Hyšpler, M. Slanařová, D. Solichová, I. Svobodová, P. Žďánský, J. Krejcarová, J. Tilšarová, S. Pokorná

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 832

Summary of 2008 results

Title of the presentation:
The evaluation of Amaranth and Hemp grain on the growth in rats

Authors: Z. Zadák, A. Tichá, R. Hyšpler, M. Slanařová, D. Solichová, I. Svobodová, P. Žďánský, J. Krejcarová, J. Tilšarová, S. Pokorná
Dept. of Metabolic Care and Gerontology

Rationale: The protein content of the amaranth grain is higher compared to common gluten-free grains except soybeans. The total protein content of hemp seed is about 65% of the globular protein edestin, which closely resembles the globulin found in human blood plasma. It is easily digested, absorbed and utilized by humans.The purpose of the study was to compare amaranth and hemp grain on the growth and nitrogen metabolism in rats.
Methods: 21 male Wistar rats were divided into 3 diet groups of 7 animal each. Amaranth, edestin and casein were tested. Daily intake of the different diets and body weight of rats were investigated during 32 days. Biochemical parameters of nitrogen metabolism in the blood and urine and total nitrogen in stercus of the terminal small intestine were determined.
Results: Results are presented as mean ± SD. Growth rate (% day) was calculated for casein group 2.78% ±0,33, edestin group 2,1% ±0,17 and amaranth group 1,75% ±0,17.
Conclusioncs: In comparison with other plant proteins, amaranth protein contains high amounts of the essential amino acid lysin (3x higher than cereals and legumes). Lower of body weight in amaranth group can be explained by the content of thermolabile antinutrients in amaranth protein. This finding deserves further study.

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Title of the project: Development of technology and produce microbe biomass as a source valuable proteins and their hydrolysates (carriers of biologically active substances)

Grant Agency: Ministry of Commerce ***Project Number:*** FI-IM5/195

Principal Investigator: Z. Zadák

Co-investigators: R. Hyšpler, A. Tichá, D. Solichová, M. Slanařová, I. Svobodová, P. Žďánský, J. Krejcarová, J. Tilšarová, S. Pokorná

Starting date: 1.1.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 325

Summary of 2008 results

Title of the presentation: Development of technology and produce microbe biomass as a source valuable proteins and their hydrolysates

Authors: Z. Zadák, R. Hyšpler, A. Tichá, D. Solichová, M. Slanařová, I. Svobodová, P. Žďánský, J. Krejcarová, J. Tilšarová, S. Pokorná
Dept. of Metabolic Care and Gerontology

Supplements developed on base microbe biomass are the source of protein and further like carrier biologically active matters. Microbe biomass is source bio-factors, above all vitamins group B, ergosterol (provitamin D2), phospholipides and other biologically active substances. Supplements containing combination manooligosacharides and beta-glucan extracted from cell wall are very interesting possibility usage biomass Torulopsis ethanolitolerans. Quality yeasty protein strain Torulopsis ethanolitolerans be full of comparable to quality proteins meat-bone powder and soyabean flour. The typical composition of yeast biomass Torulopsis ethanolitolerans (% of dry matter): protein 58.3%, fat 7.4%, nucleic acid 9.8%. Protein contains high amounts of the glutamic acid, lysin and arginin. High quality protein, B complex vitamins, minerals and immunologically active substances, including beta-glucans in yeast biomass are suitable in human nutrition.

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Title of the project: New diagnostic markers and therapeutical approaches in different periods of life with emphasis on ageing

Grant Agency: Ministry of Health ***Project Number:*** 00179906

Principal Investigator: Z. Zadák

Co-investigators: J. Bureš, V. Černý, A. Ferko, P. Hůlek, R. Hyšpler, N. Jirásková, J. Malý, B. Melichar, V. Palička, J. Petera, A. Ryška, V. Tošnerová, J. Vojáček

Starting date: 1.4.2005 ***Duration (years):*** 7

Total funds allocated for project - Kč (thousands): 48240

Summary of 2008 results

Title of the presentation: New diagnostic markers and therapeutical approaches in different periods of life with emphasis on ageing

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Prof. Bureš – The methodology of capsule endoscopy by the experimental animals was introduced using method of confocal laser endomicroskopy up.

Prof. Melichar – Extensive group of patients with breast cancer was treated with neoadjuvant chemotherapy and rate tumor predictione examined. Next it was observed neopterin like the indicator of systems immunity activation by cancer patients.

Prof. Ryška – the change of the tumor cells phenotyp was studied during chemotherapy and the role of immune system in the effectiveness of antitumour therapy. The potencial predictive markers of factors were founded, which participate in carcinogenesis of colon carcinoma.

Prof. Palička - Research team is focused on some specific areas:

- genotyping in patients with unborn errors of metabolism (neurodegenerative diseases, cystic fibrosis)
- genotype in patients with drug of abuse dependency and patients with schizophrenia. The world priority in testing EEG mismatch negativity in patients with metamphetamine dependency
- relation between HPV genotypes and head-neck cancers

- interstitial metabolism in different conditions measured by microdialysis technique
- infiltration of tumor tissue by lymphocytes for effect of therapy prediction in patients with ca mammae.

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