

**UNIVERZITA KARLOVA V PRAZE,  
LÉKAŘSKÁ FAKULTA V HRADCI KRÁLOVÉ  
A  
FAKULTNÍ NEMOCNICE V HRADCI KRÁLOVÉ**

**XII. VĚDECKÁ KONFERENCE**

**P R O G R A M**



**22. ledna 2008**

**Velká posluchárna teoretických ústavů Lékařské fakulty UK,  
Šimkova 870, Hradec Králové**

## **XII. vědecká konference LF UK a FN Hradec Králové, 22. ledna 2008**

- 10.00 - 10.15 Zahájení konference  
**prof. MUDr. RNDr. Miroslav Červinka, CSc.**  
**prof. MUDr. Vladimír Palička, CSc.**, děkan lékařské fakulty  
**doc. MUDr. Leoš Heger, CSc.**, ředitel fakultní nemocnice
- Sekce I** Předsedající: **prof. MUDr. Miroslav Červinka, CSc.**
- 10.15 - 10.30 Vliv extracelulární matrix na expresi genů jaterních myofibroblastů  
**Mgr. Alena Jiroutová**  
GA UK 86/2006 C (LF)
- 10.30 - 10.45 Studium účinků deferipronu z hlediska potenciálního protektivního působení u experimentálně navozené antracyklinové kardiotoxicity  
**Mgr. Olga Popelová**  
GA UK 89/2006 C (LF)
- 10.45 - 11.00 Spektrofluorimetrická detekce oxidačního stresu během modelového toxického poškození hepatocytů kultivovaných in vitro  
**Mgr. Tomáš Rousat**  
GA UK 90/2006 C (LF)
- 11.00 - 11.15 Porovnání sycení abdominálních struktur po aplikaci MR kontrastní látky: srovnání gadobutrolu a Gd-EOB-DTPA  
**MUDr. Ludovít Klzo**  
GA UK 142007/2007 C – 2420 (LF)
- 11.15 - 11.30 Vliv beta-hydroxy-beta-methylbutyrátu na metabolismus proteinů a aminokyselin u modelu sepse  
**Mgr. Miroslav Kovařík**  
GA UK 32207/2007 C – 7322 (LF)
- 11.30 - 12.00 *Přestávka – občerstvení*
- Sekce II** Předsedající: **prof. RNDr. Jan Krejsek, CSc.**
- 12.00 - 12.15 Ověření významu peroperačního měření tlaku v karpálním tunelu při operacích karpálního tunelu minimálně invazivními technikami  
**MUDr. Martin Kanta, Ph.D.**  
IGA MZ NR/8404-3/05 (LF)
- 12.15 - 12.30 Prediktivní a prognostické faktory u nemocných s karcinomem prsu léčených neoadjuvantní chemoterapií  
**prof. MUDr. Bohuslav Melichar, Ph.D.**  
IGA MZ NR/8392-3/05 (LF)
- 12.30 - 12.45 Imunohistochemické stanovení prognostických markerů u ovariálního karcinomu a jejich korelace s klinickým průběhem onemocnění  
**MUDr. Markéta Tomšová, Ph.D.**  
IGA MZ NR/8363-3/05 (LF)

- 12.45 - 13.00 Vztah angiogeneze k expresi ZAP-70 a klinickému průběhu u chronické lymfocytární leukémie  
**MUDr. Lukáš Smolej**  
IGA MZ NR/8373-3/05 (LF)
- 13.00 - 13.15 Nové způsoby biodozimetrie a diagnostiky u ozářených vojáků  
**doc. MUDr. Martina Řezáčová, Ph.D.**  
MO BIODOZ OBUKHK2005001 (LF)
- 13.15 - 14.45 *Přestávka na oběd*
- Sekce III** Předsedající: **prof. MUDr. Bohuslav Melichar, Ph.D.**
- 14.45 - 15.00 Aterogenní potenciál endoteliální dysfunkce a poruch metabolismu cholesterolu v procesu koronární aterosklerózy u diabetu mellitu II. typu  
**Prof. MUDr. Vladimír Bláha, CSc.**  
IGA MZ NR/8497-3 (FN)
- 15.00 - 15.15 Vliv steatózy jater na jejich regenerační schopnost po provedení částečné hepatektomie. Experimentální studie  
**Mgr. Petra Hrubá**  
IGA MZ NR/8500-3 (FN)
- 15.15 - 15.30 Rheologické, funkční a strukturální aspekty aterosklerózy: ovlivnění mikrocirkulace metodou extrakorporální hemorheoterapie  
**prof. MUDr. Milan Bláha, CSc.**  
IGA MZ NR/8505-3 (FN)
- 15.30 – 15.45 U k o n ě n í k o n f e r e n c e  
**doc. MUDr. Leoš Heger, CSc.**, ředitel fakultní nemocnice  
**prof. MUDr. Vladimír Palička, CSc.**, děkan lékařské fakulty

**SOUHRNY VÝZKUMNÝCH ÚKOLŮ  
ŘEŠENÝCH NA LF UK A VE FN V HRADCI KRÁLOVÉ  
(ABECEDNĚ)**

**Title of the project:** Energy metabolism in critically ill: The thermic effect of parenteral nutrition

**Grant Agency:** Charles University

**Project Number:** 120707/2007C

**Principal Investigator:** J. Bajnárek

**Co-investigators:** L. Sobotka, R. Hyšpler

**Starting date:** 1.1.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 231

**Summary of 2007 results**

**Title of the presentation:** The influence of the disease severity on the thermic effect of nutrition and energy substrates oxidation

**Authors:** J. Bajnárek (1), J. Maňák (1), P. Vyroubal (1), R. Mottl (1), M. Fric (1), L. Sobotka (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Metabolic Care and Gerontology (1)

**Rationale:** In spite of few previously published articles the effect of nutrition on energy metabolism is not known in ICU patients. The aim of our study was to measure the influence of parenteral nutrition (PN) on energy expenditure (EE) and substrate oxidation.

**Methods:** Nineteen patients were included into our study. After 12 hours of fasting (infusion of crystalloid) we administered total PN of subsequent composition: amino acids 1.2 mg, fat 0.5 mg, glucose 4.0 mg per minute per kilogram of ideal body weight. EE and oxidation of energy substrates were measured by indirect calorimetry (Vmax, Sensor Medics) before and subsequently in 2nd, 5th, 12th and 24th hour of PN administration. Measured parameters were compared with respect to APACHE II score.

**Results:** EE rose to maximum 5 hours after the onset of PN ( $108.12 \pm 10.8$  % of the initial value) and then it declined to fasting level. The increase was not influenced by the disease severity (APACHE II score). Lipid oxidation decreased from fasting level  $1.13 \pm 0.53$  mg/kg/min to minimum  $0.28 \pm 0.83$  mg/kg/min in 12th hour of PN administration, whereas glucose oxidation went up from  $1.19 \pm 1.34$  to  $3.29 \pm 1.80$  mg/kg/min. No lipid oxidation was apparent in patient with APACHE II > 13 after 12 hours of nutritional support.

**Conclusions:** The thermic effect of nutrition does not seem to be influenced by the disease severity. Surprisingly glucose was preferable substrate oxidized by critically ill patients 12 h after the start of nutritional support.

**Address for correspondence:** J. Bajnárek, Department of Metabolic Care and Gerontology, Charles University Teaching Hospital, Sokolská 581, 50005, Hradec Králové, Czech Republic



**Title of the project:** Chronic Diseases Induced by Impaired Function of Immune System; Their Immunopathogenesis, Early Diagnoses and Treatment

**Grant Agency:** Ministry of Education

**Project Number:** 0021620812

**Principal Investigator:** J. Bartunkova

**Co-investigators:** J. Krejsek (1), P. Kunes (2,1), V. Lonsky (2), J. Mandak (2), M. Kudlova (1), M. Kolackova (1), C. Andrys (1), K. Jankovicova (1)

**Starting date:** 1.1.2005

**Duration (years):** 7

**Total funds allocated for project - Kč (thousands):** 1818

#### **Summary of 2007 results**

**Title of the presentation:** Cardiac Surgical Operations as a Clinical Model of Inflammatory Response

**Authors:** J. Krejsek

(1) Department of Clinical Immunology and Allergy

(2) Department of Cardiac Surgery

Charles University in Prague, School of Medicine and University Hospital in Hradec Kralove  
Czech Republic

**Text:**

**Aims:** To follow selected parameters of both humoral and cellular innate immunity in patients undergoing cardiac surgical operation as a clinical model of an inflammatory response.

**Design:** Thirty-four patients, seventeen in each group, were randomly assigned to CABG surgery performed either with („on-pump“) or without („off-pump“) CPB. Blood samples were collected both during and after the operation up to the 7th day.

**Results:** Pentraxin 3 (PTX3) is a newly identified acute phase reactant with non redundant functions in innate immunity. Operations performed with the use of CPB are associated with a more pronounced release of PTX3 immediately after operation. (Scand. Cardiovasc. J., 2007; 41(3): 171-9).

The levels of lipopolysaccharide binding protein (LBP) and sCD14 are elevated in cardiac surgical patients being similar in both groups. These molecules are not produced as acute phase proteins in cardiac surgical patients. (Med. Inflam., 2007; in press). The relative and absolute number of MEM-148 positive activated myeloid cells is significantly diminished during „on-pump“ surgery. A significant increase in their number in postoperative period in both „on-pump“ and „off-pump“ patients was found. There were no significant differences between „on-pump“ and „off-pump“ patients. (Acta Med., 2007; 50(3): 187-193).

In cardiac surgical patients the expression of activation marker FcγR1 (CD64) on monocytes is increased earlier in comparison with granulocytes in both “on-pump” and “off-pump” patients. The expression of scavenger molecule CD163 on monocytes is significantly higher in “on-pump” patients. (Med. Inflam., 2007; in press).

Hsp70 level is increased in cardiac surgical patients undergoing CABG operation using CPB compared to patients operated on the beating heart. (Cor Vasa, 2007; 49(10): 356-361).

**Address for correspondence:** Jan Krejsek, Department of Clinical Immunology and Allergy, Charles University in Prague, School of Medicine and University Hospital in Hradec Kralove Czech Republic, e-mail: krejsek@fnhk.cz





**Title of the project:** Research center of cell invasion during embryonic development and cancer metastasis

**Grant Agency:** Ministry of Education

**Project Number:** LC06-invasion

**Principal Investigator:** M. Dvořák(1)

**Co-investigators:** P. Kašparová (Benešová), A. Ryška, I. Šteiner(2)

**Starting date:** 1.1.2006

**Duration (years):** 5

**Total funds allocated for project - Kč (thousands):** 4300

**Summary of 2007 results**

**Title of the presentation:** Molecules involved in cell invasion during cancer metastasis

**Authors:** P. Kašparová (Benešová), A. Ryška, I. Šteiner - The Fingerland Department of Pathology, Charles University Faculty of Medicine and University Hospital, Hradec Králové

(1) Institute of Molecular Genetics, Academy of Science

(2) The Fingerland Department of Pathology, Charles University Faculty of Medicine and University Hospital, Hradec Králové

The cellular invasion is involved in normal embryogenesis but also in cancerogenesis, playing a crucial role in metastatic potential of tumours. It is hypothesised that the regulatory mechanisms in both processes are very similar. The aim of this project is research of selected molecules and molecular mechanisms involved in embryogenesis of vertebrates and application of acquired informations into research of metastatic mechanisms.

Among many tissues studied, human malignancies (epithelial neoplasms, soft tissue tumors) are studied.

In this multicentric project with co-operation of many laboratories, the Fingerland Department of Pathology is one of participants. The archive files of our institute serves as a source of archive material (paraffin tissue blocks of tumor tissue). The appropriate specimens of primary and secondary tumours are sampled using tissue microarray method. These multitissue blocks will be subsequently studied by imunohistochemistry and other special methods.

**Address for correspondence:** benesp@seznam.cz

**Title of the project:** Rheologic, functional, and structural aspects of atherogenesis: the impact of extracorporeal hemorheotherapy on microcirculation

**Grant Agency:** Ministry of Health

**Project Number:** NR/8505-3

**Principal Investigator:** M. Bláha

**Co-investigators:** Z. Zadák, J. Malý, V. Bláha, J. Krejsek, M. Cermanová, D. Solichová

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 2140

**Summary of 2007 results**

**Title of the presentation:** Haemorheopheresis in the treatment of microcirculatory disorders

**Authors:** M. Bláha, Z. Zadák, V. Bláha, C. Andrýs, J. Krejsek, R. Malý, D. Solichová, J. Malý

Introduction: The single rheopheresis treatment simultaneously eliminates an exactly defined spectrum of high-molecular weight rheologically relevant plasma proteins (i.e.  $\alpha$ 2-macroglobulin, fibrinogen, LDL-cholesterol, Lp(a), von Willebrand factor, IgM, fibronectin, and putatively multimeric vitronectin). This results in the immediate pulsed reduction of plasma viscosity as well whole blood viscosity, which with a series of treatments can lead to sustained microcirculatory recovery, and change significantly the natural course of a disease.

Methods and patients: In the prospective trial presented here, 27 patients were treated - severe familiar hypercholesterolaemia (FH): 4 pts; non-healing lesions caused by severe ischemic diabetic foot syndrome (IDFS): 5 pts; age related macular degeneration (AMD): 13 pts; acute sensorineural hearing loss (ASHL): 3 pts; thyroid orbital endocrinopathy (TAO): 2 pts. Our own modification of rheopheresis was used: Plasma is obtained by blood cell separator (Cobe-Spectra, Denver, USA). Then it is run through the „second stage“ – a rheofilter (Evaflux 4A, Kuraray) with ethylene-vinyl-alcohol hollow fibres with holes of 0,03 micrometer. Plasma flow is continuous, anticoagulation done with heparin. The size of holes in the filter enables to retain the above mentioned high-molecular elements. Haematological, biochemical and hemorheological parameters were measured before and after procedures and after the finishing of therapeutic series (AMD 8 procedures, IDFS 10, ASHL 3, TAO 10).

Results: Haemorheopheresis appears to be a suitable adjunct therapy for diseases involving severe disturbance of microcirculation, especially when previous therapeutic options were not sufficiently effective or invasive procedures cannot be applied.

Supported by the grant IGA MH CZ NR/8505-3.

**Address for correspondence:** M. Bláha, IIInd Dept. of Medicine, Haematology, Faculty Hospital, Sokolskastreet 480, 500 05 Hradec Králové, CZ



**Title of the project:** Bioindicators for the optimization of effectivity, safety and economy of LDL-apheresis and rheopheresis during the therapy of severe familial hyperlipoproteinaemia (a comparative study)

**Grant Agency:** Ministry of Health

**Project Number:** NR/9103-4

**Principal Investigator:** M. Bláha

**Co-investigators:** M. Blažek, M. Cermanová, P. Solich, D. Šatinský, V. Bláha, D. Solichová,

**Starting date:** 1.1.2007

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 1857

#### **Summary of 2007 results**

**Title of the presentation:** The influence of extracorporeal elimination on statin levels at patients with familiar hypercholesterolemia

**Authors:** M. Bláha, L. Nováková, P. Solich, V. Bláha, M. Blažek, R. Malý, D. Solichová

**Introduction:** The patients with severe form of familiar hypercholesterolemia are treated by high, maximally tolerable doses of drugs, especially statins. During extracorporeal elimination procedure the separation of certain dose of statins could occur. This can lead to the significant decrease of effective level of drug in blood.

**Method:** Development of analytical method was very difficult considering very low levels of these compounds in biological materials. Common analytical approaches including HPLC with conventional detection techniques like UV-VIS or fluorescence detection after derivatization step failed in reaching such sensitivity.

**Results:** UPLC-MS/MS method for the sensitive and selective determination of simvastatin and atorvastatin in biological materials was developed. Its sensitivity is very high. A possibility to measure the concentrations of statins in very wide concentration range (10<sup>-6</sup> to 10<sup>-11</sup> mol/l) is also a great advantage. Three blood samples were collected and subsequently analyzed at 12 patients with familiar hypercholesterolemia in a long-time extracorporeal elimination treatment. The results were reproducible and they showed substantial decrease of statin levels after the procedure.

**Conclusions:** The method is ready for use and for the adjustment of individual treatment plan of treated patients.

Project was supported by the research task IGA MH CZ NR/9103-4.

**Address for correspondence:** M. Bláha, IInd Dpt. of Medicine, Haematology, Faculty Hospital, Sokolskastreet 408, 500 05 Hradec Králové.

**Title of the project:** Atherogenic mechanisms in renal replacement therapy

**Grant Agency:** Ministry of Health

**Project Number:** NR/9259-3

**Principal Investigator:** V. Bláha

**Co-investigators:** D. Solichová, S. Dusilová-Sulková, M. Bláha, C. Andrýs, L.Sobotka

**Starting date:** 01.01.2007

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1562

**Summary of 2007 results**

**Title of the presentation:** Individually targeted intervention decreasing lipopolysaccharide binding protein (LBP) in end stage renal disease patients with atherosclerosis

**Authors:** V. Bláha (1), E. Mistrík, S. Dusilová-Sulková (1), C. Andrýs (2), D. Solichová (1), J. Zahradník (1), B. Hájková (1), L. Sobotka (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. Metabolic Care and Gerontology (1), Dept. Immunology (2)

Text:

Background: Among cellular signaling pathways of specific host immune/inflammatory responses to atherogenesis, LBP represents a link between cardiovascular disease and chronic inflammation caused by endotoxin producing microorganisms.

Aim: Because end stage renal disease (ESRD) patients are well known to suffer greatly from cardiovascular diseases (CVD) caused by excessive atherosclerosis, we measured LBP in ESRD patients after intervention to improve their inflammatory and nutritive status.

Methods: 97 patients were studied. Among them were 43 patients with ESRD (61 y (39, 82), 21 women, 22 men) on chronic hemodialysis without any apparent acute disease or infection. The controls were 54 healthy individuals (33 men and 21 women, 33 (22-65) y) without any CVD or atherosclerotic risk factors. The 3 months intervention consisted in improving the performance of dialysis or sanation of asymptomatic chronic infective foci.

Results: The serum LBP in ESRD was significantly higher than in controls ( $13.88 \pm 6.32$  vs.  $4.94 \pm 3.2$  ug/ml,  $p < 0.001$ ), and significantly decreased after the intervention ( $5.32 \pm 3.2$  ug/ml,  $p < 0.001$ ). There was a significant increase in HDL cholesterol. There was no significant change in CRP (before /after intervention  $6.26 \pm 3.37 / 7.17 \pm 4.12$  mg/l) nor serum lipoproteins (total cholesterol, LDL, TAG). We found no significant correlation between LBP and CRP or any of the serum lipoproteins.

Conclusion: Therapeutic intervention in ESRD patients with CVD and low-level chronic inflammation might improve the course of atherosclerosis marked by serum LBP.

Supported by project IGA Ministry of Health Czech Republic No. NR/9259-3.

**Address for correspondence:** V. Bláha, Department of Metabolic Care and Gerontology, University Hospital Hradec Králové, Sokolská 581, 50005 Hradec Králové, Czech Republic.

**Title of the project:** Oral health of pre-school children, fluoride intake and parental attitudes and behavior towards prevention of dental caries of deciduous dentition.

**Grant Agency:** Ministry of Health

**Project Number:** NR 8331-3/05

**Principal Investigator:** Z. Broukal

**Co-investigators:** R. Ivančaková, J. Dušková, E. Lenčová

**Starting date:** 1.1. 2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1089

**Summary of 2007 results**

**Title of the presentation:** Longitudinal Study of Oral Health in Pre-school Children: Two Years Study of Caries Experience.

**Authors:** R.Ivančaková (1), Z.Broukal (2), E.Lenčová (2)

Fac.Med., Charles Univ., Hr. Králové, Dept. of Dentistry (1). Institute of Dental Research GFH, Prague (2).

Early onset of caries disease and widespread caries experience in pre-school children call for profound evaluation of risk factors and implementation of effective preventive care. The longitudinal intervention study has been launched in pre-school children aimed at ascertaining eating habits, fluoride intake background and parental oral health attitudes and behaviour. An onset examination of dental status has been conducted in a cohort of 3 to 5 year old pre-school children in Prague (P) and Hradec Králové (HK). The criteria for including the child in the study were an informed consent of parents and no systemic disease of the child. The standard methodology recommended by WHO has been used and following parameters were calculated: % caries free, dt, dmft, ri, sci and presence of dental plaque in upper incisors. The total count of examined children was 144. 98 (55 boys/43 girls) in P and 46 (21 boys/25 girls) in HK. The average age of the children was 3.77 (SD  $\pm$  0.29). The mean count of dt in both cohorts was 1.44 (1.58 in boys and 1.39 in girls). There was no statistical difference in dt between P and HK cohorts of children. The mean dmft amounted to 1.75 (dt 81.2%) The dmft was statistically lower in HK (1.48) compared to P (2.05). Restorative index (ri) was 17.3 (no statistical differences). Significant caries index (sci) for both cohorts amounted to 5.96 (significant difference between HK 5.33 and P 6.58). Detectable amount of dental microbial plaque on upper incisors was found in 83 children (61.9 %). No differences between both cohorts. Results of the onset examination showed urgent need of the effective primary preventive intervention based on the next findings. Supported by grant of the Int. Grant Agency of the Min. of Health (CZ) No. NR/8331-3.

**Address for correspondence:** R. Ivančaková, Dept. of Dentistry, University Hospital and Faculty of Medicine in Hradec Králové, 500 05 Hradec Králové.

E-mail address: ivancakovar@lfhk.cuni.cz

**Title of the project:** Evaluation of the Crohn 's disease activity with use of dynamic contrast enhanced ultrasound.

**Grant Agency:** Charles University

**Project Number:** 123307/07 C

**Principal Investigator:** J. Brožík

**Co-investigators:** O. Pozler, P. Dědek, T. Douda, J. Cyrany, P. Rejtar, L. Ungermann,Z. Bělobrádek, V. Tyčová, V. Mašín

**Starting date:** 1.6.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 201

#### **Summary of 2007 results**

**Title of the presentation:** Evaluation of the Crohn 's disease activity with use of dynamic contrast enhanced ultrasound (CEUS).

**Authors:** J. Brožík (1), O.Pozler (2), P. Dědek(2), T.Douda(3), J.Cyrany(3), P.Rejtar (1), L.Ungermann (1),Z. Bělobrádek(1), V. Tyčová (4), V. Mašín (5)

Fac.Med. Charles University and University Hospital, Hradec Králové: (1) Department of Radiology, (2) Department of Pediatrics, (3)Department of Internal Medicine , (4) Department of Pathology (4), Department of Biophysics (5)

Crohn 's disease (CD) is characterised by remitting and relapsing episodes and a common problem in the management of CD is discrepancy between patient 's symptoms and objective signs of disease activity. There is no established gold standard in evaluation disease activity. High- resolution bowel ultrasound with newly introduced ultrasound contrast media of second generation with low mechanical index real time harmonic sonography allows to evaluate extent of neovascularisation of the bowel wall which corresponds with activity of disease. In our study we perform examinations with a linear 7.5 MHz transducer after administration of contrast agent. Every examination is recorded and the pattern of bowel wall enhancement as well as a E/W ratio ( thickness of enhancing wall and thickness of the whole wall) are evaluated by two independent radiologists. Obtained data are correlated with laboratory and clinical values as well as with magnetic resonance enterography findings or in patients after ileocecal resection with histopathological findings.

We have performed in total five examinations so far, in all patients were present signs of CD activity and corresponded with clinical, laboratory, endoscopical and imaging findings.

According to the literature, CEUS has the ability to detect subclinically ongoing disease activity and thus improve management of patients with CD. This ability and other matters concerning CD activity will be further studied.

Literature: C. Serra et al.: European Journal of Radiology 62, 114-121, 2007

D. Robotti et al.: Abdom Imaging 29, 648-52, 2004

**Address for correspondence:** J. Brožík, Radiology Department, University Hospital, Sokolská 581, Hradec Králové, 500 05, Czech Republic





**Title of the project:** Following occurrence heparin resistance near population ill before operation heart in extra-corporeal circulation

**Grant Agency:** Ministry of Health

**Project Number:** 1A/8590-4

**Principal Investigator:** V. Brzek

**Co-investigators:** V. Lonský, S. Jiška, M. Volt, J. Kubíček

**Starting date:** 1.9.2005

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 2509

**Summary of 2006 results**

**Title of the presentation:** THE INCIDENCE OF HEPARIN RESISTANCE IN PATIENT OPERATED WITH THE USE OF EXTRACORPOREAL CIRCULATION

**Authors:** V. Brzek, V. Lonský, S. Jiška, M. Volt, J. Kubíček

University Department of Cardiac Surgery, University Hospital, Hradec Králové, Czech Republic

**Purpose:** Heparin resistance (HR) is described as a common (10-20 %) feature in cardiac operations requiring cardiopulmonary bypass. HR is defined as the need of higher than usual doses of heparin in order to achieve required level of anticoagulation. Four predictors for HR were identified: age  $\geq 65$  years, platelet count  $\geq 300000$  cels/mm<sup>3</sup>, preoperative heparin treatment and AT III levels  $\leq 60$  %. Combination of risk factors increases the probability of HR. The purpose of our study was to identify the incidence of HR in our patients. Resistance to heparin was determined to be prolong the ACT to more than 480 s.

**Methods:** Until now 332 consecutive patients scheduled for cardiac surgery with the use of cardiopulmonary bypass were enrolled in this prospective trial.

**Results:** we following groups of 332 patients. 114 patients (34%) has higher postoperative blood losses than average. From groups of 114 patients has 88 patients (77%) one or more risk factors inductive of heparin resistance.

**Conclusions:** our data confirm the higher incidence postoperative bleeding in patients with heparin resistance.

The study is supported with the grant No. 1A/8590-4 of IGA of Czech Ministry of Health

**Address for correspondence:** V. Brzek, University Department of Cardiac Surgery, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic.

**Title of the project:** Hemorheopheresis in treatment of severe thyroid associated orbitopathy, the role of changes in selected pathogenetic indicators.

**Grant Agency:** Ministry of Health

**Project Number:** NR 9181-3/07

**Principal Investigator:** J. Čáp

**Co-investigators:** M. Bláha, V. Ceeová, M. Škácha, P. Rezek, P. Vlček

**Starting date:** 1.1.2007

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 5223

#### **Summary of 2007 results**

**Title of the presentation:** Hemorheopheresis in treatment of severe thyroid associated orbitopathy - preliminary results.

**Authors:** V. Ceeová (1), M. Bláha (1), M. Škácha (3), P. Rezek (3), P. Vlček (2), J. Čáp (1)

Fac. Med., Charles Univ. and University Hospital, Hr. Králové: (1) Second. Dept. of Internal Medicine; (2) 2<sup>nd</sup> Fac. Med, Charles Univ. Prague; (3) University Hospital Motol, Prague, Dept. of Nuclear Medicine and Endocrinology.

Introduction: Thyroid associated orbitopathy (TAO) is an autoimmune disease characterized by inflammatory swelling and cellular infiltration of orbital tissues. The treatment of choice for severe active disease has been corticosteroids. The effect of other immunosuppressive and somatostatine analogues treatment has not been demonstrated clearly. For the minority of patients with progressive disease threatening eye functions new therapeutic options is desired. Methods and patients: We have developed our modification of cascade rheopheretic method using blood-cell separator Cobe-Spectra and Evaflux 4A filters with pores capable to separate majority of immunoglobulins, including autoantibodies. Three patients with severe active TAO have been treated.

Results: The elimination of an exactly defined spectrum of high-molecular weight proteins including fibrinogen resulted in the reduction of blood and plasma viscosity. Changes of basic haematological and biochemical parameters were without clinical importance. The majority of changes were due to hemodilution (by 5-10%). The method proved to be safe and well tolerated. Severe side-effects were not observed, only transient headache and hypotension occurred in 2 procedures and could be easily controlled.

Conclusion: Treatment procedures lead to decrease in disease activity evaluated both clinically (measured by Clinical Activity Score) and biochemically (drop in antibodies against TSH receptor, thyroperoxidase and thyrogloblin). Whether this will improve outcome of severe active TAO can only be evaluated in larger patients sample.

The project was supported by the grant IGA MH NR/9181-3

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**Title of the research project:** The use of experimental and clinical models of metabolic processes, nutrition and pharmacotherapy for the advancement of knowledge, clinical practice and quality of life improvement

**Grant Agency:** Ministry of Education

**Project Number:** 021620820

**Principal Researcher:** M. Červinka

**Joint Researchers:** J. Cerman, Z. Červinková, V. Geršl, M. Holeček, J. Martínková, J. Mokřý, L. Sobotka, P. Tomšík, A. Žák,

**Starting date:** 1.1.2005

**Duration (years):** 7

**Funds allocated for project -Kč (thousands):** 34833

#### **Summary of 2007 results**

**Title of the presentation:** Nutritional and pharmacological intervention on metabolic processes.

**Authors:** M. Červinka (1), J. Cerman (2), Z. Červinková (3), V. Geršl (4), M. Holeček (3), J. Martínková (4), J. Mokřý (5), L. Sobotka (6), P. Tomšík (2), A. Žák (7) Fac. Med., Charles Univ., Hr. Králové, Depts: Med. Biol. (1), Med. Biochem. (2), Physiology (3), Pharmacol. (4), Histology (5), Internal Medicine (6) and 1st Medical Faculty Charles Univeristy, Prague, 4th Department of Internal Medicine (7)

During 2007, all planned results of the current research project were achieved; in total 18 original papers were published in journals with impact factor and another 60 in peer-reviewed journals. All individual working groups within the project team produced the aforementioned papers.

Interactions of selected signalling pathways (p53-dependent) after administration of micronutrients (selenium, zinc and inositol hexaphosphate) were studied with help of advanced morphological and molecular techniques (blotting, immunofluorescence) in colon cancer cells (HCT-116, HT-29, SW480 and SW620).

We isolated distinct mesenchymal stem cell populations from the bone marrow and the dental pulp and provided their phenotypic characterization using a panel of 22 antibodies. We also succeeded in isolation of stem cells from periodontal ligaments. The latter cells represented a homogeneous cell population with a high proliferative potential and expressed markers of mesenchymal stem cells (CD29, CD44, CD73, CD 90, CD105). The expression levels differed from markers expressed by bone marrow and dental pulp stem cells which confirms isolation of a unique and distinct cell population.

In vitro experiments performed on rat hepatocytes in suspension and/or in culture were focused on the study of possible protective effect of S-adenosylmethionine against acetaminophen-induced injury. Beside markers of cell viability also parameters of functional capacity, ROS production, reduced and oxidized glutathione content and activity of glutathione reductase were measured. Increased sensitivity of mitochondrial permeability transition pore after triiodothyronine administration in vitro was documented. Expression of uncoupling protein 2 RNA was found in the liver in various intervals after partial hepatectomy.

Study of leukaemia cells MOLT-4: Ionizing radiation initiated an ATM-kinase-dependent pathway resulting in upregulation of p53 and induction of apoptosis. Activation of p53 on serine 15 was dose-dependent up to 3 Gy. Valproic acid administered both before and after the irradiation has a radiosensitizing effect.

We found that L-carnitine administered with mitoxantrone in mice may protect against drug-induced toxicity. Furthermore, a new, syngenic mammary tumour model growing in NMRI

(continue)

mice was characterized with selected cytostatic drugs both *in vitro* and *in vivo*. We described in detail the pharmacokinetics and, for the first time, the biliary excretion of melibiose and rhamnose in healthy and cholestatic rats. Moreover, we showed that the dual-sugar permeability test can characterize the alteration of the blood–biliary barrier in acute extrahepatic cholestasis.

Study of liver myofibroblasts: Individual components of extracellular matrix in a culture medium (fibrin, collagen I) modified the cell morphology and selectively influenced gene expression. Hence, there may be a feedback between myofibroblasts and their products.

To obtain deeper insight into the role of cardiac troponins as biomarkers of anthracycline cardiotoxicity, the release kinetics of cTnT and cTnI were studied using an *in vitro* model of isolated rat neonatal ventricular cardiomyocytes (daunorubicin 0.1 – 3  $\mu$ M). On the rabbit model of daunorubicin cardiotoxicity, it was revealed that novel iron chelator deferipron is unable to protect myocardium against oxidative stress and heart failure (in contrast to *in vitro* findings found in the literature).

To understand the pathogenesis and to find new treatment possibilities of muscle wasting in cachexia we studied the effect of proteasome inhibitors (PIs) and leucine metabolite  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) on protein metabolism of septic rats. PIs bortezomib or belactosin significantly reduce myofibrillar proteolysis and inhibit chymotrypsin-like activity (CHTLA) of proteasome in skeletal muscle while cathepsin activity did not change. HMB treatment induced significant decrease in proteolysis, CHTLA and leucine oxidation both in red and white type of muscle.

The influence of –493G/T polymorphism of the gene promotor for microsomal triglyceride transfer protein was studied in 86 persons with metabolic syndrome and 184 controls. The T allele carriers differed from G homozygotes only in the group of men with MS by raised concentrations of insulin, nonesterified fatty acids, cholesterol, triglycerides and decreased content of polyunsaturated fatty acids in plasma phospholipids.

We developed a new modification of hemorheopheresis to treat severe atherosclerotic disease wounds. Our pilot trial confirmed the assumption that a series of hemorheopheresis treatments have a benefit for patients with macular degeneration. 2-Using Laser Doppler Line Scanner we demonstrated, that skin blood flow changes were dependent on several parameters of malnutrition inflammation syndrome. 3-The effect of hyaluronan-iodine complex was studied on 18 patients suffering from complicated foot diabetic wounds and complete healing was evident in 15 patients within 9-20 weeks after the start of treatment.

Biomarkers showed that amiodarone-treated rats exhibited 3.3-fold decrease in renal clearance of conjugated bilirubin and reduction of MTX biliary clearance of methotrexate to 72% with a significant increase in plasma concentration. In man, methamphetamine can partially impair visual processing. In a rat model of lipopolysaccharide-induced pulmonary inflammation, pentoxifylline administered intratracheally reduced nitrosative and oxidative stress as documented by exhaled nitric oxide as well as by other cellular and biochemical markers of inflammation.

Project was supported by the Ministry of Education Research Project, No. MSM 021620820.

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

**Grant Agency:** Charles University

**Project Number:** 24807/2007 C

**Principal Investigator:** J. Dusová

**Co-investigators:** D. Hejčmanová

**Starting date:** 1.1.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 160

**Summary of 2007 results**

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

**Authors:** J. Dusová, D. Hejčmanová

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  $518\mu\text{m}$  to  $374\mu\text{m}$  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  $476\mu\text{m}$  to  $309\mu\text{m}$ . 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  $536\mu\text{m}$  to  $244\mu\text{m}$  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.

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**Title of the project:** Creation of virtual and material anatomic models by „rapid prototyping“ method.

**Grant Agency:** Ministry of Education

**Project Number:** 817/2007F3a

**Principal Investigator:** P. Hájek

**Co-investigators:** L. Čapek

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 180

**Summary of 2007 results**

**Title of the presentation:** Creation of virtual and material anatomic models by „rapid prototyping“ method.

**Authors:** P. Hájek (1), L. Čapek (2)

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

Fac. of Mechanical Engineering, Technical Univ. of Liberec, Dept. of Engineering Mechanics (2)

Text:

Our teaching experiences show that 3D imagination is one of the most difficult point of view in the anatomy education. We decided to create a database of 3D virtual and physical models. The data acquired from CT are processed in software 3D-Doctor, which allows to create 3D virtual copy. Computer corrections and conversion to 3D models in wrl format are possible. Wrl models can be rotated on monitor by simple movement of PC mouse. These virtual models are put on the web sites of our departments subsequently and produced on CD for self-study. Moreover these virtual models serve as an input for Rapid Prototyping systems. In Rapid Prototyping method the output leads to a machine (3D printer) which stratifies special powder by coordinates and joins it by adhesive paste. Material is not toxic, it fits to be painted and drilled, models can be washed.

The benefit is also in representation of small difficult structures, for instance labyrinthus osseus. Expenses of our method are significantly reduced contrary to buying commercial models. Material models will be used in practical classes in Department of Anatomy.

This work is supported by grant of Ministry of Education No 817/2007

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**Title of the project:** Use of biocompatible materials with shape memory in construction of temporary or permanent human tissue scaffolds

**Grant Agency:** Ministry of Commerce

**Project Number:** FT-TA/097/04

**Principal Investigator:** J. Hanuš

**Co-investigators:** J. Záhora, A. Bezrouk

**Starting date:** 1.1.2004

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 11650

**Summary of 2007 results**

**Title of the presentation:** Physical model of the spiral stent

**Authors:** A. Bezrouk, J. Hanuš, J. Záhora

Fac. Med., Charles Univ., Hr. Králové: Dept. of Medical Biophysics

The miniinvasive methods of the application of scaffolds (stents, stentgrafts) for various indications are known for their high safety of a medical treatment. These methods also have some disadvantages, the most typical are leakage, shift, and crack of the scaffold. It is caused by the design of the scaffold most frequently. The aim of our research was to develop a complex mathematical model of the spiral stent and to develop and realize the measurement methods and set-ups. The mathematical model should describe the deformation properties of the spiral stent. The measurement methods should enable the spiral stents to be custom designed according to the biomechanical properties of the biological system. This should be possible in the connection with the mathematical model and simulation process at the place of application of the spiral stent. We developed such an “exact” mathematical model. Our model enables the prediction of the behavior of the spiral stent and it gives the possibility of the spiral stent to be custom designed for a concrete patient. Designed model enables us also to perform the easier and more exact measurement of the axial force depending on the total length of the spiral stent. Then, using the proper transformations, the model determines the radial force and the radial pressure depending on the total radius of the spiral stent. The spiral stent acts on the tissue by the radial pressure. Our model is also the keystone of the corresponding research about the relationships between the spiral stent and the tissue.

Literature: J. Záhora, A. Bezrouk, J. Hanuš: Models of stents – Comparison and applications, *Physiol. Res.* 2007, 56 (Suppl. 1): In press

Project was supported by the Ministry of Commerce Grant Agency , No FT-TA/097

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**Title of the project:** Combination of ketamine-dexmedetomidine in experiment and clinical practice

**Grant Agency:** Ministry of Health

**Project Number:** NR/8508-3/05

**Principal Investigator:** L. Hess

**Co-investigators:** J. Málek, J. Schreiberová

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1092

**Summary of 2007 results**

**Title of the presentation:** Dexmedetomidine-ketamine-midazolam combination for sedation in endovascular embolizations of cerebral arteriovenous malformations and carotid stenting

**Authors:** J. Schreiberová (1), L. Hess (2)

Charles Univ. in Prague, Fac. Med. and Univ. Hospital Hradec Králové, Dept. of Anesth. and Intensive Care Medicine (1), Institute for Clinical and Experimental Medicine, Prague (2)

Sedation is indicated in endovascular interventional procedures to secure immobility, analgesia, anxiolysis, and amnesia of the patient. Hemodynamic stability, absence of respiratory depression, preserved patient responsiveness and cooperation are required. We used theoretically promising dexmedetomidine-ketamine-midazolam combination and compared with routine sufentanil-midazolam sedation. After local ethic committee approval, 62 ASA 1-3 patients were randomly allocated into 2 groups for sedation. Group A: bolus application of dexmedetomidine 0,5 µg/kg, ketamine 0,25 mg/kg, midazolam 0,02 mg/kg i.v. over 5 minutes, followed by infusion of dexmedetomidine 0,25 µg/kg/h and ketamine 0,25 mg/kg/h. Infusion rate was altered to keep the patient sedated, but responsive and cooperative. Group B: bolus of sufentanil 10 µg and midazolam 0,02 mg/kg followed by further bolus increments as needed. Blood pressure (BP), ECG, sat O<sub>2</sub>, paCO<sub>2</sub>, Ramsay sedation score, complications, rescue medication, and recovery were recorded. Patient cooperation was assessed by the radiologist (blind to the method used) as excellent-sufficient-poor at the end of each procedure. Statistical analysis: t-test,  $\chi^2$  test, ANOVA for repeated measures. Decreases of BP and heart rate were deeper in A group ( $p < 0,001$ ), though the number of episodes of hypotension or bradycardia did not differ significantly. Intraindividual variability of BP was lower in A group ( $p < 0,05$ ). Cooperation was better in A group ( $p < 0,001$ ). No clinically significant complications were recorded. Dexmedetomidine-ketamine-midazolam sedation seems to be safe method for endovascular neurointerventions securing excellent cooperation and hemodynamic stability without respiratory depression.

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**Title of the project:** Hepatic steatosis and its influence on regeneration of the rat liver after partial hepatectomy. Experimental study

**Grant Agency:** Ministry of Health

**Project Number:** NR/8500-3

**Principal Investigator:** P. Hrubá-Mocková

**Co-investigators:** P. Živný (1), H. Živná (2), D. Feřtek (1), V. Palička (1)

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1508

**Summary of 2007 results**

**Title of the presentation:** Modulation of rat liver regeneration after partial hepatectomy by dietary cholesterol

**Authors:** P. Živný, H. Živná, P. Mocková, V. Palička, L. Pavlíková, J. Cermanová, S. Mičuda (1) Institute of Clinical Biochemistry and Diagnostics, University Hospital, Charles University, Hradec Králové, Czech Republic (2) Radioisotope Laboratories and Vivarium, Medical Faculty, Charles University, Hradec Králové, Czech Republic

Objective. The aim of study was to evaluate impact of long-term dietary cholesterol overload on the cholesterol homeostasis and liver regeneration in rats. Materials and Methods. Serum lipid parameters, tissue <sup>14</sup>C-cholesterol incorporation, liver DNA synthesis and selected protein expression was determined in partially hepatectomized (PH) rats fed with a standard (SLD) or hypercholesterolemic (CHOL) diet. Results. 29-day continual intake of high cholesterol diet in rats before PH produced increase in serum total cholesterol, LDL-cholesterol, and triglyceride concentration. PH caused decrease in serum total cholesterol, HDL-cholesterol and triglyceride concentration in both SLD and CHOL groups. Hepatic DNA synthesis was increased after PH in both dietary groups, but less in CHOL animals. Long-term CHOL diet produced insignificant up-regulation of Acyl-CoA: cholesterol acyltransferase-2 protein expression (ACAT-2). PH was associated with insignificant changes of 3-hydroxy-3-methylglutaryl coenzyme A reductase, with marked increase of LDL receptor and ACAT-2 and decrease in CYP7A1 protein expression in both dietary groups. In conclusion, the present study is one of the few describing the mechanism of impaired liver regeneration after high cholesterol dietary intake. We assumed, that optimal liver regeneration was initiated by successive uptake of exogenous extrahepatic cholesterol and its esterification by ACAT-2, followed by gradual increase of LDL receptor protein expression with decrease of cholesterol degradation to bile acid through decrease of CYP7A1 protein expression. These mechanisms were less marked or delayed in hypercholesterolemic groups.

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**Title of the project:** Creating of the microscope-operated educational dental centre.

**Grant Agency:** Ministry of Education

**Project Number:** 1100/07 A a

**Principal Investigator:** V. Hubková

**Co-investigators:** R. Slezák, R. Ivančaková

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 1165

**Summary of 2007 results**

**Title of the presentation:** Creating of the microscope-operated dental centre targeted for the undergraduate clinical education in the dentistry.

**Authors:** V. Hubková, R. Slezák, R. Ivančaková, M. Záhořík

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

The aim of the project was to gain the clinical microscope-operated centre using so called operative mobile stereomicroscope OPMI PROergo (Carl Zeiss) at the Dept. of Dentistry, Faculty of Medicine in Hradec Králové. The device enables to improve the quality of the educational clinical process in various branches of modern dentistry, e. g., diagnosis and treatment of various diseases affecting teeth, periodontal tissue and oral mucous membrane in the area of restorative dentistry and endodontics, preventive and paediatric dentistry, and oral medicine and pathology (microdentistry). The centre helps undergraduate students of dentistry to be familiar with principles of microdentistry, since the device has been used more often by private dental practitioners and dental specialists, as well. Using another financial sources the transmission of the optical signal from the microscope into the large TV monitor has been realized in the same year. The training of undergraduate students in microscopically-assisted periodontal and oral mucosa selected diagnostic and therapeutic procedures has been started. Additional multifunctional device (MediLive MindStream, Carl Zeiss) will be obtained within the next year 2008, allowing to record, edit, cut and archive all realized microscopically-assisted procedures and create consequently various modern educational tools (pictures, videos). The last step would be the connection of the microscope with the local computer network.

The project was supported by the Ministry of Education (FRVS), No 2B06104.

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**Title of the research project:** Hemodynamic, clinical and biochemical monitoring of patients before and after transjugular intrahepatic portosystemic shunt (TIPS), part III

**Grant Agency:** Ministry of Health

**Project Number:** NR/8419-4/05

**Principal Researcher:** P. Hůlek

**Joint Researchers:** V. Šafka, J. Štefánková, R. Pudil, T. Fejfar, V. Koblížek

**Starting date:** 1.1.2005

**Duration (years):** 4

**Funds allocated for project - total in Czech crowns:** 1168

**Summary of 2007 results**

**Title of the presentation:** Hemodynamic, clinical and biochemical monitoring of patients before and after transjugular intrahepatic portosystemic shunt (TIPS), part III

Ministry of Health, NR 8419-4

**Authors:** <sup>1</sup>Petr Hůlek, <sup>2</sup>Václav Šafka, <sup>1</sup>Jozefína Štefánková, <sup>1</sup>Radek Pudil, <sup>1</sup>Tomáš Fejfar, <sup>1</sup>Vladimír Koblížek, <sup>1</sup>Miroslav Solař, <sup>3</sup>Antonín Krajina, <sup>4</sup>Jaroslav Vižďa, <sup>3</sup>Leoš Ungermann, <sup>5</sup>Jan Kremláček

<sup>1</sup>Dept. of Internal Medicine, <sup>2</sup>Dept. of Physiology, <sup>3</sup>Dept. of Diagnostic Radiology, <sup>4</sup>Dept. of Oncology and Radiotherapy, <sup>5</sup>Dept. of Pathological Physiology, Charles University in Prague,

Faculty of Medicine in Hradec Králové.

The project follows with previous successful projects aiming to improve the care for patients with serious portal hypertension treated with TIPS. It concentrates on the problem of hepatopulmonary syndrome and portopulmonary hypertension. It deals with prevalence, diagnosis, etiopathogenesis and eventual impact of TIPS. Methods consist in catheterization measurement of the parameters of pulmonary circulation and clinical evaluation of respiratory functions, comparing this findings with results of scintigraphic and ultrasonographic investigation, and also in their comparison with measured parameters of portal circulation and plasmatic levels of selected hormones and mediators in specific compartments of circulation. Another aim is finalizing and verifying the method to predict the impact of TIPS on liver circulation using scintigraphy of liver perfusion, started in previous project. The third aim is to follow with previous results of glucose metabolism research in liver disease with portal hypertension and study influence of TIPS on the altered nutrition status in these patients. In the year 2006 17 patients were evaluated according to the study protocol. Total number of patientes since starting date of the project is 46.

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**Title of the project:** Physiological modeling of nitric oxide bioavailability and effects in lung diseases

**Grant Agency:** Ministry of Education

**Project Number:** 1P05OC066

**Principal Investigator:** J. Chládek

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

**Starting date:** 1.1.2005

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 700

#### **Summary of 2007 results**

**Title of the presentation:** Methodological aspects of the online measurement of exhaled lower respiratory nitric oxide in children with allergy and asthma: a detailed analysis of single-breath profiles and two different analyzer

**Authors:** J. Chládková, Z. Havlínová, J. Chládek, J. Martínková

Exhaled nitric oxide (eNO) measurement is a valuable tool in the management of patients with asthma. In this study, the results of eNO measurement in children with allergy and asthma using an electrochemical hand-held analyzer (NIOX MINO) were compared to those from a chemiluminescence analyzer (CLD88sp). Both analyzers use two different modes of eNO evaluation for children >12 yr and for younger children.

**Methods.** A total of 82 children and young adults (4.9–18.7 years; 44 males), 58 with atopy and 59 with asthma were included. The mean of three approved exhalations at the flow rate of 50 ml/s in CLD88sp and the first approved measurement were compared to the first approved result from NIOX MINO. Moreover, a detailed analysis of single-breath eNO profiles from CLD88sp was performed in order to select a stable plateau eNO value according to the current guidelines.

**Results.** Success rate was high ( $\geq 91\%$ ) in both analyzers. The FENO values ranged from 4.4 to 255 ppb in children >12 yr and from 2.5 to 42.6 ppb in children <12 yr, respectively. The repeatability of the eNO value from triplicate measurements was markedly better in older children compared to younger children [the geometric mean (range) of the percent intra-CV 4.8 (0.3-15) % vs. 14.8 (3.8-51) %]. In children >12 yr, the expected median ratio (CLD88sp to NIOX MINO) of 1.11 indicated a small bias (the eNO values obtained using CLD88sp were 11% higher). In children <12 yr, the expected median ratio of eNO values of 0.89 showed that results obtained using CLD88sp are 11% less. In children >12 yr, the guideline-derived plateau eNO values agreed very well with the eNO plateau values obtained as a result of automatic evaluation by the CLD88sp. Contrary to that, the guideline-derived plateau eNO values of younger children were 25% higher compared to the results automatically calculated. Supported by 1P05C066.

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**Title of the project:** The histopathology of middle ear with the main topic of middle ear inflammation

**Grant Agency:** Ministry of Health

**Project Number:** NR/8376-3

**Principal Investigator:** V. Chrobok

**Co-investigators:** A. Pellant, P. Rothrockel, E. Šimáková, V. Juttnerová, B. Ježek, K. Antoš

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 119

#### **Summary of 2007 results**

**Title of the presentation:** Hearing loss and the Middle Ear Risk Index (MERI)

**Authors:** V. Chrobok (1), A. Pellant (1), M. Meloun (2), K. Pokorný (1), E. Šimáková (3)  
Dept. of ORL, Regional Hospital of Pardubice, Faculty of Health Studies, University of Pardubice (1), Faculty of Chemical Technology, University of Pardubice (2), Dept. of Pathology, University Hospital, Charles University, Hradec Králové (3)

**Objectives/Hypothesis:** The aim of this study was an observation of the impact of prognostic factors (the Middle Ear Risk Index [MERI] by Becvarovski and Kartush) on hearing of patients surgically treated for chronic otitis media. Furthermore, the aim of the study was to determine which of the monitored factors were linked to greater hearing impairment.

**Methods:** The level of hearing was assessed for each preoperative prognostic factor category in a total of 155 patients surgically treated for chronic otitis media. A pre-op and post-op statistical analysis of each patient's hearing was conducted by pure-tone audiometry using the MERI.

**Results:** Patients with a generally lower MERI had better pre-op and post-op air and bone conduction than patients with a higher MERI ( $p < 0.05$ ). Among patients with a MERI of 0 to 3 (minor disorder), there was a post-op improvement in air conduction at frequencies of 0.5 to 3 kHz by 4 to 6 dB. In a group with a MERI of 4 to 6 (a moderate disorder), air conduction dropped at frequencies of 3 and 4 kHz by 4 and 5 dB, and among patients with a MERI of 7 or higher (a severe disorder), air conduction declined at all frequencies by 1 to 7 dB.

**Conclusions:** An evaluation of pre-op and post-op hearing revealed that highly significant pre-op negative prognostic factors were the presence of cholesteatoma, the presence of perforation of the tympanic membrane, ossicular status, previous surgery, and the overall sum of the MERI. A less significant negative factor was smoking. Minor prognostic factors included the presence of middle ear granulations and otorrhoea. Patients with a higher overall MERI have a more severe impairment of the hearing threshold of air and bone conduction pre-op and post-op than patients with a lower MERI.

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**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):** 2

**Total funds allocated for project - Kč (thousands):** 505

**Summary of 2007 results**

**Title of the presentation:** Gene expression in liver myofibroblasts affected by compounds of extracellular matrix.

**Authors:** A. Jiroutová, J. Kanta, L. Majdiaková, M. Hajzlerová

Fac. Med., Charles Univ., Hradec Králové, Dept. of Medical Biochemistry

Liver fibrosis may be defined as an excessive accumulation of extracellular matrix (ECM). In past years the attention has been paid to the fibrogenic liver cells - hepatic stellate cells (HSC) and liver myofibroblasts (MFB). In the present study we investigated the influence of extracellular matrix environment (fibrin and collagen gel) on gene expression in MFB. We isolated nonparenchymal rat liver cell population. Liver myofibroblasts were obtained by passaging these cells 5 times. cDNA array analysis showed changes in the expression of a number of genes when MFB were cultivated in gels. Real-time RT-PCR was used for the verification of the array results. Expression of metalloproteinases was stimulated by the matrix in which the cells were embedded. Metalloproteinases play an important role in the remodeling of connective tissue that precedes the formation of a scar. These changes can be supported by a decrease in the level of plasminogen activator inhibitor mRNA and by an increase in the level of transforming growth factor beta1 mRNA. The changes in the expression of some protein components of ECM (osteopontin, thrombospondin 2) may have been affected by the altered level of certain growth factors and mediators. Hyaluronan facilitates cell migration and the increased expression of hyaluronan synthase 2 mRNA may support this process.

We can conclude that ECM plays an important role in the regulation of cell behavior (proliferation, migration, protein synthesis). The present work shows that two important components of extracellular matrix (fibrin and collagen type I) in three-dimensional contact with liver myofibroblasts can stimulate their remodelling activities. Our results require further studies on protein level.

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**Title of the project:** Prove of significance of pressure measurements in carpal tunnel during the carpal tunnel syndrome minimally invasive technique surgery

**Grant Agency:** Ministry of Health

**Project Number:** NR/8404-3/05

**Principal Investigator:** M. Kanta

**Co-investigators:** E. Ehler, D. Lastovicka, J. Adamkov

**Starting date:** 10.4.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1863

**Summary of 2007 results**

**Title of the presentation:** Intracarpal pressure measurement in endoscopic carpal tunnel syndrome surgery – an analysis of EMG findings, pressure values and patients ratings

**Authors:** M. Kanta<sup>1</sup>, E. Ehler<sup>2</sup>, D. Lastovicka<sup>1</sup>, J. Adamkov<sup>1</sup>

<sup>1</sup>Department of Neurosurgery, University Hospital, Hradec Králové, Faculty of Medicine in Hradec Kralove, Charles University in Prague

<sup>2</sup>Department of Neurology, Regional Hospital, Pardubice

Endoscopic carpal tunnel syndrome surgery is a modern minimally invasive method of carpal tunnel decompression. To further improve and optimize results of endoscopic surgery we used an intracarpal pressure sensor made by Codman to verify the effect of carpal tunnel decompression. We investigated two groups of patients. The first group was done by 29 subjects (3 males) aged 27 to 78 with a median age of 57 years. The second group done by 32 subjects (5 males) aged 24 to 83 with a median age of 55 years. The EMG measurements were performed prior to and 3 and 6 months after surgery. The following parameters were evaluated: DML, ACMAP, SCV and ASNAP. In both groups, we observed similarly significant improvements in all conduction parameters. No difference in postoperative EMG between the two groups was observed. Patients' ratings were significantly improved in both groups (satisfaction, postoperative pain, full hand function return, length of work inability). Pressure measurement during CTS surgery enables new insight and provides the surgeon with valuable information such as the distal border of the carpal ligament, better control over decompression and objectivization, enabling more repeatable results. As far as postoperative EMG is concerned, improvement occurred in both groups, but a statistically significant difference between these two groups was not found, i.e. pressure measurement during CTS surgery did not have a significant effect on postoperative EMG improvement and on the patients rating too.

**Address for correspondence:** Dr. M. Kanta, Ph.D., Department of Neurosurgery, University Hospital of Charles University, Hradec Králové, e-mail: kantam@lfhk.cuni.cz

**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):** 1493

#### **Summary of 2007 results**

**Title of the presentation:** Neural cell differentiation of dental pulp stem cells

**Authors:** J. Karbanová (1), T. Soukup (1), J. Mokřý (1), S. Mičuda (2), J. Suchánek (4), R. Ivančáková (4), R. Pytlík (5)

(1) Dept. of Histology and Embryology, (2) Dept. of Pharmacology, Fac. Med., Charles Univ. Hr. Králové, (3) Dental Clinic, Univ. Hospital in Hr. Králové, (2) 1st Dept. of Internal Medicine, 1st Fac. Med., Charles Univ., Prague, Czech Republic

Dental cavity encloses tissue material, which is assumed to be of ectomesenchymal origin. This incavated dental pulp from third molars we utilized for isolation of stem cells (SCs). Using the innovated culture method based on low serum content medium supplemented with epidermal and platelet-derived growth factors we obtained stem cell population which showed excellent culture characteristics and immature phenotype. With respect to neural crest origin of these dental pulp stem cells (DPSCs) we decided to examine their phenotype and differentiation properties towards neural cells. Acquired DPSCs expressed markers typical for the mesenchymal SCs including STRO-1, stem cell associated markers (hTERT, nucleostemin, CXCR4,  $\beta$ 1-integrins, weakly VEGFR2, Bcrp1 and MDR1) and also nestin. In expansion medium cells did not reveal immunoreactivity to majority of neural cell markers. When the cells were placed into medium commonly used for cultivation of neural SCs supplemented with serum, DPSCs generated spheroids while exhibiting positivity for markers of immature neural cells – nestin, A2B5, RC2, O4 and SSEA-1. Exclusion of serum from media facilitated cell differentiation and expression of neuronal (Pan NF,  $\beta$ -III tubulin) and glial (GFAP) markers. The number of cells expressing neuronal markers increased when supplemented medium with BMP2 which is known to instruct neural crest cells into autonomic neurons. When the valproic acid was added to serum free neural SC medium cells become elongated and strongly positive for nestin, beta-III tubulin and Pan NF. Our experiments showed that DPSCs could be induced in vitro to exhibit neural cell phenotype, which suggests their neural crest origin but another experiments including those in vitro remains further to evaluate it. Nevertheless DPSCs seems to become a promising easily accessible source of SCs for tissue engineering.

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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/8037-5/04

**Principal Investigator:** M. Kaška

**Co-investigators:** T. Grosmanová, E. Havel, D. 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 2007 results**

**Title of the presentation:** The Impact of Preoperative Fasting on Elective Surgical Patients.

**Authors:** M. Kaška (1), T. Grosmanová (2), E. Havel (1), D. Bareš (1), M. Brtko (1), R. Hyšpler (1), V. Tošnerová (1), Z. Petrová (2), B. Schusterová (2), M. Sluka (2), L. Pyszková (2)

1 - LF a FN HK, 2 - FN Olomouc

The group of 161 patients had been treated surgically for chronic malignant or benign disease of the colon. That was divided into three subgroups: A-56 patients basically fasting, B-50 patients secured by parenteral intake, and 55 patients drinking special drink preoperatively. The monitored biochemical parameters in A, B, and C were roughly the same for electrolytes, glucose, C-reactive protein, and albumin serum concentration. In B and C there was a relative decrease in the serum concentration of insulin, myoglobin, and creatin kinase. At the beginning of surgery patients' stomach condition were the best in C. The muscular power and its recovery was better in B and C than in A, a flow velocity through mitral valve was higher in B, and an ejection fraction was better in B and C postoperatively. Unpleasant feelings like thirst, hunger, nervousness, fear, were reduced preoperatively in C essentially and in B partially.

Some of this above mentioned results were published as a poster at 29<sup>th</sup> Congress of ESPEN in Prague (September, 2007) and in the magazine Clinical Nutrition: Kaska M, Grosmanova T, Havel E, Petrova Z et al. Influence of preoperative fasting on elective surgical patients. Clinical Nutrition 2007, 26:2(Suppl. 2), 143. ISSN 1744-1161. The original article with all actual results of our four years investigation for special magazine is preparing during this days. One lecture was performed at the Burn Injury Department (April, 2007), Teaching Hospital - Královské Vinohrady in Prague and one at the Medical Faculty, Palacký University, Olomouc (May, 2007). Next one poster was performed at 11<sup>th</sup> Scientific Conference of the Medical Faculty and the Teaching Hospital in Hradec Králové.

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**Title of the project:** Dynamic and delayed contrast enhancement in upper abdominal MRI studies: comparison of gadoxetic acid and gadobutrol

**Grant Agency:** Charles University

**Project Number:** 142007/2007C

**Principal Investigator:** L. Klzo

**Co-investigators:** J. Zizka, V. Kynosová, O. Dašek

**Starting date:** 20.4.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 160

**Summary of 2007 results**

**Title of the presentation:** Dynamic and delayed contrast enhancement in upper abdominal MRI studies: comparison of gadoxetic acid and gadobutrol

**Authors:** L.Klzo, J. Zizka, V. Kynosová, O. Dašek

Univ. hospital and Fac. Med., Charles Univ., Hr. Králové: Dept. of Radiology

Hepatospecific contrast agents demonstrated development during last years, improved sensitivity and specificity for detection of focal liver lesions compared to other diagnostic methods was approved. In 2005 new contrast agent - gadoxetic acid - was approved for clinical use. The aim of our study was to prospectively compare contrast properties of clinically used extracellular contrast agent (gadobutrol) and hepatospecific (gadoxetic acid) contrast agent in upper abdominal MRI studies. Patients were examined using standardized (0.1 ml/kg) dose of both contrast agents (5 and 5 subjects). MR signal intensity changes (SIC) between precontrast scans and arterial phase, portal venous phase, equilibrium, and delayed scans at 10 and 20 min were measured and compared. In abdominal aorta, the mean SIC in the arterial phase did not differ between both contrast agents. In portal vein, the mean SIC in the portal venous phase significantly differed between gadobutrol (262%) and gadoxetic acid (171%). Liver parenchyma enhancement was higher for gadobutrol than for gadoxetic acid in both arterial phase (26 versus 12%) and portal venous phase (79 versus 43%). On the contrary, gadobutrol reached lower mean SIC in the liver on delayed scans at 10 min (46 versus 55%) and 20 min (38 versus 64%), as well as in common bile duct at 10 min (54 versus 133%) and 20 min (57 versus 457%). In the spleen, mean SIC for gadobutrol was higher at all phases. Although signal intensity increase after bolus injection of gadoxetic acid was inferior when compared to gadobutrol, gadoxetic acid showed adequate enhancement of upper abdominal structures during perfusion phases. Possibility of late hepatospecific imaging during single examination makes this contrast agent unique among other hepatospecific contrast agents. Project was supported by the Charles University Grant Agency, No 142007/2007.

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**Title of the project:** Examination of function and ultrastructure of respiratory cilia of adult patients suffering from chronic obstructive pulmonary disease - part II (comorbidity data from The CILIARY STUDY)

**Grant Agency:** Ministry of Health

**Project Number:** NR/8407-4/05

**Principal Investigator:** V. Koblížek

**Co-investigators:** F. Salajka, D. Pohnětalová, M. Tomšová, J. Chládek, H. Vaníček

**Starting date:** 1.1.2005

**Duration (years):** 4

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

**Summary of 2007 results**

**Title of the presentation:** COPD and its comorbidities - prospective study (partial results of CILIARY study).

**Authors:** Vladimír Koblížek (1), František Salajka (1), Dagmar Pohnětalová (2), Markéta Tomšová (2), Jaroslav Chládek (3), Hubert Vaníček (4), Miroslav Lánský (5).  
Fac.Med., Charles Univ., Hr.Králové: Dept.of Pulmonary Medicine (1), Dep. of Pathology (2), Dept.of Pharmacology (3), Dept.of Pediatric Medicine (4), ENT Dep

Introduction: COPD pts often have a variety of other diseases related to smoking or aging or significant systemic effects of COPD. Aim: To investigate comorbidity rates of our COPD pts. Methods: Prospective study of our COPD pts. (CILIARY STUDY) with the assessment of the comorbidity and mortality rates (during 2 years of follow up). Material: From Jan 04 to Feb 07 we have performed clinical observation of 134 COPD pts: stage I 13 pts (age 66,8), II 41 pts (67,5), stage III 35 pts (69.1), stage IV 45 pts (66.3), 15 adult cystic fibrosis (CF) (27.7) and 10 healthy volunteers (63.0). Results: 46% of the COPD stage IV pts suffered from four and more comorbidities, 31% had two or three comorbidities and 22% had zero or one comorbidity. In stage III we have found 31% ( $\geq 4$  comorb.), 37% (2-3 c.) and 31% pts (0-1 c.). Among stage II we have noted 26% ( $\geq 4$  c.), 46% (2-3 c.) and 26% pts (0-1 c.). In the I stage we have recorded 7% ( $\geq 4$  c.), 69% (2-3 c.) and 23% pts (0-1 c.). Results in the control groups have been 46% ( $\geq 4$  c.), 53% (2-3 c.) and 0% pts (0-1 c.) in CF pts 10% ( $\geq 4$  c.), 40% (2-3 c.) and 50% 0-1 c.) in healthy volunteers. Mortality rates were noted 22%, 11%, 5%, 0% (COPD stage IV, III, II, I), 20% (CF) and 0% (volunteers). The differences between comorbidity and mortality frequency were assessed by Fisher exact test and Chi-square test. Assessment of comorbidity frequency: the differences were found between COPD stage I and COPD stage IV pts ( $p=0.021$ ) and between CF pts and healthy volunteers ( $p=0.006$ ). Evaluation of mortality frequency: the difference was revealed between COPD stage II and COPD stage IV pts ( $p=0.028$ ). Conclusion: Our data showed that comorbidity occurrence and mortality rates of COPD pts increased with severity of disease. Possible generalization of these results would require further investigation and analysis.

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

**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 2006 results**

**Title of the presentation:** Compartmentalization of anti-tumour cell-mediated response in patients with renal cell carcinoma

**Authors:** O.Kopecky (1, 4), Š. Lukešová (1, 4), V. Vroblová (2), D.Hlávková (2), C. Andrýs (2), P. Morávek (3), M., D. Vokurková (2), H. Šafránek (3)

University Hospital, Hradec Králové, 2nd Dept. of Internal Medicine(1), Inst. of Clinical Immunology and Allergy (2), Dept. of Urology (2), Dept. of Pathology (3), District Hospital Náchod, Dept. of Oncology (4)

**Introduction:** Views concerning the role of T lymphocytic subpopulations and NK cells in an anti-tumour response are not established. The aim is to determine the phenotype and activation of lymphocytic cells and to compare their representation in tumour stroma (TIL), peripheral blood (PBL) and renal vein blood in patients with RCC.

**Patients and methods:** The samples of peripheral blood taken from the cubital and renal veins and tumour stroma cells were obtained from 60 patients in the course of their surgeries carried out due to primary RCC. TILs were isolated from mechanically disintegrated tumour tissue. Immunophenotype multiparametric analysis of PBL and TILs was carried out. Results: CD3+ T lymphocytes (70.4%) were the main population of TILs. The number of CD3+/8+ T lymphocytes was significantly higher in TILs, 39.7% ( $p < 0.01$ ), while CD4+ T lymphocytes were the majority population in peripheral blood, 41.35% ( $p < 0.001$ ). The representation of CD3+/69+ T lymphocytes was significantly higher in TILs, 32.05%, compared to PBL ( $p < 0.001$ ). On the contrary, the numbers of CD3+/25+, CD8+/57+ and CD4+/RA+ (naive CD4+ T lymphocytes) were higher in PBL ( $p < 0.001$ ). The differences in representation of (CD3-/16+56+) NK cells and CD3+/DR+ T cells in TILs and PBL were not significant.

**Conclusion:** The above-mentioned results prove that the characteristics and intensity of anti-tumour responses are different in compared compartments (tumour/PBL). CD3+/CD8+ T lymphocytes are the dominant lymphocytic population of TILs.

Project were supported by the Internal Grant Agency of Ministry of Health, No. NR/8914-4

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**Title of the project:** Effects of beta-hydroxy-beta-methylbutyrate treatment on protein and amino acid metabolism in model of sepsis

**Grant Agency:** Charles University

**Project Number:** 32207/2007 C

**Principal Investigator:** M. Kovařík

**Co-investigators:** M. Holeček, L. Šišpera, T. Muthný

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 272

**Summary of 2007 results**

**Title of the presentation:** Effects of beta-hydroxy-beta-methylbutyrate treatment on protein metabolism in rat skeletal muscle

**Authors:** M. Kovařík (1), T. Muthný (2), L. Šišpera (1), M. Holeček (1)

Charles University, Faculty of Medicine, Department of Physiology, Hradec Králové (1) and Faculty of Pharmacy, Department of Pharmacology and Toxicology, Hradec Králové (2)

Beta-hydroxy-beta-methylbutyrate (HMB) is the leucine metabolite, which potentially decreases proteolysis and activates protein synthesis in skeletal muscle and could contribute to reversing AIDS- and cancer-related cachexia. The aim of our study was to determine changes after HMB treatment in protein metabolism in skeletal muscle of intact and septic rat. Animals were implanted with osmotic pump with or without HMB content (0.5 g/kg/day); sepsis was induced by single endotoxin administration (5 mg/kg) ( $n \geq 9$  for each group). After 24 hours extensor digitorum longus (EDL) and soleus (SOL) muscles were isolated and used for determination of total and myofibrillar proteolysis (PL), protein synthesis (PS), leucine oxidation (OL), chymotrypsin like activity (CTLA) and expression of alpha subunits of proteasome. Sepsis induced a stimulation of PL, CTLA and OL in both types of muscles and an attenuation of PS in EDL only. In HMB treated septic animals we observed a decrease in OL in both types of muscles, myofibrillar PL and CTLA in SOL and total PL in EDL only. In intact rats treated with HMB we found a decrease in OL and CTLA in SOL, in EDL an increase in PS and total PL and reduction in myofibrillar PL. No changes in expression of alpha subunits of proteasome were observed. The results indicate positive effect of HMB treatment on protein metabolism both in intact and septic animals. This effect is muscle type dependent and is caused by attenuation of proteasome activity and protein breakdown, not by stimulation of PS or changes in proteasome expression.

Project was supported by the Charles University Grant Agency, No 7322/07/C.

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**Title of the project:** Innovation in practical lessons from ophthalmology – introduction of the lecture “Electrophysiological methods in ophthalmology and their clinical importance.

**Grant Agency:** Ministry of Education

**Project Number:** 155/2007 A a

**Principal Investigator:** H. Langrová

**Co-investigators:** J. Kvasnička, H. Dvořáková, J. Kremláček

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 1750

**Summary of 2007 results**

**Title of the presentation:** Innovation in practical lessons from ophthalmology – introduction of the lecture “Electrophysiological methods in ophthalmology and their clinical importance.

**Authors:** H. Langrová, J. Kvasnička, H. Dvořáková, J. Kremláček

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

Text:

Students of medical faculties are being prepared for work with clinical praxis. Within the course of their practical training, they should therefore become familiar with modern diagnostic devices employed at the workplace. Our goal was to introduce the presentation “Electrophysiologic methods in ophthalmology and their clinical significance”, as part of the ophthalmology lecture program. Using grant means, the multifocal electrophysiologic diagnostic system “RETI-port gamma plus multifocal REG” and the program “mf ERG Science” (Roland Consult, Germany) were purchased. They allow complex, objective and non-invasive evaluation of functions of the entire optic system. Its use in diagnosis and the evaluation of the obtained results complement practical training in ophthalmology.

Introducing this modern examination technique as part of the practical training of students will increase their theoretical and practical knowledge in the region of functional testing of the optic system and this modernised examination method will be part of the ophthalmologic examination.

Project was supported by Ministry of Education grant, No A155/07

**Address for correspondence:**

H. Langrová, Dept. of Ophthalmology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

**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/8421-4/2005

**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):** 1227

#### **Summary of 2007 results**

**Title of the presentation:** Motion-onset visual evoked potentials - testing of applications in dyslexia, neuroborreliosis and cerebral visual impairment

**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; <http://www.lfhk.cuni.cz/ELF>

The project contributes to extension of applications of visual evoked potentials (VEPs) in diagnostics of functional disorders of the visual pathway and central nervous system via implementation of new variants of VEPs, in particular with the use of visual moving stimuli - „motion-onset VEPs.“

A review of existing reports to a visual pathway deficit in dyslexia was published with a summary of our results from motion-onset VEPs studies testing the magnocellular system function in dyslexics (1).

Comparison of the pattern-reversal and motion-onset VEPs in patients with Multiple Sclerosis and Neuroborreliosis suggests that Neuroborreliosis affects predominantly the magnocellular system/dorsal stream of the visual pathway compared to Multiple Sclerosis involving mostly the parvocellular system/ventral stream (2).

Our pilot study in children with a history of preterm birth and perinatal post-hemorrhagic hydrocephalus has shown that the combined pattern and motion-related VEPs examinations can detect a hidden cortical/cerebral visual impairment (3).

#### **References:**

1. Kubová Z. Visual Pathway Deficit in Dyslexia - VEPs Testing. 2006, Brno, MSD, ISBN 80-86633-63-2, 87 p.
2. Szanyi J., Kubová Z., Kuba M., et al. Comparison of visual evoked potentials in patients with Multiple Sclerosis and Neuroborreliosis. Čs. Neurol. Neurochir., 2007, vol. 70/103, p. 658-664.
3. Kuba M., Liláková D., Hejmanová D. et al. Visual pathway functions after perinatal CNS involvement in preterm children tested with VEPs. Doc. Ophthalmol. - submitted to publ.

*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 – 4 a

**Principal Investigator:** M. Kuba

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

**Starting date:** 1.1.2005

**Duration (years):** 5

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

**Summary of 2007 results**

**Title of the presentation:** Effect of ageing, adaptation/habituation and fatigue on visual evoked potentials

**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; <http://www.lfhk.cuni.cz/elf>.

In 133 healthy volunteers at age of 19 - 83 years ageing effect was studied on three different levels of visual information processing with the use of three types of visual evoked potentials (VEPs). We examined reactions of the primary visual cortex (to pattern-reversal stimuli), responses of extrastriate areas to motion-onset and P300 wave in visual cognitive task (recognition of digits and letters). The most distinct change of VEPs parameters was a prolongation of P300 latency representing highly significant delay of cognitive processes toward elderly (2 ms/1 year of age,  $r = 0.71$ ), which was more expressed in men. Motion processing (activity of the magnocellular system/dorsal stream) seems to be more influenced by ageing compared to the parvocellular system/ventral stream function. VEPs might contribute as an objective tool for recognition of physiological and pathological functional changes of the ageing brain (1).

We explored also the effect of repeated visual stimulation on motion-onset VEPs during 25 min recording sessions in 10 subjects. The aim of the experiment was to suggest an optimal recording design for clinical examination. N2 peak latency was prolonged by 10 ms and amplitude was attenuated by 30% with respect to the start of the experiment. An exponential model was employed to describe the dependency. The model can be used to reduce intra-individual variability during examination (2).

**References:**

1. Kuba M., Kremláček J., Langrová J. et al. Ageing effect at various levels of visual information processing -VEPs testing. Perception, 2007, vol. 36 Suppl., p. 14.
2. Kremláček J., Kuba M., Kubová Z. et al. One session intra-individual reproducibility of motion-onset VEPs - Effect of adaptation/habituation or fatigue on N2 peak amplitude and latency. Doc. Ophthalmol., 2007, vol. 115, p. 95-103.

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

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**Title of the project:** Innovation in ophthalmology education using multimedia presentation

**Grant Agency:** Ministry of Education

**Project Number:** 156/2007F3a

**Principal Investigator:** H. Langrová

**Co-investigators:** P. Rozsival, G. Kyprianou

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 175

**Summary of 2007 results**

**Title of the presentation:** Innovation in ophthalmology education using multimedia presentation

**Authors:** H. Langrová, P. Rozsival, G. Kyprianou

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

Within the frame of modernization of ophthalmology education, the computer program 3D Eye Office (Eyemaginations, USA) was purchased from the grant funds. This program presents animations from the anatomy, physiology and pathophysiology of the entire optic system, including basic ophthalmologic diagnostic and treatment steps. It allows for the creation of various sequences of animations according to individual requirements. The animations are presented using a data projector and managed by a touch-screen notebook which directly allows graphic intervention by the lecturer during the animation. Further, the camera system Stream View (Vaddio, USA) was purchased for the automated projecting and recording the course of diagnosis or treatment. It allows direct digital access for the connection of 4 devices with four visual and one audio access. It is therefore possible during a lecture to view on-line an operation, a diagnostic or treatment process. Another advantage of this system is the direct access to the internet that allows the online visualization of data through the internet.

Project was supported by Ministry of Education grant, No G156/07

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**Title of the project:** Infections in pathology and clinical practice.

**Grant Agency:** Ministry of Education

**Project Number:** 27/2007 F3 b

**Principal Investigator:** J. Lesná

**Co-investigators:** A. Ryška, I. Šteiner

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 150

**Summary of 2007 results**

**Title of the presentation:** Infections in pathology and clinical practice.

**Authors:** J. Lesná (1), A. Ryška (2), I. Šteiner (2)

Fac. Med., Charles Univ., Hr. Králové: Department of clinical Microbiology (1)

Fingerlands Department of Pathology(2)

This project is based on the application of medical microbiology knowledge to pathologic anatomy and to clinical practice. The study material is mainly addressed to 3rd year medicine students after having already passed Medical Microbiology exam and before having passed Pathologic Anatomy exam. New facultative subject consists of five seminars within studying pathologic anatomy, based on the study text and digital photographs. Chapters are divided according to five seminars, infections in pathology are classified into respiratory, gastrointestinal, urogenital, central nervous and skin infectious diseases. This project has been realized using e-learning course and presented by way of internet application. Thanks to internet application authors can advantage of easy creation, actual data reconstruction and editing of study materials. The number of students can be easily controlled by server administrator. Test results and summary of participating students are very important for course lector. There is a possibility to insert pictures, animations, videosequences, new tests and new scientific materials addressed to students.

Project was supported by Charles University Ministry of Education

**Address for correspondence:**

J. Lesná, Dep. of Clinical Microbiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Teaching hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic

**Title of the project:** Pathogenesis, prevention and treatment of severe neuropsychiatric disorders

**Grant Agency:** Ministry of Education

**Project Number:** 0021620816 - 4 c

**Principal Investigator:** J. Libiger

**Co-investigators:** I. Tůma, J. Hons

**Starting date:** 1.1.2005

**Duration (years):** 5

**Total funds allocated for project - Kč (thousands):** 642

**Summary of 2007 results**

**Title of the presentation:** Advances in diagnosis and treatment of psychotic disorders

**Authors:** Libiger J., Masopust J., Urban A., Hons J., Tuma I.

Research has been focused on the same topics as in the previous year. The electrophysiological part of the research programme resulted into a well documented evidence of the deficit in " mismatch negativity "( MMN) in the visual domain. This is a novel finding with the implications for understanding the very early perceptual and cognitive processes in schizohrenia. The MMN is associated with the deficit form of schizophrenia and may be either a marker of a subtype of schizophrenia or of the clinical state and progression of the brain changes. The results were successfully presented at several international meetings. The differences in early information processing were detected also in metaphetamine abusers with psychosis, which provides an insight into linkage of neurochemical ( excessive dopaminergic stimulation) and physiological processes associated with psychosis. The work on electrophysiological markers of disturbed information processing is funded from additional grant sources. The data set on the serum levels of excitatory amino acids were collected in a large sample of patients with shizophrenia and it is being analyzed . The data on psychopathology and cognitive performance are available as well. The preliminary results do not confirm the scant previous reports of the association between low levels of d- serine and schizophrenia. The exploratory analyses in the experimental population of 50 patients focus on detecting the differences between subgroups of schizophrenia, namely the deficit subtype and the disorder with the predominance of positive symptoms. The third point of interest are the less frequent, but serious adverse effects of antipsychotic treatment. Dr. Masopust and his collaborators publish and report on the links between antipsychotic treatment and thromboembolism, its pathophysiology and the prevention in psychiatric patients.

**Address for correspondence:** J. Libiger, Dept. of Psychiatry, Charles University Prague, Faculty of Medicine Hradec Králové, Fakultní nemocnice 500 05, Hradec Králové, Czech Republic

**Title of the project:** Quality of vision in premature children

**Grant Agency:** Charles University

**Project Number:** 36007/2007 C

**Principal Investigator:** D. Liláková

**Co-investigators:** D. Hejčmanová

**Starting date:** 1.1.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 230

**Summary of 2007 results**

**Title of the presentation:** Quality of vision in premature children

**Authors:** D. Liláková, D. Hejčmanová

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

The developments in neonatology have resulted in an increasing number of deliveries of extremely immature infants that are associated with high morbidity. The main cause of eye disorders is retinopathy of prematurity and neurological diseases. Eye disorders related to the retinopathy of premature infants may be presumed and examined very early after birth. A lot of the eye disorder appear, however, later in life.

The aim of this study is to document the quality of visual functions in premature infants and to compare them with the visual functions in a group of full-term healthy children.

The research in this year was carried out on 50 children at the age of 6 years, who were born prematurely with a weight below 1500 g. The visual acuity, contrast sensitivity, strabismus and refraction errors were examined. Similar qualities of vision were examined in the group of full-term born children of the same age.

We found an increased prevalence of refraction errors and strabismus in premature infants. Myopic correction had 13% of premature children, hypermetropic correction had 28% of premature children. Astigmatism was found in 12% of premature children. Strabismus was found in 19% of premature children. The contrast sensitivity was in the group of premature infants significantly lower. The ophthalmological follow-up of the premature infants should include the children both with and without ROP.

Project was supported by Charles University Grant Agency, No 7360/2007

**Address for correspondence:** D. Liláková, Dept. of Ophthalmology, University Hospital, Hradec Králové, 50005

**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. Mand'ák, 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):** 748

**Summary of 2007 results**

**Title of the presentation:** MINI CPB VS. CONVENTIONAL CPB – DIFFERENT SYSTEMS -DIFFERENT CLINICAL OUTCOMES?

**Authors:** V.Lonský(1), V.Svitek, J.Mand'ák, M.Bartoš(2), J.Kubíček, M.Volt, P.Valentová, V.Brzek, J.Harrer,

University Departments of Cardiac Surgery and Anesthesiology(2), University Hospital, Olomouc(1)and Hradec Králové, Czech Republic, 50005

Minimizing of the current ECC systems seems to be the logical evolution in the field of cardiopulmonary bypass technology research. The new completely closed circuits consists of a centrifugal pump, oxygenator, reduced tubing length and biocompatible surface.

Cardiotomy reservoir and conventional suction devices are eliminated. The purpose of our study was to evaluate potential clinical benefits of it. 54 patients who underwent primary isolated CABG at our institution were prospectively studied. 26 patients (MINI) were operated upon using a closed circuit IDEAL/SYNERGY, Sorin, Italy, second group of 28 patients (CPB) were operated with the use of conventional extracorporeal circulation with hardshell reservoir. Analysis of various demographic, preoperative, intraoperative, perfusion and postoperative parameters was performed. Patients demographics, preoperative, main operative and postoperative data did not differ between both groups. There was a significantly reduced prime and hemodilution during CPB in MINI group 1100 ml (1100 – 1400) m=1100 vs. 1605 ml (1000 - 2100) m=1600;  $p<0,0001$ ; Htc 0,32 (0,28-0,41) m=0,32 vs. 0,25 (0,19-0,31) m=25;  $p<0,0001$ ). There was no difference in postoper.drainage blood loss, length of ventilation, transfusion requirement and other postoperative complications. Despite of markedly reduced inflammatory reaction of mini systems documented in various studies we did not observe any clinical benefit for this low risk group of patients. Further studies are necessary to v verify whether a specific group of patients with preexisting organ dysfunctions could benefit from the new systems.

**Address for correspondence:** V.Lonský, Dept.of Cardiac Surgery, Palacký University in Olomouc, I.P.Pavlova 6, Olomouc 77520, Czech Republic

**Title of the project:** Optimalization of the therapy of 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 2007 results**

**Title of the presentation:**

The new Registry of Monoclonal Gammopathies - RMG

**Authors:**

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

2<sup>nd</sup> Dept. of Medicine - Clin. Haematology, Faculty Hospital Hradec Králové (1), 1<sup>st</sup> Dept. of Medicine, General Faculty Hospital Prague (2), Hemato-oncological Dept., University Hospital Brno (3), 3<sup>rd</sup> 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 new Registry of Monoclonal Gammopathies was started from 1. 5. 2007. Currently it is registered 640 patients with monoclonal gammopathies totally: 240 patients with multiple myeloma and 400 patients with monoclonal gammopathies of undetermined significance (24 patients with MM and 99 with MGUS in Hradec Králové).

**Address for correspondence:** V. Maisnar, 2<sup>nd</sup> Dept. of Medicine - Div. of Clinical Haematology, Charles University in Prague, Faculty Hospital in Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

**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. Mandáček

**Co-investigators:** V. Lonský, V. 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 2007 results**

**Title of the presentation:** Biochemical monitoring of metabolic changes in skeletal muscle during cardiac operations with and without cardiopulmonary bypass. A microdialysis study.

**Authors:** J. Mandáček (1), M. Pojar (1), N. Cibíček (2), V. Lonský (1), V. Palička (2), V. Živný (2), J. Kubíček (1), D. Kakrdová (2)

Faculty of Medicine and University Hospital, Charles University, Hradec Králové, Czech Republic: Dept. of Cardiac Surgery (1), Inst. of Clinical Biochemistry (2)

Text:

Serious complication of cardiac surgery using extracorporeal circulation, cardiopulmonary bypass (CPB), could be a hypoperfusion of peripheral tissues. The aim of this study was to compare the metabolism changes in the skeletal muscle during the cardiac operations in CPB and operations without CPB by means of interstitial microdialysis. 20 patients (group On-pump) were operated using CPB, 20 patients (group Off-pump) without CPB. Glucose, lactate, pyruvate and glycerol were measured in dialysates. 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. 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. 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 cardiopulmonary bypass compromises peripheral tissue, skeletal muscle, energy metabolism.

This project continues and is supported by the Internal Grant Agency of the Ministry of Health, NR/ 8944-3, and by Research Project MZO 00179906.

**Address for correspondence:** J. Mandáček, Dept. of Cardiac Surgery, University Hospital, Sokolská 581, 50005 Hradec Králové, Czech Republic

**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<sup>1</sup>, S. Ježek<sup>2</sup>, B. Koukola, E. Ondřejová<sup>3</sup>, T. Svatoš<sup>4</sup>, M. Dostálek, M. Petriščíková<sup>5</sup>

**Starting date:** 1.1.2006

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1136

#### **Summary of 2006 results**

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

**Authors:** J. Mareš (1), M. Rybářová (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)

<sup>1</sup> Charles University, Faculty of Medicine

<sup>2</sup> Masaryk University, Faculty of Social Studies

<sup>3</sup> Silesian University, Faculty of Arts and Sciences

<sup>4</sup> University of Hradec Králové, Faculty of Education

<sup>5</sup> Hospital in Litomyšl

During the second year of the project we pursued our goals at three different levels. In the theoretical part we have carried out several review studies which were focused on various topics: posttraumatic growth in children and adolescents, children's quality of life related to family, children's quality of life with regard to school. We have also reviewed state of the art knowledge on quality of life in newborn babies who experience pain.

In the methodological part we carried out a review of qualitative methods which are used internationally for quality of life assessment in children and adolescents. Particular attention was paid to current trends in computer based diagnostics of quality of life using interactive computer animation.

In the empirical part we have accomplished research studies as follows: exploration of child's preconception of the term „quality of life“ in focus group discussion, at which 71 pupils of elementary schools participated; exploration of preconception of the term „quality of life“ in group of 110 pre-service teachers for primary schools. Using ComQol-S5 questionnaire, we studied quality of life in 277 healthy adolescents, who lived in diverse sociocultural milieu (children's home, village, housing estate in a big city). Using PedsQL 4.0 we studied quality of life in 526 students of nursing school. With BSFC questionnaire we surveyed 55 ill children with diagnosis of scoliosis so that to explore their perceived benefit of chronic illness experience for their life.

Literature: Mareš, J. et al. Quality of life in children and adolescents II. Brno: MSD 2007, 260p. ISBN 978 - 80 - 7392 - 008 - 1

#### **Address for correspondence:**

J. Mareš, Dept of Social Medicine, Charles University Faculty of Medicine Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech republic



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

**Grant Agency:** Ministry of Education

**Project Number:** 1P05OC062

**Principal Researcher:** J. Martínková

**Joint Researchers:** S. Mičuda, J. Cermanová, J. Chládek, L. Fuksa, E. Brčáková

**Starting date:** 1.1.2005

**Duration (years):** 4

**Total funds allocated for project - Czech crowns (thousands):** 2800

**Summary of 2007 results**

**Title of the presentation:** Influence of cholestasis on the expression of hepatic and renal transport proteins and cytokines in rats *in vivo*.

**Authors:** E. Brčáková, L. Fuksa, J. Cermanová, J. Osterreicher, J. Mokry, F. Staud, J. Chládek, M. Hroch, G. Kolouchová, J. Martínková, S. Mičuda

Dept. of Pharmacology, Charles Univ. in Prague, Faculty of Medicine in Hradec Králové

The purpose of the present project is to examine the influence of cholestasis on the activity of main canalicular drug transporters - multidrug resistance-associated proteins 2 (mrp2), organic anion transporting polypeptide 2 (Oatp2), breast cancer resistance protein (Bcrp) and reduced folate carrier 1 (Rfc1). The further aim of the project is to evaluate cholestasis-induced changes in the expression of cytokines (e.g. IL-1 $\beta$ , IL-6, IL-10, TNF- $\alpha$ ) - the basic signal molecules during this pathological process. The mRNA and protein expression of transporters in the liver and kidney was evaluated by Western blot and qRT-PCR. In the liver, down-regulation of Mrp2, Bcrp, and Oatp2, but up-regulation of Mrp3 as the compensatory mechanism for the decreased Mrp2 content was observed during extrahepatic cholestasis. In response to these changes, the expression of renal Mrp2 and Mrp3 was increased. Cytokine expression changes during acute (bile duct obstruction for 1 day) and chronic extrahepatic cholestasis (bile duct obstruction for 7 days) was evaluated by antibody array. Decreasing tendency of the proinflammatory cytokine expression was observed during acute cholestasis and no significant changes in their concentration were found seven days after bile duct obstruction. In conclusion, our findings may help to explain mechanisms of impaired drug elimination during cholestasis, the pathological state which may commonly occur in clinical practise.

This study was supported by a grant from the Ministry of Education of the Czech Republic No. 1P05OC062 (COST B25.002).

**Address for correspondence:** Jiřina Martínková, Dpt. of Pharmacology, Faculty of Medicine in Hradec Králové, Šimkova 870, PO Box 38, 500 38 Hradec Králové, Czech Republic, e-mail: micuda@lfhk.cuni.cz

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

**Grant Agency:** Ministry of Health

**Project Number:** 1A/8671-5/05

**Principal Investigator:** J. Martínková

**Co-investigators:** P. Pokorná

**Starting date:** 10.10.2005

**Duration (years):** 5

**Total funds allocated for project - Kč (thousands):** 543

**Summary of 2007 results**

**Title of the presentation:** THERAPEUTIC DRUG MONITORING (TDM) OF GENTAMICIN IN CRITICALLY ILL NEONATES

**Authors:** Iva Selke-Krulichová, Jirina Martínková, Pavla Pokorná, Jiri Záhora, Jaroslav Chládek.

First dose plasma concentrations of gentamicin (GE) in neonates during the first week of postnatal age seem to be predictive for steady state concentrations in neonates without co-variates significantly modifying pharmacokinetics of GE. However, the results of Bland-Altman analysis indicate a lack of agreement between predicted and monitored GE concentrations Cpeakss and Ctroughss in neonates administered i.v. furosemide (>0.7 mg/kg.h<sup>-1</sup>), and/or suffering from serious systemic capillary leak syndrome. The unexplained variability between observed and predicted values may originate from an effect of co-administered drugs and/or more serious clinical status of neonates. The variability requires further analysis.

**Address for correspondence:** prof. Jirina Martínková, M.D., Ph.D, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, Hradec Králové, Czech republic

**Title of the project:** Renewal And Expansion Of The Computer Equipment In The Labs Of The Faculty of medicine in Hradec Kralove

**Grant Agency:** Ministry of Education

**Project Number:** 1639/2007 A b

**Principal Investigator:** V. Mašín

**Co-investigators:**

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 1741

**Summary of 2007 results**

**Title of the presentation:** Renewal And Expansion Of The Computer Equipment In The Labs Of The Faculty of medicine in Hradec Kralove

**Authors:** MUDr. Vladimír Mašín, Ph.D.

The aim of this project was to renew and expand computer equipment in the labs of the Departments of Anatomy, Histology and Embryology, Physiology, Biochemistry, Pathological Physiology and Biophysics.

We fulfilled our original aim and built new computer labs or renewed existing computer labs in all before mentioned departments.

The computer lab of the Department of Anatomy was equipped with 3 new computers, designated to multimedia presentation, the microscopy lab of the Department of Histology was equipped with 1 presentation computer and 12 student computers for virtual microscopy and 1 server computer, which is hosting the virtual microscopy program. The computer lab of the Department of Pathological Physiology was expanded by 5 new computers and a networked printer.

We also built two completely new computer labs - one with 20 PCs and one networked printer in the Department of Physiology and one with 9 PCs and one networked printer in the Department of Medical Biochemistry. Both these labs will be used during practical lessons in the respective departments.

Finally, we also updated equipment of the computer lab No. 2 in the Department of Medical Biophysics, where we replaced 20 outdated computers with new ones. This lab is used in the computer science lessons and outside lessons is open for student's self-study activities.

All these updated or newly build labs are connected to our faculty local area network and users can log on with the same credentials anywhere. All the computers are via LAN connected to the internet through a shared 1 Gbps line.

Because of volume discounts we were able to significantly upgrade the specifications of the new computers, compared to their originally planned specifications.

**Address for correspondence:**

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Dept. of Medical Biophysics, Charles University in Prague, Faculty of Medicine  
Šimkova 870, 500 38 Hradec Králové

**Title of the project:** Predictive and prognostic factors in patients with breast carcinoma treated with neoadjuvant chemotherapy

**Grant Agency:** Ministry of Health

**Project Number:** NR/8392-3/05

**Principal Investigator:** B. Melichar

**Co-investigators:** A. Ryška, D. Solichová, M. Tomšová, P. Benešová, P. Veselý, P. Králíčková, D. Vokurková, H. Urminská, J. Mergancová, P. Jandík

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 7412

#### **Summary of 2007 results**

**Title of the presentation:** Urinary neopterin and hemoglobin in breast carcinoma patients treated with dose-dense chemotherapy

**Authors:** . Melichar (1), L. Urbánek (2), L. Krčmová (2), H. Kalábová (1), K. Melicharová (2), P. Veselý (1), E. Malířová (3), H. Hornychová (4), A. Ryška (4), D. Solichová (2)

Departments of Oncology & Radiotherapy (1), Gerontology & Metabolic Care (2), Nuclear Medicine (3), and Pathology (4), Charles University Medical School & Teaching Hospital, Hradec Králové, Czech Republic

In the present study, we evaluated urinary neopterin, hemoglobin and peripheral blood cell count in breast carcinoma patients treated with dose-dense combination of doxorubicin, cyclophosphamide and sequential paclitaxel. Iron metabolism was also evaluated in a subgroup of patients. Urinary neopterin increased significantly during the chemotherapy. The increase in urinary neopterin was accompanied by a gradual decrease in hemoglobin concentrations.

Among 161 patients treated with primary chemotherapy, the pathological response was evaluable in 150 cases. Pathological response was observed in 37 cases (25%). No differences were observed in urinary neopterin concentrations between 37 cases with pathological complete response and remaining 113 cases. Patients who subsequently had pathological complete response had significantly lower baseline hemoglobin concentrations and lower urinary neopterin at the end of therapy, but no other consistent differences were observed between the patients with or without pathological complete response in urinary neopterin, hemoglobin and peripheral blood cell counts during therapy. In patients with pathological complete response, significantly lower serum ferritin and significantly higher iron binding capacity were observed.

Reference

Melichar B, Solichová D, Melicharová K, Cermanová M, Urminská H, Ryska A. Systemic immune activation, anemia and thrombocytosis in breast cancer patients treated by doxorubicin and paclitaxel. *Pteridines* 2006, 17, 107 – 114.

**Address for correspondence:** B. Melichar, Dept. Oncology and Radiotherapy, Charles University Medical School and Teaching Hospital, Sokolská 581, 500 05 Hradec Králové



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

**Grant Agency:** Ministry of Health

**Project Number:** NR/9096-4/06

**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):** 13975

**Summary of 2007 results**

**Title of the presentation:** Serum Alpha-tocopherol, Retinol and Neopterin during Paclitaxel/carboplatin Chemotherapy

**Authors:** B. Melichar (1), H. Kalábová (1), L. Krčmová (2), L. Urbánek (2), R. Hyšpler (2), Eva Malířová (3) and Dagmar Solichová (2)

Departments of Oncology & Radiotherapy (1), Gerontology & Metabolic Care (2), and Nuclear Medicine (3), Charles University Medical School & Teaching Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Disorders of antioxidant balance are thought to be involved in the toxicity associated with radiotherapy or chemotherapy. Serum alpha-tocopherol and retinol were determined, by high performance liquid chromatography, before and during therapy with a combination of paclitaxel and carboplatin in 28 patients with breast and ovarian cancer. Serum neopterin and cholesterol were measured using a radioimmunoassay and enzymatic colorimetric method, respectively. Compared to pretreatment concentrations, a significant increase was observed in serum alpha-tocopherol and retinol concentrations during therapy that was associated with decreased serum neopterin concentrations. Serum alpha-tocopherol concentrations were significantly higher during therapy in patients who did not experience serious toxicity. We conclude that an increase in alpha-tocopherol and retinol during therapy with combination paclitaxel/carboplatin may be explained by inhibition of systemic immune activation secondary to control of the tumor with effective chemotherapy. Lower alpha-tocopherol concentrations were associated with the toxicity of therapy.

Reference

Melichar B, Kalábová H, Krčmová L, Urbánek L, Solichová D. Serum alpha-tocopherol during paclitaxel/carboplatin chemotherapy. Anti-Cancer Research, in press.

**Address for correspondence:** B. Melichar, Dept. Oncology and Radiotherapy, Charles University Medical School and Teaching Hospital, Sokolská 581, 500 05 Hradec Králové

**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, G. Kolouchová

**Starting date:** 1.1.2005

**Duration (years):** 4

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

#### **Summary of 2007 results**

**Title of the presentation:** Influence of amiodarone on renal and hepatic elimination of rhodamine-123 in rats.

**Authors:** L. Fuksa, J. Cermanova, E. Brčakova, G. Kolouchova, F. Staud, M. Hroch, J. Martinkova, S. Micuda

Dept. of Pharmacology, Charles Univ. in Prague, Fac. Med. in Hradec Kralove

Amiodarone, a widely used antiarrhythmic agent, is a suspected inhibitor of drug transporting proteins in the liver. Therefore, the aim of present project was the evaluation of amiodarone's influence on the pharmacokinetics of rhodamine-123 (Rho123), a model substrate for drug transporters in the liver. We have used a rat model to follow renal and biliary elimination of Rho123 during its constant-rate i.v. infusion and concomitant single bolus i.v. injection of amiodarone (25 mg/kg). In addition, influence of 4-, 7-, and 14-days amiodarone pretreatment (25 mg/kg orally) on the Rho123 pharmacokinetics was evaluated using the same model. Rho123 mean steady-state plasma concentration of  $0.65 \pm 0.05$   $\mu\text{mol/L}$  was reached and the total clearance achieved  $29.6 \pm 2.3$  ml/min/kg. Administration of amiodarone decreased the biliary excretion and clearance of Rho123 to 37.9% and 33.9% of the control values ( $p < 0.001$ ), respectively. In addition, cyclosporine, a model inhibitor of cation transporters, decreased the biliary excretion and clearance of Rho123 to 15.9% and 13.9% of the control values ( $p < 0.001$ ), respectively. No effect of amiodarone on any pharmacokinetic parameter was seen after either duration of pretreatment. Interestingly, amiodarone concentrations measured in bile were similar in all groups, while its concentrations in plasma were measurable only in intravenous bolus-administered rats. In conclusion, these data suggest that the inhibition of Rho123 transport does not occur at the canalicular transporters but rather at the basolateral membrane of hepatocytes.

This study was supported by a grant from the Ministry of Education of the Czech Republic No. 1P05OC061 (COST B25.001).

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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:** NR9182-3/07

**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):** 2904

**Summary of 2007 results**

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

**Authors:** T. Soukup, J. Suchánek, R. Ivančaková, J. Mokrý

Our aim was to isolate stem cells from the dental pulp (DPSCs) and carry out their phenotypic characterization to distinguish whether DPSCs represent a uniform or heterogeneous population. In the first year of the study, we isolated 10 DPSC lines derived from the third molars or first premolars. 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, 2%FCS and ITS. While DPSCs were cultured over 50 population doublings (PD), their cell doubling time increased from 12-50 hrs for initial 43 PD to 60-90 hrs after 55 PD. In the course of the long-term cultivation, DPSCs did not show any signs of degeneration or spontaneous differentiation and cell viability reached 96%. DNA analysis proved that 42% cells occurred in SG2 phase of the cell cycle. Flow cytometry phenotypic analysis proved that DPSCs isolated from subodontoblastic and perivascular compartments differed in expression of their surface markers. Mesenchymal stem cell markers CD29, CD44 and CDS90 were expressed in high levels in DPSCs derived from both compartments. However, subodontoblastic DPSCs expressed CD16 in medium intensity and CD11b, CD11c, CD49a,c,d,e, CD105, CD117 and CD166 were expressed in low levels. On the contrary, perivascular DPSCs expressed CD63 in medium level and CD49a,c,d,e, CD105, CD146 and CD166 were expressed in low levels. Our findings gave evidence that stem cells in the human dental pulp were not identical which might support our hypothesis that subodontoblastic and perivascular DPSCs were of different embryonic origins.

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

**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:** Study on effects of deferiprone from the viewpoint of its potential cardioprotective action in experimental anthracycline-induced cardiotoxicity.

**Grant Agency:** Charles University

**Project Number:** 89/2006/C

**Principal Investigator:** O. Popelová

**Co-investigators:** M. Štěřba, I. Gunčová, Y. Mazurová, V. Geršl

**Starting date:** 1.4.2006

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 590

#### **Summary of 2007 results**

**Title of the presentation:** Study of potential cardioprotective effect of deferiprone in chronic anthracycline cardiotoxicity in rabbit.

**Authors:** O. Popelová (1), M. Štěřba (1), T. Šimůnek (2), I. Gunčová (3), Y. Mazurová (3), M. Adamcová (4), V. Geršl (1). Charles Univ. in Prague, Fac. Med., Hr. Králové, Dept. of Pharmacology (1), Dept. of Histology and Embryology (2), Dept. of Physiology (4), Fac. Pharm., Hr. Králové, Dept. of Biochemical Sciences (3).

Recent in vitro studies have strongly suggested that novel iron chelator deferiprone (L1) can afford significant cardioprotection against anthracycline (ANT) cardiotoxicity. The present study was aimed to assess whether L1 can provide effective protection against chronic ANT cardiotoxicity in vivo. First, using HL-60 leukemic cell line, we have demonstrated that L1 (1-300 uM) did not attenuate the antiproliferative efficacy of anthracyclines. Moreover, in clinically relevant concentrations, L1 (>10uM) augmented the antiproliferative action of daunorubicin (DAU). Consecutively, rabbits were injected once weekly for 10 weeks with DAU (3 mg/kg, i.v., n=11), saline (control group, 1 ml/kg, i.v., n=8), L1 with DAU (10 or 50 mg/kg, p.o., 45 min prior to DAU) and L1 alone (50 mg/kg, p.o., n=6). Chronic DAU treatment induced 18% premature mortality, left ventricular (LV) dysfunction, troponin T rise and typical histological changes in the LV myocardium. Surprisingly, in both studied doses, L1 was unable to reduce anthracycline-induced mortality. Moreover, the higher L1 dose (50 mg/kg) led to earlier deaths. Furthermore, L1 (in both doses) failed to afford significant protection of the myocardium against lipid peroxidation, LV cardiac damage and congestive heart failure induced by DAU. In conclusion, the results of the present study revealed that oral treatment with L1 has no beneficial effect on the chronic anthracycline cardiotoxicity in rabbits. The lack of cardioprotective effect of L1, together with our previous findings, suggest that the role of iron chelation in anthracycline cardiotoxicity is more complex and/or other mechanisms unrelated to iron-catalysed ROS production are involved in this pathology. Project was supported by the Charles University Grant Agency, No. 89/2006/C.

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

**Title of the project:** Multimedial atlas of dental variations, anomalies and pathologies

**Grant Agency:** Ministry of Education

**Project Number:** 581/07/F3 d

**Principal Investigator:** B. Pospíšilová

**Co-investigators:** O. Procházková

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 184

**Summary of 2007 results**

**Title of the presentation:** Multimedial atlas of variations, anomalies and pathologies of teeth:  
The e-learning study guide for dental anatomy

**Authors:** B. Pospíšilová, O. Procházková

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

Department of Anatomy of Charles University in Prague, Faculty of Medicine in Hradec Králové, Czech Republic disposes with extensive collection of human dry skulls which essentially originated from the Broumov Ossuary (13<sup>th</sup>-18<sup>th</sup> century). In the crania, considerable number of variations, anomalies and pathologies of teeth was diagnosed. All dental findings were systematically organized and photographically documented. In the part of them, X-ray examination was also performed. Photographies and/or radiographs were didactically classified and incorporated in the "Multimedial atlas of dental variations, anomalies and pathologies". For the presentation, the Microsoft PowerPoint form was used. By creation of "Atlas" authors benefited from the established cooperation with the Austrian Pathological Museum in Vienna. In the Czech Republic, "Multimedial atlas of dental variations, anomalies and pathologies" presents unique e-learning study guide which is primarily intended in the teaching of anatomy for medical students. It will to take advantage in the magister dental study program, where the time allocated for face-to-face teaching of anatomy is very reduced. "Multimedial atlas of dental variations, anomalies and pathologies" is available on the internet pages [www.lfhk.cuni.cz/anatomie](http://www.lfhk.cuni.cz/anatomie) and in the CD-ROM.

References:

1. Schneid R. C. Woelfel's Dental Anatomy: Its Relevance to Dentistry. 7<sup>th</sup> ed. Philadelphia: Lippincott Williams and Wilkins, 2007:533.
2. Hillson S. Teeth. 2<sup>nd</sup> ed. Cambridge University Press, 2005:373.

Project was supported by the Ministry of Education Grant Agency, No 581/07.

**Address for correspondence:** B. Pospíšilová, Dept of Anatomy, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic. [pospisil@lfhk.cuni.cz](mailto:pospisil@lfhk.cuni.cz)



**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:** A. Potáčová

**Co-investigators:** M. Adamcová, O. Popelová, M. Štěřba

**Starting date:** 1.1.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 525

#### **Summary of 2007 results**

**Title of the presentation:** Changes of matrix metalloproteinases, cardiac troponins and soluble collagen on the model of chronic anthracycline cardiotoxicity in rabbits

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

Matrix metalloproteinases (MMPs) play an important role during the cardiac remodeling. The temporal pattern of MMPs activation may be unique to the type of pathological stimulus. Hence, in this pilot study we for the first time aimed to investigate the contribution of MMPs to anthracycline-induced chronic cardiotoxicity. Chronic daunorubicin cardiomyopathy was induced in rabbits in a previously validated schedule (daunorubicin 3 mg/kg, i.v., once weekly). Animals were sacrificed 24 hours and 7 days after different cumulative dose. At the beginning (week 1 and week 2) the activity of MMP-2 determined by zymography was slightly elevated by 9 and 12 %, respectively, as compared with the initial values. Significant drop in MMP-2 occurred in the 5th week (-17 %) and then the activity of MMP-2 gradually increased reaching the value +16 % at the end of experiment (week 11). The changes of MMP activity corresponded with the alteration of soluble collagen in the left ventricles (LV). Furthermore, the daunorubicin-induced cardiomyopathy was associated with a progressive increase of both cTnT (Roche) and cTnI (Abbott). Although the correlation between cTnT and cTnI cumulative release (AUCs) was found ( $R=0.81$ ;  $P<0.01$ ) and both cardiac troponins corresponded well with the echocardiographically-assessed LV systolic dysfunction ( $R=0.83$  and  $0.81$  for cTnT and cTnI, respectively;  $P<0.001$ ), the first significant increase in cTnI levels was observed earlier (at a cumulative daunorubicin dose of 200 mg/m<sup>2</sup>) than with cTnT (350 mg/m<sup>2</sup>). In agreement with literature data, we have described 3 different stages of MMP activities during the process of heart failure development.

Project was supported by the Charles University Grant Agency, No. 7531/2007/C.

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

**Title of the project:** Compliance of azathioprine therapy in children and adolescent with inflammatory bowel disease

**Grant Agency:** Ministry of Health

**Project Number:** NR/9255-3/07

**Principal Investigator:** O. Pozler<sup>1</sup>

**Co-investigators:** J. Malý<sup>1</sup>, P. Dědek<sup>1</sup>, M. Hroch<sup>2</sup>, J. Chládek<sup>2</sup>

**Starting date:** 27.4.2007

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 1644

#### **Summary of 2007 results**

**Title of the presentation:** HPLC method for the quantitation of azathioprine metabolites and thiopurinmethyltransferase activity in patients with Crohn disease.

**Authors:** M. Hroch, J. Chládek

<sup>1</sup>Teaching hospital, Hradec Kralove

<sup>2</sup>Charles University in Prague, Faculty of medicine in Hradec Kralove

Introduction

Crohn disease (CD) is inflammatory autoimmune disease of unknown etiology, which affects gastrointestinal tract. Azathioprine (AZA) is a preferred drug for more serious forms of CD. After oral administration, AZA is non-enzymatically converted to methylnitroimidazole and 6-thiopurine (6-TP), which is further metabolized in a cascade of enzymatic reactions to 6-tioguanine (6-TG) nucleotides as major effective substances of immunosuppressive therapy. Enzymes xanthinoxidase (XO) and thiopurinmethyltransferase (TPMT) furthermore transform 6-TP to inactive metabolites – thiouric acid and 6-methylthiopurine (6-MeTP). Occurrence of severe side effects (leucopenia, hepatotoxicity) associated with AZA pharmacotherapy is influenced by TPMT metabolic activity. With regard to TPMT genetic polymorphism, there is a need for patient phenotype determination before treatment of CD with AZA and effective clinical monitoring of AZA metabolites concentrations during therapy.

Method

For 6-TG, 6-TP and 6-MeTP monitoring and TPMT activity determination in red blood cells (RBC) hemolyzate, new liquid chromatographic assay was developed. AZA metabolites are separated after acid hydrolysis on reversed phase MERCK Purosphere RP-18e column with gradient elution in methanol-phosphate buffer system. TPMT activity is determined from 6-MeTP concentration rise after 6-MP incubation with RBC hemolyzate. Analysis of reaction mixture is carried out under similar conditions as for metabolites assay.

Results

In a cohort of children with CD (n=53), TPMT activity in RBC hemolyzate was in the range of 6.2 - 44.4 nmol/hour/mL with average activity  $27.8 \pm 7.7$  nmol/hour/mL. After five weeks of therapy, average concentrations of AZA metabolites in RBC was  $5.5 \pm 4.0$   $\mu$ mol/L 6-TG and  $15.1 \pm 13.2$   $\mu$ mol/L 6-MeTP. Concentrations of 6-TP were undetectable (less than 0.6  $\mu$ mol/L).

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**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:** 66107/2007 C

**Principal Investigator:** L. Procházková

**Co-investigators:** P. Rozsival, M. Závorková

**Starting date:** 1.1.2007

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 296

**Summary of 2007 results**

**Title of the presentation:** Contribution of OCT VISANTE for an examination of eyes with secondary glaucoma after pars plana vitrectomy

**Authors:** L. Procházková (1), E. Andrenacciová (1), M. Závorková (1), P. Rozsival (2)

Masaryk Hospital, Ústí nad Labem, Dept. of Ophthalmology (1), Fac. Med., Charles Univ., Hradec Králové, Dept. of Ophthalmology (2)

**Purpose:** Measuring the structures of the anterior segment in patients with particular types of secondary glaucoma after pars plana vitrectomy (PPV).

**Methods:** The OCT Visante device is used to visualise and measure the structures of the eye's anterior segment, to biometrical measurements of cornea and to measure the anterior chamber's angle. The whole anterior segment is viewed as if in a histological cut also with the data of the angle. This method is a non-invasive one, contactless and without mydriasis. The examination is carried out in a horizontal of 0-180°, in a vertical of 90-270° and in inclination of 45-225° and 135-315°. We measured the anterior segment in patients suffering different types of secondary glaucoma after the PPV, for example with a neovascular glaucoma, with silicon oil in the anterior chamber, or after draining the silicon oil, etc.

**Results:** Snapshots of anterior chamber from the OCT will display both normal and exceeding depth. They can also clearly show the accretions in anterior chamber angle and micelle of the emulsificated silicon oil. A completely adherent angle and thickening cornea in neovascular glaucoma can be observed as well.

**Conclusion:** Instead of describing the angle it is possible to measure it and visualise it in numerous profiles, while studying the timeline of the findings. In patients with silicon oil in the anterior chamber the device has not been used yet.

Project was supported by the Charles University Grant Agency, No 66107/2007 C

**Address for correspondence:** L. Procházková, Dept. of Ophthalmology, Masaryk hospital, Sociální péče 12A, 401 13 Ústí nad Labem, Czech Republic

**Title of the project:** Protein biomarkers in patients with hypertrophic 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 2007 results**

**Title of the presentation:** Protein biomarkers in patients with hypertrophic cardiomyopathy

**Authors:** J. Lenčo (1), A Fučíková (1), V. Tambor (1), M. Hubálek (1), J. Stulík (1), R. Pudil (2)

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

(2) Charles University in Prague, Faculty of Medicine Hradec Kralove, 1st Dept.of Medicine, 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 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  $58,1 \pm 13.5$ , 15 male, 7 female). According these statistical parameters a cohort of healthy donors was designed. So far, 23 of 30 proposed samples have been obtained. Further, we focused on the optimization of proteomic methods to be used in the project. Using a standard protein sample we revealed that much more proteins may be identified by means of combination of HPLC with MALDI-TOF/TOF tandem mass spectrometer (492 identified proteins) than by means of ESI-QTOF instrument (130 identified proteins). In addition, a novel separation approach based on isoelectric focusing of peptides was successfully introduced and is planned to be used in future experiments in order to decrease the sample complexity. In the following year we are going to employ the optimized methods in the proteome analysis of collected samples.

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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):** 440

**Summary of 2007 results**

**Title of the presentation:** An effect of hemorheopheresis to stop defelopment of dry to wet form of age related macular degeneration.

**Authors:** E. Rencová,(1), M. Bláha (2), J. Studnička (1), M. Blažek (2), V. Bláha(3), Solichová, D (3)

Fac. Med., Charles Univ., Hradec Králově: Dept. of Ophthalmology (1), Dept. of Clinical Hematology (2) of Medicine Clinic, Dept. of Gerontology and Metabolism (3)

Text:

Dry form of age related macular degeneration (AMD) with soft druses tends tu progress into the wet form with development of disciform scar. It is an oft cause of legat blindness. The aim of this work was to stop the progress from dry form to wet form.

13 patients were treated by rheopheresis (8 procedures in 10 weeks, one procedure was added in the 12th month).

The cascade method of treatment (hemorheopheresis) was used. Plasma was pumped into the rheofilter: high molecular plasma components were retained. Lipoprotein fractions and important rheologic factors (fibrinogen, blood and plasma viscosity) were examined. Retina examinations: at the beginning, after 4 and 8 weeks, than every 3 month in the first year.

Results: Lipoproteins decreased significantly (total cholesterol 62.9%, apo-B 73,7%. LDL cholesterol 74,7%. Rheologic factors decreased as well. Eye examinations:disappearance of soft druses in 9 eyes of 7 patients. In 4 eyes od 3 patients nonvascular RPE detachment reduction. Visual improvement in 4 eyes of 4 patients (one line of ETDRS charts).Improvement of hemodynamic of choroid in 3 studied eyes. All eyes remained without submacular neovascularisation.

Literature: Rencová, E., Bláha,M., Studnička, J. . et al.: Supplemnt to the European Journal of Ophhtalmology 17, 2007, 64

Supported by IG MHCR NR/ 9118-3

**Address for correspondence:** E. Rencová, Dept. of Ophthalmology, University Hospital, Sokolská 583, 500 05 Hradec Králové

**Title of the project:** BIDOZOZ – New ways of biodosimetry and diagnostics at irradiated soldiers

**Grant Agency:** Ministry of Defense

**Project Number:** OBUKHK05001

**Principal Investigator:** M. Řezáčová

**Co-investigators:** J. Vávrová, D. Vokurková, J. Cerman, S. Filip, D. Zášková, A. Tichý, Z. Vilasová, E. Lukášová

**Starting date:** 1.10.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 2477

**Summary of 2007 results**

**Title of the presentation:**  $\gamma$ H2A.X and p21: perspective biodosimetric markers in peripheral lymphocytes

**Authors:** Martina Řezáčová (1), Jiřina Vávrová (3), Doris Vokurková (2), Jaroslav Cerman (1), Darina Zášková (1), Aleš Tichý (1), Zdena Vilasová (3), Emilie Lukášová (4)  
Fac. Med. and Univ. Hosp., Charles Univ., Hr. Králové: Dept. Med. Biochem. (1), Clin. Immunol. Allergol. (2); Fac. Milit. Health Sci., Univ. Defense, Dept. Radiobiol. (3); CAS Inst. Biophys. (4)

We search for a suitable biodosimetric indicator of gamma radiation (IR) dose, which would be based on analysis of human peripheral lymphocytes. We proved that histone H2A.X is quickly phosphorylated in response to IR and this phosphorylation occurs in dose-dependent manners 1 h after the irradiation. From three possible methods tested the best for the quantification of the phosphorylation is flow-cytometry. The biggest disadvantage of biodosimetric usage of the phosphorylation of H2A.X is the rapidity of the process (2 min – 4 h after irradiation), in later intervals phosphorylation of H2A.X in live cells decreases. Interesting finding was a lack of p53 and its phosphorylated forms (on serines 15 or 392) in the quiescent lymphocytes after irradiation. Phosphorylation of p53 on serine 15 is detectable and dose-dependent only in PHA-stimulated proliferating lymphocytes. However, cell-cycle inhibitor protein p21 increases after exposure to IR also in quiescent lymphocytes. This increase is dose-dependent and detectable by ELISA method 24 and 40 h after the irradiation. This interval and method are convenient for the biodosimetric purpose. It should be possible to isolate peripheral lymphocytes from the blood sample taken within 6 h after the irradiation and measure p21 levels after subsequent in vitro incubation. This should prevent phagocytosis of damaged lymphocytes in vivo.

Project was supported by Ministry of Defense, project no. OBUKHK2005001

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Hradec Králové, Czech Republic, rezacovam@lfhk.cuni.cz

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

**Grant Agency:** Charles University

**Project Number:** 90/2006 C

**Principal Investigator:** T. Roušar

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

**Starting date:** 1.1.2006

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 517

**Summary of 2007 results**

**Title of the presentation:** Optimization of spectrofluorimetric assay of glutathione in hepatocyte cultures

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

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

Glutathione, one of the most important intracellular antioxidants, exists in two forms - the reduced (GSH) and the oxidized (GSSG). Therefore, the analysis of its levels is crucial to evaluate the oxidative stress. Various types of methods have been used recently; the HPLC methods have been preferred mostly, although spectrophotometric and spectrofluorimetric methods could be used too. The aim of our work was to optimize and describe fluorimetric method based on the reaction between glutathione and o-phthalaldehyde. Following this reaction, the isoindole compound, which possesses high fluorescence, is formed. The main disadvantage of this method described in literature has been related to low specificity of the detected fluorescence and consequently to enhancement of glutathione levels in biological material. On the other hand, our preliminary results showed no interference, and therefore we focused on optimizing of detection and reaction conditions, especially on optimizing excitation and emission wavelength, temperature of detection, pH value. Our results: GSH assay - calibration ( $c = 0-500 \mu\text{M}$ ;  $R^2 = 1.00$ ), intra-assay ( $c = 20 \mu\text{M}$ ;  $\text{CV}\% = 3.67\%$ ,  $c = 100 \mu\text{M}$ ;  $\text{CV}\% = 0.92\%$ ); GSSG assay - calibration ( $c = 0-70 \mu\text{M}$ ,  $R^2 = 0.99$ ), intra-assay ( $c = 3 \mu\text{M}$ ;  $\text{CV}\% = 4.30\%$ ,  $c = 7 \mu\text{M}$ ;  $\text{CV}\% = 2.80\%$ ). We found great correlation of GSH and GSSG levels, respectively, between HPLC and optimized method ( $r = 0.99$ ). As the results show, we have developed fast, simple and low-cost glutathione assay comparable with HPLC methods.

Literature: A. Pastore et al.: Clin. Chim. Acta 333, 19-39, 2003.

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:** 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. Jiráček, 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 2007 results**

**Title of the presentation:** Monitoring and analysis of sleep disorders, sleep breathing disorders and mental disorders in patients with neurodegenerative diseases.

**Authors:** P. Smolík

The basic part of our contribution to the large multicentric and multidisciplinary study targets to the observational, longitudinal study of psychiatric patients with neurodegenerative diseases and vice versa, with neurological patients with comorbidity of neurodegenerative and psychiatric diseases. During the first, preparatory year of the whole study the organizational and methodological background of particular components has been prepared. Our individual participation has started with the choice of the set of observational instruments that covers the representative investigative spectrum of methods relevant to the basic tasks. The large literature search has been provided, evaluated and used for two chapters of the new text book about disorders of sleep and waking and the compendious publication about chronobiology in psychiatry as the contribution to the journal Psychiatry for Practice. The design of the study has been discussed continuously with co-investigators and, with the local managements of the departments of psychiatry, neurology and pneumology.

Project was supported by the Ministry of Education, Youth and Sports, No 0021620849 via Charles University Prague, Hradec Kralove Medical School, No 52030

**Address for correspondence:** P. Smolík, Dpt. of Psychiatry, Charles University Prague, Faculty of Medicine Hradec Králové, Sokolovská 581, 500 05 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 2007 results**

**Title of the presentation:** Selenium inhibits growth of malignant colonic cells

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

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

Colon cancer is a major cause of cancer-associated mortality in the Czech Republic. Therefore it is necessary to broaden chances of anti-cancer therapy. Several selenium compounds have been studied in in vitro models as potential anti cancer agents. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effect of selenium.

We studied the effect of sodium selenite, seleno-L-methionine and Se-(Methyl)selenocysteine on proliferation, metabolic activity and apoptosis in three colorectal cell lines with different malignant potential (HT-29, SW 480 and SW 620). Proliferation was measured as BrdU incorporation, total protein amount using Brilliant Blue staining and colorimetric WST-1 assay. Cytotoxicity was assessed by neutral red test. Induction of apoptosis was measured as caspase-3 activity fluorescence assay. Changes in cell morphology were studied by phase-contrast microscopy. Cells were exposed to selenium in concentrations 0-240  $\mu$ M. Brilliant Blue and Neutral red results indicated that Se-(Methyl)selenocysteine was the most potent in grow inhibition of HT29, SW480 and SW620 cells. The most potent compound in induction of apoptosis was Se-(Methyl)selenocysteine.

This work was supported by Grant Agency of the Czech Republic (grant 301/06/P047) and Ministry of Education of the Czech Republic (grant MSM0021620820)

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**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:** 20.04.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 265

#### **Summary of 2007 results**

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

**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:** The present project is focused on extending the knowledge of wound repair in the model of Zucker Diabetic Fatty (ZDF) rat suffering from obesity and type II diabetes mellitus. Our main concern is a description of local changes in the wounds of diabetic obese animals.

**Methods :** Two groups of experimental animals were created : healthy rats (CONT) and obese diabetic rats possessing homozygous mutation in leptin receptor (DIAB). Circular wounds were created by full thickness excision of skin. The wounds were periodically photographed and measured. The healing tissue was removed after 10 days. The granulation tissue was analyzed for hydroxyproline content and for mRNA expression of 111 genes using DNA array.

**Results :** We observed a significant reduction in wound healing during early phases in the DIAB group. The differences were more pronounced in females. Excessive formation of fibrin clot was clearly shown in the DIAB group with underlying excessive polymorphonuclear inflammation. The wounds in CONT group contained significantly more hydroxyproline which suggests better progress in repair comparing to DIAB. The expression of certain genes mRNA was slightly changed, the expression of IL6, IL1b, iNOS, PAI, SLPI was increased, and the IAP4 and elastin expression was decreased in DIAB male group.

**Conclusion :** The wound repair in ZDF animal is impaired, the formation of collagen diminished and inflammatory response is aberrant in DIAB animals due to obesity and hyperglycemia.

Project was supported by the Charles Univ. Grant Agency: n. 7402/07/C, and CPN: n. D11-14

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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<sup>1</sup>, V. Buchta, M. Förstl, R. Ivančaková, D. Kopecká, O. Krs, L. Ryšková, D. Slížová, A. Šimůnek<sup>2</sup>

**Starting date:** 1.1.2006

**Duration (years):** 5

**Total funds allocated for project - Kč (thousands):** 305

#### **Summary of 2007 results**

**Title of the presentation:** Photosensitizers in Dentistry in 2007 updated.

**Authors:** R. Slezák(1), M. Karásková (2), V. Buchta (3), D. Slížová (4), R. Ivančaková (1), J. Rakušan (2), L. Ryšková (3), O. Krs (4)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Dent. (1), Dept. of Clinical Microbiology (3), Dept. of Anatomy (4), Výzkumný ústav organických syntéz a. s., Pardubice (2)

<sup>1</sup> Výzkumný ústav organických syntéz, a. s., Pardubice

<sup>2</sup> Lékařská fakulta UK v Hradci Králové

The research followed all main directions planned in the year 2007. New types of phthalocyanine (PC) photosensitizers have been synthesized and selected for further research, i. e., azoPC, PC substituted with biomolecule, silver PC, metal free tetrasulphonated PC and PC substituted with sulphonamidic group. The method for the modification of titanium oxide nanoparticles using various types of PCs has been developed and samples were referred for testing of their antimicrobial properties. The method for the preparation of silver nanoparticles with average size 10 - 30 nm was also worked out. Method of processing of undecalcified hard tissue (e. g., tooth, bone, titanium) was elaborated. The most suitable approach was the slicing of specimens into smaller blocks and thick sections using the diamond saw microtome, successive milling on the heavy duty microtome, equipped with the ultramiller. Milled section may be stained and studied using light microscope and stereomicroscope. Gold-coated samples could be observed in the SEM. New laser-operated microbiological laboratory and the original method for testing of antibacterial properties of FCs have been developed. Initial laboratory testing of antibacterial activity of 8 FC samples with and without their laser initiation has started using *S. aureus* strain 5887 and *E. coli* strain 5276. Significant antibacterial effect was confirmed in 3 types of FCs referred for additional laboratory testing of their biological toxicity. A clinical laser department and the original device designed as oral simulator simulating oral cavity condition have been created and tested.

The project has been supported by the Ministry of Education (NPV II MŠMT), No 2B06104.

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**Title of the project:** Immunohistochemical quantification of prognostic factors in ovarian cancer and correlation with clinical history of the disease

**Grant Agency:** Ministry of Health

**Project Number:** NR/8363-3/05

**Principal Investigator:** M. Tomšová

**Co-investigators:** I. Šteiner, B. Melichar, I. Sedláková

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 731

**Summary of 2007 results**

**Title of the presentation:** Immunohistochemical prognostic factors in ovarian cancer

**Authors:** Markéta Tomšová (1), Ivo Šteiner (1), Bohuslav Melichar (2), Iva Sedláková (3)  
Charles University in Prague, Faculty of Medicine and Faculty Hospital Hradec Králové:  
Fingerland Department of Pathology (1), Department of Oncology and Radiotherapy (2),  
Department of Obstetrics and Gynecology (3)

The aim of study was to find immunohistochemically detectable significant prognostic factors for epithelial ovarian cancer. There were six areas of research: (1) the expression of hormonal receptors; (2) the assessment of cell growth kinetics by examination of proliferation activity; (3) the assessment of angiogenic potential by means of immunohistochemical detection of vascular endothelial growth factor and by quantification of microvessel density at sites of the highest angiogenic activity; (4) the expression of apoptosis-related oncoproteins; (5) the expression of the oncoprotein HER-2/neu and (6) presence of CD3+ tumour-infiltrating T lymphocytes (TIL). We retrospectively evaluated tissue blocks of 116 consecutive EOC patients who underwent primary surgery. The following pannel of immunohistochemical reactions was applied: estrogen (ER) and progesterone (PR) receptors, proliferation antigens Ki-67 and topoisomerase II $\alpha$ , CD3, oncoproteins p53, p21, bcl-2, bax, HER-2/neu and VEGF. The tumor angiogenic capacity determined by density of the capillaries was visualized by antibodies against antigens FVIII and CD34.

In univariate analysis, age ( $p=0.02$ ), stage ( $P=0.000004$ ), residual tumor ( $P=0.0002$ ), grade ( $P=0.02$ ), Ki-67 ( $P=0.005$ ), topoisomerase II $\alpha$  ( $P=0.04$ ), PR ( $P=0.0008$ ), CD34 mean microvessel density ( $P=0.01$ ), CD34 maximal microvessel density ( $P=0.05$ ) and TIL ( $P=0.00005$ ), were associated with survival. In multivariate analysis, the factors that were independent predictors of survival were only age ( $P=0.01$ ), residual tumor ( $P=0.0004$ ), stage ( $P=0.02$ ), CD34 mean microvessel density ( $P=0.004$ ) and TIL ( $P=0.0000$ ). The presented data suggest that the immunohistochemically detectable significant prognostic marker for ovarian carcinoma is microvessel density by using antigen CD34 and number of intraepithelial TIL. Supported by the Czech Ministry of Health grant NR8363-3/2005

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**Title of the project:** Smoking and oral health

**Grant Agency:** Ministry of Health

**Project Number:** NR/8781-3/06

**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 2007 results**

**Title of the presentation:** Influence of smoking and other variables on periodontal health and dental status.

**Authors:** J. Šmejkalová (1), E. Čermáková (2), R. Slezák (3), V. Jacob (1), L. Hodačová (4), S. Vellappally (1), Z. Fiala (1)

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

A cross-sectional population-based study concerning the influence of smoking habits on oral health was conducted. The study was performed on a representative sample of 1327 subjects (379 current smokers, 208 former smokers and 740 non-smokers) within the age span of 30 to 69 years. The influence of smoking and other chosen independent variables on CPITN and DMFT indices were analysed. Taking the highest CPITN score from six sextants into consideration, non-smokers (NS) exhibited a higher percentage of healthy periodontium and a higher percentage of bleeding on probing but showed a lower percentage of pockets compared to smokers ( $p < 0.001$ ). Among other variables age, preventive dental visits and brushing frequency ( $p < 0.001$ ) and education ( $p < 0.01$ ) had a significant influence on the periodontal status where as gender of respondents did not have any significant influence ( $p = 0.245$ ). The mean number of decayed as well as missing teeth was higher in smokers compared to NS (1.2 vs 0.8;  $p < 0.001$  and 5.9 vs 4.6;  $p < 0.001$  respectively), where as the mean number of filled teeth was higher in NS (10 vs. 9;  $p = 0.024$ ). Gender had an influence on the mean number of filled teeth, which was significantly higher in females ( $p < 0.01$ ) and age had an impact on the mean number of missing teeth ( $p < 0.001$ ). Participation on preventive check-ups influenced mainly the mean number of filled teeth ( $p < 0.001$ ) and decayed teeth ( $p = 0.003$ ). Tooth brushing frequency had an impact mainly on the mean number of missing teeth ( $p = 0.007$ ). Conclusion: Our study confirmed the negative influence of smoking on periodontal health and dental status.

Supported by the Ministry of Health Grant Agency, No. NR8781-3/06.

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**Title of the project:** Relationship of angiogenesis to ZAP-70 expression and clinical course in chronic lymphocytic leukaemia.

**Grant Agency:** Ministry of Health

**Project Number:** NR/8373-3/05

**Principal Investigator:** L. Smolej

**Co-investigators:** J. Krejsek, C. Andrýs, P. Kašparová, P. Žák

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 722

#### **Summary of 2007 results**

**Title of the presentation:** Markers of angiogenesis and their correlation with ZAP-70 expression and clinical course of chronic lymphocytic leukaemia.

**Authors:** Lukáš Smolej (1), Jan Krejsek (2), Ctirad Andrýs (2), Petra Kašparová (3), Pavel Žák (1), Monika Hrudková (1), Jakub Novosad (2), Vladimíra Vroblová (2) University Hospital and Medical School, Hradec Králové: (1) 2nd Dept. Of Internal Medicine, Dept. of Clin.Haematology, (2) Institute of Clin. Immunology and Allergology, (3) Fingerland Dept. of Pathology

Several studies have shown that angiogenesis is increased in chronic lymphocytic leukaemia (CLL) and may potentially serve as a new prognostic factor. Zeta-associated protein of 70 kilodaltons (ZAP-70) is an intracellular tyrosin kinase belonging to modern powerful prognostic factors in CLL with significant impact on clinical course. To assess potential relationship of ZAP-70 and angiogenic signaling in CLL, we quantified plasma concentrations of angiogenic activators VEGF, and bFGF in 73 patients with untreated CLL (43 males, 30 females, median age, 65 years, range, 31-88) and 80 controls. ZAP-70 expression was measured using flow cytometry in 32 patients and compared to the levels of circulating angiogenic factors. bFGF as well as VEGF were significantly increased in CLL group ( $p < 0.0001$  for both cytokines). No differences in angiogenic factors were noted between subgroups with low vs. intermediate vs. high-risk clinical stage according to modified Rai staging or males vs. females. Similarly, there was no association of bFGF or VEGF levels with progression-free survival. Interestingly, bFGF and VEGF correlated inversely with percentage of ZAP-positive cells (bFGF,  $r = -0.43$ ,  $p = 0.014$ ; VEGF,  $r = -0.39$ ,  $p = 0.025$ ) and were significantly elevated in ZAP-negative versus positive patients (bFGF,  $p = 0.021$ ; VEGF,  $p = 0.035$ ). In conclusions, angiogenic activators were elevated in CLL; furthermore, ZAP-70 expression correlated inversely with plasma levels of bFGF and VEGF. Our findings underline the importance of angiogenic signaling in CLL and point to possible association with ZAP-70 expression. Further investigations on possible impact of angiogenesis on overall survival is clearly warranted. Supported by Ministry Of Health Grant Agency, NR/8373-3.

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Hradec Králové, 500 05, Czech Republic

**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/8768-3/06

**Principal Investigator:** J. Tošner

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

**Starting date:** 1.3.2006

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 3412

#### **Summary of 2007 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.Měříčka (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.

The aim of the project is to evaluate usefulness of the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide chemosensitivity test (MTT test). Assessment of results obtained from fresh and defrost tumor tissue is of essential importance for chemoresistance testing in practice as frozen samples could be transported to specialized laboratory from whole republic.

During the year 2007 (second year of project) cells from solid tumours of 18 patients were tested in Hradec Králové and 14 samples from ascites. Cooworkers in Olomouc have tested , 26 samples from tumours and 13 from ascites. 12 tumors were frost by special procedure in Tissue Bank of University Hospital. VEGF and CA125 were investigated in 30 patients as well as phenotyping. 39 patients were investigated for LRP at ht Departemnt of Pathology Tumour infiltrating lymphocytes and tumor markers were investigated in all patients with ascites.

Fresh tumor tissue samples were tested mainly against cisplatinum, paclitaxel, carboplatinum, doxorubicin, gemcitabin, docetaxel and topotecan.

Previous results are expected at the end of the year 2008.

**Address for correspondence:** J.Tošner, Dept.of Gynecology and Obstetrics, Charles University in Prague, Faculty of Medicine Hradec Králové, Nova nemocnice, Sokolska 581, 50005 Hradec Kralové, Czechia E-mail [tosner@fnhk.cz](mailto:tosner@fnhk.cz)

**Title of the project:** Therapy intensification of patients with Diffuse Large B Cell Lymphomas based on early FDG-PET positivity (prospective analysis) and retrospective evaluation of the changes in lymphoma therapy and outcome in Czech Republic within last 15 years

**Grant Agency:** Ministry of Health

**Project Number:** NR/9453-3

**Principal Investigator:** M. Trněný (1)

**Co-investigators:** D. Belada (2), L. Boudova (3), I. Kantorova (4), I. Vasova (5), T. Papajik (6), M. Jankovska (7), M. Matuska (8)

**Starting date:** 1.1.2007

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 2185

#### **Summary of 2007 results**

**Title of the presentation:** Therapy intensification of patients with Diffuse Large B Cell Lymphomas based on early FDG-PET positivity (prospective analysis) and retrospective evaluation of the changes in lymphoma therapy

**Authors:** Marek Trneny, David Belada, Ludmila Boudova, Kantorova Ivana, Vasova Ingrid, Papajik Tomas, Prausova Jana, Jankovska Milada, Matuska Milan, Lysy Milan

General Hospital Prague, Dept.of Internal Medicine (1),2-nd Clinic of Internal Medicine, Charles University and Teaching Hospital, Haematology Dept., Hradec Kralove (2), Fac.Hospital Plzen (3), Hospital Na Homolce, Prague (4), Fac.Hospital Brno (5), Fac.Hospital Olomouc (6), Fac.Hosp.Kralovske VInohrady, Prague (7), Fac.Hosp.Ostrava (8)

Czech Lymphoma Study Group (CLSG) consist of the majority of University Hospitals in Czech Republic (CR) which are focused on lymphoma management as well as of the number of regional hematology or oncology centers. The proposed multicentric project is based on the current level of collaboration in this field in CR and is focused on: 1. analysis of selected subgroups of Non-Hodgkin's lymphomas (follicular, marginal zone, T cell) registered in CLSG registry (from 1999) and to describe the situation in CR - clinical features, therapy, outcome 2. analysis of changes in therapy and outcome in CR within last 15 years (diagnosis 1990-2004) (The number of patients analyzed in both parts is estimated to 4000) 3. prospective multicentric study of the impact of tailored therapy based on early FDG-PET evaluation of younger patients (<65y) with low IPI risk with Diffuse Large B Cell Lymphomas based on early FDG-PET positivity. The importance of proposed project is seen in the addressing of unanswered question (prospective study) as well as in the description of situation in lymphoma field in CR. The aim of the project is the analysis of different lymphoma subgroups (follicular, marginal zone, peripheral T cells) and the description of clinical features, therapeutic approaches and the outcome of patients in Czech Republic. In 2007, based on retrospective analysis of follicular lymphoma patients, molecular genetic data has been published. New therapeutical recommendations has been also published including new insights in diagnostic as well as therapeutical algorithms for lymphoma patients in Czech Republic. Project was supported by Internal Grant Agency of Ministry of Health NR/9453-3.

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**Title of the project:** Visual mismatch negativity among patients with schizophrenia

**Grant Agency:** Charles University

**Project Number:** 24207/2007 C

**Principal Investigator:** A. Urban

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

**Starting date:** 20.4.2007

**Duration (years):** 2

**Total funds allocated for project - Kč (thousands):** 135

**Summary of 2007 results**

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

**Authors:** A. Urban (1), J. Kremláček (2), J. Masopust (1), J. Libiger (1)

Faculty of Medicine in Hradec Králové, Charles University in Prague and Faculty Hospital Hradec Králové, Czech Republic: Dept. of Psychiatry (1), Institute of Pathological Physiology (2)

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 interpreted as the marker of progression in schizophrenia. The objective of our study is to test the presence of the impaired generation of mismatch negativity after visual motion stimuli among patients with schizophrenia and schizophrenia related psychosis. 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 vMMN in 24 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. The results of our vMMN study suggest that there is a deficit in generation of mismatch negativity induced by deviant visual motion stimuli among patients with schizophrenia. This impairment appears analogous to the impairment of MMN in the auditory domain and is probably related to early visual information processing. The origin and stability of the visual MMN deficit and its relationship to cognitive functions require further study.

Project was supported by the Charles University Grant Agency, No 24207/2007.

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**Title of the project:** Finish line exposure exercising during preclinical prosthetic instruction

**Grant Agency:** Ministry of Education

**Project Number:** 675/2007 F3a

**Principal Investigator:** D. Vahalová

**Co-investigators:** L. Vavříčková, D. Dufková

**Starting date:** 1.1.2007

**Duration (years):** 1

**Total funds allocated for project - Kč (thousands):** 106

**Summary of 2007 results**

**Title of the presentation:** Finish line exposure exercising during preclinical prosthetic instruction

**Authors:** D. Vahalová, L. Vavříčková, D. Dufková

Charles Univ. Prague, Fac. Med. Hradec Králové, Dept. of Dentistry

A successful manufacturing of single-crowns and fixed partial dentures depends on many steps during clinical and laboratory stages. One of a very important clinical step is an exposure of a preparation finish line, that in a form of chamfer or shoulder creates a border line between prepared and non-prepared surface of a tooth. This border line has to be replicated on the stone model of the tooth. It means that an impression material can flow into the widely opened gingival crevice below the border line and take the precise impression of these areas. A result of this impression is a precise stone model; technician knows now, where to finish the modelling of the crown and, of course, together with the precise work during other stages of crown or bridge fabrication a very important marginal fit of the crown will be achieved. There are several instruments and devices available on the dental market which are able to remove the gingiva gently from the tooth away. The procedure needs appropriate retraction cords, special packing instruments and the proper technique how to pack the retraction cord into the gingival sulcus. In the past the students met this procedure at the beginning of the 3<sup>rd</sup> schoolyear and were very disappointed, because it seemed that it was impossible to place the cord into the narrow crevice; they have never learnt about gingiva retraction before. That's why we decided to buy special models with compressible gingiva and all types of retractions cords available on our dental market and different types of packing instruments. This instruction takes place during the subject "Preclinical Dentistry III" in the winter term of the 2nd schoolyear and our first experience with this method is excellent. Project was supported by the Ministry of Education Grant Agency, No.675/F3/2007

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

**Grant Agency:** Ministry of Health

**Project Number:** NR/9174-3/07

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 2007 results**

**Title of the presentation:** Resistance to antiplatelet therapy

**Authors:** H.Ševčíková (1), J.Vojáček (1), R.Pudil (1), J.Malý (2), P.Dulíček (2), I.Fátorová (2), R.Ševčík (1)

Fac. Med., Charles Univ., Univ. Hosp. Hr.Králové: Dept. Int. Med. I (1), Dept. Int. Med. II (2)

Despite documented efficacy of acetylsalicylic acid (ASA) and clopidogrel some patients on this therapy suffer from thrombotic cardiovascular events due to nonresponsiveness or partial responsiveness to these drugs. A mechanism of the resistance to antiplatelet therapy is still not completely understood. It is influenced by genetic, cellular and clinical factors. We set following targets for our project. First an evaluation of the prevalence and clinical significance of a resistance to antiplatelet drugs, second detection of the group of nonresponders and its characteristics and finally we want to compare the incidence of coronary events and prognosis in responders and nonresponders to antiplatelet effect. Inclusion criteria and study scheme were defined during the first year of our study. Blood samples are taken according to the following 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). So far we discovered the resistance to antipatelet therapy in two main groups of patients (group A - patients with acute coronary syndromes and group B - patients with stable coronary artery disease). Optical aggregometry with cationic propylgallate and adenosidiphosphate has been used for monitoring ASA and clopidogrel efficacy. Multiple Platelet Function Analyser (MULTIPLATE) has been used for monitoring efficacy of clopidogrel in patients during the first week after coronary stenting. 42 patients were included into the study on January 4th (27 of them were patients with acute coronary syndromes). Clopidogrel efficacy was determined by MULTIPLATE in 214 patients after coronary stenting and in 34 healthy volunteers. The results will be statistically analysed in all groups of patients.

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

**Grant Agency:** Ministry of Health

**Project Number:** NR 9176-3/07

**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):** 3855

**Summary of 2007 results**

**Title of the presentation:** Importance and time course of plasma metalloproteinases, soluble 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: 1<sup>st</sup> Dept. of Int. Medicine(1), Dept. of Immunology (2), Dept. of Clin Biochemistry (3), 2<sup>nd</sup> 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 hypothesize 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. Proposed project anticipates assessment of time course of plasma level of circulating metalloproteinases -2, -9, PAPP-A, sICD40, TF, hsCRP, TNF-alfa. Inclusion criteria and study scheme were defined during the first year of our study. Blood samples are taken according to the following 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). In all patients clinical data will be obtained and long-term follow up will be carried out. 42 patients were included into the study on January 4th (27 of them were patients with acute coronary syndromes). The results will be statistically analysed in all groups of patients.

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:** Paraendoscopic intuitive computer assisted operating system (acronym PICO)

**Grant Agency:** FP3-2002-SME 1 EU

**Project Number:** COOP CT 508231

**Principal Investigator:** Centre of Competence for Minimally invasive medicine and Technology Tuebingen,Germany(MITT)

**Co-investigators:** ORL clinic FN Hradec Králové, Czech Republic(FN HK), Dept. of Neurosurgery, Katharinenhospital,Stuttgart,Germany(KH-NC), Dept.of Neurosurgery, Ernst-Moritz-Arndt University, Greifswald, Germany(EMAU), Neurosurgical center St.Radboud, Nijmegen, Holland, Next 4 SME and 4R&D partners and one large Enterprise

**Starting date:** 1.12.2004

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 342

#### **Summary of 2007 results**

**Title of the presentation:** Application of intuitive holder of endoscope(PICO) in endonasal endoscopic surgery

**Authors:** J.Vokurka

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

Text:

The problem of current paraendoscopic endonasal sinus surgery and surgery using the same approach and instrumentation e.g. endonasal pituitary surgery, skull base surgery etc. is restricted possibility of manipulation in the operating field. The surgeon is "one" handed, because his/her left hand is holding the endoscope usually placed in suction-irrigation handle. Current most common solution of this problem is "three, or four handed surgery". Though using more hands than one looks like simple, realization in the narrow nose is not simple. The other solution of "one handed surgery" in endonasal endoscopic surgery is using of holder of endoscope. This problem was mentioned in more lectures in the 7th European Skull base society congress (1). We need flexible, moveable holding of endoscope, move with it in case of bleeding, cleansing of endoscope, introduction of instruments paraendoscopically etc. PICO intuitive holder should be appropriate solution when surgeon could use bimanual manipulation in the operating field without holding of endoscope (2). Intuitive endoscope holder is one of possible solution of the problem of current FESS. In ORL/HNS there are indication for using of PICO system of holder:

- Endonasal surgery of intranasal CSF (cerebrospinal fluid leakage)
- Intrasphenoidal operation namely in lateral recess of the sinus
- Bleeding in posterior part of nasal cavity etc.

1.Presentation abstracts of the 7th European Skull base congress. Skull Base 2005, 15, suppl.2, 1-84. ISSN 1531-5010

2. Vokurka, J. et al.:Management of endoscopic surgery of skullbase-current problems. Abstracts of 6th. Joint workshop of Middle-German and Czech ENT specialist. 2007., ISBN 978-80-86472-29-4

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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 2007 results**

**Title of the presentation:** Disturbances of synthesis of cholesterol in intensive care

**Authors:** P. Vyroubal (1), Z. Zadák (2), R. Hyšpler (1), A. Tichá (1), J. Cerman (3), E. Havel (3), J. Samek (4)

(1)Dept. of Gerontology and metabolism, Faculty Hospital, Hradec Králové, (2)Center of Research and Development, Charles Univ., Hradec Králové, (3)Dept. of Surgery, Faculty Hospital, Hradec Králové, (4)Dept. of Cardiosurgery, Faculty Hospital, Hradec Králové

The aim of the project: Cholesterol synthesis is a complicated and energy-demanding process which requires many enzymes. Due to complicated synthesis of cholesterol and its high requirements in critical disease, a human organism can be deprived of cholesterol. The aim of the project is the elucidation of the role and importance of hypocholesterolemia during the intensive care.

Methods: Probands are the critically ill patients with acute pancreatitis, polytrauma, after elective abdominal surgery and after elective cardiac surgery. We performed the determination of sterols and their precursors in the blood serum. Sterols are extracted by the Abell-Kendall procedure and analysed by gas chromatography/ Mass spectrometry. We determined also insulin, interleukin IL-6, stimulation ACTH test and functional capacity of granulocytes (oxydative burst test).

Results: In total 45 probands (25 males, 20 females) were studied. We proved declining cholesterol level despite increased endogenous cholesterol production (that we proved at the same time). Suppression of stimulation "burst test" correspond to the hypocholesterolemia. In the event of hypocholesterolemia granulocytes are functionally deficient. A cortisol level and a synacten test weren't significantly modified except patients with extremely low cholesterol level.

Conclusion: Above mentioned results illustrate, that the cholesterol is probably a conditionally essential nutrient in patients with stress.

Project was supported by the IGA Ministry of Health ČR, NR 8921-3

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**Title of the project:** Effect of humic substances in vivo. Study of chemical and physical characteristics of humic substances and their biological activity, mechanism of effect on microorganisms, plant cells and animal cells in the dependence on the humic substances structure.

**Grant Agency:** Ministry of Industry and Trade      **Project Number:** FT-TA/038

**Principal Investigator:** Z. Zadák

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

**Starting date:** 1.1.2004

**Duration (years):** 5

**Total funds allocated for project - Kč (thousands):** 3300

#### **Summary of 2007 results**

**Title of the presentation:** The method of the cholesterol absorption estimation in vivo

**Authors:** Hyšpler R., Tichá A., Kriesfalusyová L., Ježková D., Zadák Z.

**Text:**

The reduction of cholesterol absorption from the gut is a one of therapeutic approaches in hypercholesterolaemia treatment. It is possible to use pharmaceuticals, plant sterols or other substances (e.g. humic acids, dietary fibre).

The aim of the study was to evaluate the cholesterol absorption reduction by phytosterol formulas and humic acids.

**Method:** Laboratory mice C57B16 were divided into five groups (ten animals in each group). Group A -controls, B - ezetimibe, C-humic acid, D- LipoPhytol (non-esterified phytosterols), E - Flora Pro.Activ. The tracer dose 5 uCi (185 MBq) of <sup>3</sup>H-cholesterol was applied to each animal by gastric probe. The tritium activity in liver and blood extracts was determined by liquid scintillation 48 hours later. The results were processed by software Analyse-It.

**Results:** Results are presented as median ± interquartile interval of the detected percentage of applied tracer dose. The lower cholesterol absorption was found in group B (0,047±0,017), C (0,178±0,027), D (0,36±0,019),E (0,21±0,013) compared to control group A (0,423±0,014).

The level of statistical significance is p<0,01.

**Conclusions:** The efficiency of nutritional supplements was evaluated. Phytosterol esters are more efficient in cholesterol absorption reduction in comparison with free phytosterols.

Humic acids are promising nutritional supplement.

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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. Beran, 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):** 55453

#### **Summary of 2007 results**

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

**Authors:** Z. Zadák (1), J. Beran (2), J. Bureš (3), V. Černý(4), A. Ferko(5), P. Hůlek (6), R. Hyšpler (7), N. Jirásková (8), J. Malý (9), B. Melichar(10), V. Palička (11), J. Petera (12), A. Ryška (13), V. Tošnerová (14), J. Vojáček (15)

Faculty of Medicine and Teaching Hospital, Hradec Králové:

(1) Dpt. of Research and Development; (2) Dpt. of Infectious diseases; (3) Dpt. of Gastroenterology; (4) Dpt. of Anesteziology; (5) Dpt. of Surgery; (6) Dpt. of Hepatology; (7) Dpt. of Gerontology and Metabolism; (8) Dpt. of Oftalmology; (9) Dpt. of Hematology; (10) Dpt. of Oncology; (11) Dpt. of Biochemistry;(12) Dpt. of Oncology; (13) Dpt. of Pathology; (14) Dpt. of Rehabilitation; (15) Dpt. of Internal Medicine

J. Bureš – Bio-indicators in Gastroenterology

The objective of the study was to evaluate the early stage of healing after the use of two different solutions for submucosal injection for endoscopic mucosal resection (EMR). Submucosal injection of glycerol, glucose and Patenblau V (compared to hydroxypropyl methylcellulose) provides significantly superior histochemical signs of early healing of the gastric EMR in experimental pigs.

R. Hyšpler – Determination of cholesterol metabolites in breath and indirect calorimetry.

Biological and nutritional quality of dietary fibre evaluation (breath tests). Metabolic changes in critical diseases and during aging.

Isoprene (2-methyl-1, 3-butadiene) arises from the non-enzymatic decomposition of dimethylallyl diphosphate, an intermediate in the synthesis of cholesterol and other isoprenoids. According to reaction mechanism of isoprene origin, the average enrichment of body NADPH pool could be estimated. The hypothesized equation is  $IE_{isoprene} = 2 * IE_{NADPH\ pool} + IE_{body\ water}$ .

Hypocholesterolemia has recently gained importance as a possible indicator of the prognosis in critically ill patients. The FSR (Fractional Synthesis Rate) of cholesterol in the liver tissue was much higher in the septic group compared to controls ( $0,425 \pm 0,151$  vs.  $0,185 \pm 0,022$ , respectively). Despite hypocholesterolemia, the cholesterol synthesis rate in the liver tissue was significantly increased.

J. Malý – Bio-indicators in Medicine

Activity of primary hemostasis plays an important role in the process of atherosclerosis. This fact led us to hypothesize that the investigation of primary hemostatic activity might be a good marker for monitoring LDL-apheresis efficacy. Modified platelets aggregation seems to be more suitable to determine the optimal intensity of individual LDL-apheresis with statistically significantly improved results.

B. Melichar – Biological therapy of cancer

Cetuximab is a chimeric antibody registered for the therapy of advanced colorectal carcinoma after failure of standard chemotherapy. Rare infusion reactions that resulted in the cessation of therapy have been described after cetuximab administration. We present here two new cases. The present experience indicates that cetuximab can be administered in patients who experience infusion reactions. Surveillance in the intensive care unit is mandatory during re-administration of the drug.

References:

1. Bureš, J. et al. Endoscopy 2007, 39, suppl 1: A346
2. Hyšpler, R. et al. Clinical Nutrition 2007, 2, suppl 2: 29
3. Tichá, A. et al. Clinical Nutrition 2007, 2, suppl 2: 93
4. Melichar, B., Cerman, J., Malířová, E. Successful management of infusion reaction accompanying the start of cetuximab therapy. Supportive Care in Cancer 2007, 15, 445-449.

Supported by the Research project MZO 00179906.

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**Title of the project:** Atherogenic role of endothelial dysfunction and disorders of cholesterol metabolism in coronary atherogenesis in diabetes mellitus type II

**Grant Agency:** Ministry of Health

**Project Number:** NR/8497-3

**Principal Investigator:** V. Bláha

**Co-investigators:** D. Černohorský, D. Solichová, C. Andrýs, F. Musil, R. Hyšpler, A. Tichá, Z. Zadák

**Starting date:** 1.1.2005

**Duration (years):** 3

**Total funds allocated for project - Kč (thousands):** 622

**Summary of 2007 results**

**Title of the presentation:** Effect of atorvastatin on soluble CD14, CD40 Ligand, sE- and sP-selectins and MCP-1 in patients with type 2 diabetes mellitus: relationship to cholesterol turnover

**Authors:** V. Bláha (1), C. Andrýs (2), D. Černohorský (1), D. Solichová (1), R. Hyšpler (1), Z. Zadák (1), A. Tichá (1), F. Musil (1) Faculty Hospital, Charles Univ., Dept. Gerontol. Metabol. Care (1), Dept. Immunology (2)

**Text:**

Metabolic abnormalities frequently associated with Type 2 diabetes, feature besides endothelial dysfunction a novel factor of low cholesterol absorption and high cholesterol synthesis, thus predisposing advanced atherosclerosis. We studied 75 patients: 30 with type 2 diabetes, 30 non-diabetic subjects with a history of cardiovascular disease, and 15 healthy subjects with and without atorvastatin therapy. Plasma levels of hsCRP were significantly higher in patients with diabetes than in healthy subjects. There was no difference in hsCRP between patients with cardiovascular disease and diabetes compared to patients without diabetes. In diabetic individuals without statin therapy there were significantly higher plasma levels of sP-selectin. In diabetic individuals on statin therapy there were significantly higher plasma levels of sE-selectin. Plasma levels of MCP-1 were significantly higher in patients with cardiovascular disease than in the healthy subjects regardless of the presence of diabetes. In atorvastatin treated diabetic patients, significantly lower levels of lathosterol as well as index lathosterol/cholesterol, and significantly higher levels of campesterol as well as index sitosterol/cholesterol and campesterol/cholesterol were found. In diabetic patients, a positive significant correlation of sE-selectin ( $p=0.0138$ ) with sitosterol and sitosterol/cholesterol ratio disappeared with statin treatment. The diabetic patients without statin therapy showed a negative significant correlation of sP-selectin with the lathosterol ( $p=0.0335$ ), which disappeared with statin treatment. MCP-1 significantly correlated with lathosterol ( $p=0.0266$ ). We conclude that in obese diabetic type 2 patients the endothelial dysfunction (such as sP-selectin and sE-selectin) relate to cholesterol synthesis and absorption, which may be influenced by reverse cholesterol transport.

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**Title of the project:** The development and application of new biotechnological methods to study relationship of phenotypes, genotypes and response to statin therapy upon lipoprotein metabolism

**Grant Agency:** Ministry of Health

**Project Number:** 1A/8689-4

**Principal Investigator:** P. Solich

**Co-investigators:** D. Šatinský, L. Nováková, V. Bláha, D. Solichová

**Starting date:** 1.9.2005

**Duration (years):** 4

**Total funds allocated for project - Kč (thousands):** 4899

#### **Summary of 2007 results**

**Title of the presentation:** Pharmacodynamic of statins – Development of the method for their determination in biological material

**Authors:** Nováková, L., \*Bláha, V., Sadílek P., Šatinský D., Solich P., \*Bláha, M., \*Solichová, D., Malý, R., \*Blažek, M., \*Filip, S., \*Malý, J.

Department of Analytical Chemistry, Faculty of Pharmacy, Charles University, Hradec Králové, Czech Republic

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Introduction: Patients with severe form of familiar hypercholesterolemia (FH) are treated with maximally tolerable doses of statins besides of diet and lifestyle change. A group of 12 patients has been observed in a long-term study ( $7.2 \pm 2.96$  years). Moreover repeated extracorporeal elimination of LDL-cholesterol was necessary at these patients. Such procedures could influence a blood level of statins and thus reduce their therapeutic effect. The aim of this work was to develop a method for their determination in biological fluids. Method: Two modifications of HPLC method (high performance liquid chromatography) with various types of detection (UV spectrophotometric and fluorescence) were tested and subsequently one GC (gas chromatography) approach and UPLC-MS/MS method (hyphenation of ultra performance liquid chromatography with tandem mass spectrometry) were examined.

Results: UPLC-MS/MS method using specific SRM (selected reaction monitoring) experiment enables to achieve high sensitivity and selectivity of the determination with excellent reproducibility during a very short period of time.

Conclusion: A new UPLC-MS/MS method for the determination of atorvastatin and simvastatin as most widely used drug in the treatment of FH was described. The method is available, fast and precise. It is convenient, after some modifications, for the determination of other statins, for pharmacodynamic studies and dosage adjustments in order to reach the best treatment effect as well.

The work was supported by the project IGA MH CZ no. 1A/8689-4.

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