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

XV. VĚDECKÁ KONFERENCE

P R O G R A M

26. ledna 2011

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

Program XV. vědecké konference Lékařské fakulty v Hradci Králové a Fakultní nemocnice Hradec Králové (2011)

XV. vědecká konference Lékařské fakulty Univerzity Karlovy v Hradci Králové a Fakultní nemocnice Hradec Králové

Středa 26. ledna 2011 - budova teoretických ústavů LF, Šimkova 870

09.00 - 09.15	Z a h á j e n í k o n f e r e n c e **prof. MUDr. Radek Pudil, Ph.D.** **prof. MUDr. RNDr. Miroslav Červinka, CSc.** děkan lékařské fakulty **prof. MUDr. Roman Prymula, CSc., Ph.D.** ředitel fakultní nemocnice
Sekce I	Předsedající: **prof. MUDr. Zuzana Červinková, CSc.**
09.15 - 09.30	Vliv anesteziologických technik na mikrocirkulaci orgánů **MUDr. Zdeněk Turek** GA UK 135809 (LF)
09.30 - 09.45	Ovlivní ztukovatění jater nealkoholového původu průběh regenerace jater po částečné hepatektomii? **MUDr. Tomáš Garnol** GA UK 126109 (LF)
09.45 - 10.00	Mechanismus ukončení jaterní regenerace po parciální hepatektomii **MUDr. David Rychtrmoc** GA UK 94509 (LF)
10.00 - 10.15	Asociační studie v genetice za použití sekvencí DNA a jejich biologických vlastností **Mgr. Ondřej Libiger** GA UK 134609 (LF)
10.15 - 10.30	Studium potenciálního terapeutického významu pravastatinu v terapii jaterního poškození během akutní a chronické extrahepatální cholestázy a biliární cirhózy u potkanů **Mgr. Gabriela Kolouchová** GA UK 122408 (LF)
10.30 - 11.00	*Přestávka – občerstvení*
Sekce II	Předsedající: **prof. MUDr. Aleš Ryška, Ph.D.**
11.00 - 11.15	Kvantitativní exprese dopaminergních receptorů v klinicky afunkčních nádorech hypofýzy **MUDr. Filip Gabalec** GA UK 79008 (LF)
11.15 - 11.30	MIO versus ORIF intraartikulárních zlomenin patní kosti **MUDr. Tomáš Holeček** GA UK 95708 (LF)
11.30 - 11.45	Studium vlivu zinku na proliferaci a buněčnou smrt buněk kolorektálního

karcinomu in vitro
Tomáš Briatka (MUDr. Stanislav John)
GA UK 132808 (LF)

11.45 - 12.00 Cytotoxicita dentálních materiálů
MUDr. Lenka Vavřičková
GA UK 81508 (LF)

12.00 - 12.15 Koroze protetických materiálů v dutině ústní
MUDr. Lenka Vavřičková
IGA MZ NS/9744-3/2008 (LF)

12.15 - 13.30 *Přestávka na oběd*

Sekce III Předsedající: **prof. RNDr. Jan Krejsek, CSc.**

13.30 - 13.45 Jsou játra postižená nealkoholickým ztukovatěním (non-alcoholic fatty liver disease, NAFLD) citlivější vůči toxickému poškození?
MUDr. Otto Kučera, Ph.D.
GA ČR 305/08/P184 (LF)

13.45 - 14.00 Studium faktorů mikroprostředí ovlivňujících procesy reparace kosterní svaloviny
prof. MUDr. Stanislav Filip, Ph.D., DSc.
GA ČR 309/08/0329 (LF)

14.00 - 14.15 Změny chemorezistence/chemosensitivity ovariálních nádorových buněk
MUDr. Iva Sedláková, Ph.D.
IGA MZ NS/9737-3/2008 (LF)

14.15 - 14.30 Mohou statiny zmírnit rozvoj cholestatického jaterního poškození?
doc. MUDr. Halka Lotková, Ph.D.
IGA MZ NS/9739-3/2008 (LF)

14.30 - 14.45 Katetrizační léčba nemocných s těžkou aortální stenosou
prof. MUDr. Jan Vojáček, DrSc.
IGA MZ NS/9741-3/2009 (LF)

14.45 - 15.15 *Přestávka – občerstvení*

Sekce IV Předsedající: **prof. MUDr. Jaroslav Malý, CSc.**

15.15 - 15.30 Využití vydechovaného oxidu dusnatého jako biochemického markeru zánětu v dýchacích cestách nemocných se sezónní alergickou rýmou a průduškovým astmatem
MUDr. Jiřina Chládková, Ph.D.
IGA MZ NS/9692-3/2008 (FN)

15.30 - 15.45 Nové terapeutické postupy základní a rozšířené neodkladné resuscitace na zvířecím modelu fibrilace komor
MUDr. Anatolij Truhlář
IGA MZ NS/10383-2 (FN)

15.45 - 16.00 Fotosenzibilizátory v zubním lékařství
doc. MUDr. Radovan Slezák, CSc.
MŠMT 2B06104 (LF)

16.00 - 16.15 U k o n č e n í k o n f e r e n c e
prof. MUDr. Roman Prymula, CSc., Ph.D. ředitel fakultní nemocnice
prof. MUDr. RNDr. Miroslav Červinka, CSc. děkan lékařské fakulty

SOUHRNY VÝZKUMNÝCH ÚKOLŮ

ŘEŠENÝCH NA LF UK A VE FN V HRADCI KRÁLOVÉ

(ABECEDNĚ)

Title of the project: The Centre of cellular Invasion in Embryonal Development and Tumour Metastasis

Grant Agency: Ministry of Education ***Project Number:*** LC06-invasion

Principal Investigator: M. Dvořák

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

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

Total funds allocated for project - Kč (thousands): 3171

Summary of 2010 results

Title of the presentation: The search for „cancer genes“: humanizing the chicken research

Authors: M. Dvořák (1), P. Pajer (1), P. Benešová (2), A. Ryška (2), I. Šteiner (2)

(1) Institute of Molecular Genetics , Academy of Sciences

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

The genes showing mutation and/or altered expression actively contributing to oncogenic transformation are denoted as „cancer genes“.We use a model of MAV2-induced chicken nephroblastoma to analyze genes involved in the development and progression of renal tumors. MAV2 is an oncogenic retrovirus, inducing nephroblastomas in chicken. The resulting tumors are clonal with tumor-inducing genetic lesions (cancer genes) marked by retroviral integrations. We have analyzed a series of more than 300 independent chicken nephroblastomas. To date, viral integration sites (VIS) from more than 150 unique tumor samples were identified and assigned a position in the chicken genome. About 50 genomic loci were classified as common viral integration sites, seven of them have been shown previously to participate in malignant transformation of different human cell types. Thus, the experimental chicken nephroblastoma model is a useful model for studying cancer genes playing a role in pathogenesis of various human malignancies. Several human tissue microarrays have been assembled each containing tens matched samples of tumor tissue and normal (control) tissue. The use of tissue microarrays is highly effective as the large number of tissue samples can be checked simultaneously, repeatedly and highly reproducibly for the expression of individual candidate genes previously identified in chicken nephroblastoma model. Various human malignancies were chosen from the archive of paraffin-embedded tissue samples to select subset of human malignancies with potentially deregulated candidate genes. Our results link a parallel between the molecular basis of cell transformation in man and chicken and demonstrate the usefulness of the animal retroviral model for the identification of human cancer genes.

Address for correspondence: Petra Kašparová, The Fingerland Department of Pathology, University Hospital, Sokolská, Hradec Králové, CZ 50005

Title of the project: Pathophysiology of malnutrion in renal failure and impact of therapeutical intervention

Grant Agency: Ministry of Health ***Project Number:*** NS/9743-4

Principal Investigator: V. Bláha

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

Starting date: 30.6.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 6115

Summary of 2010 results

***Title of the presentation*:** Malnutrition, inflammation, atherosclerosis, inflammation in hemodialysis (HD) patients: the impact of intervention.

Authors: Bláha V, Mistrík E, Dusilová-Sulková S, Bláha M*, Andrys C+, Sobotka L. Dept Metabolism and Gerontology, *IInd Dept Internal Med, + Dept Immunology, University Hospital, Hradec Kralove; Charles University, Prague, Czech Republic

Introduction and aim. ESRD patients are well known to suffer from high mortality often caused by MIAC (malnutrition, inflammation, atherosclerosis, inflammation) syndrome. We measured parameters of MIAC syndrome (high sensitivity C-reactive protein (hsCRP), total iron binding capacity (TIBC), pregnancy associated plasma protein A (PAPP A) and fetuin A in ESRD patients after intervention aimed to improve their inflammatory status and dialysis adequacy. **Methods:** 49 ESRD patients (22f, 27m, 67.5 y (range 39-90)) on chronic hemodialysis (23.5 (10-34) months) without any apparent acute disease or infection were treated with 3 months intervention. The group was divided into N-group with no intervention needed to be done (n=18), F-group with sanation of chronic infective foci (n=17) and D-group with improvement of hemodialysis adequacy (n=16). After testing for normality we used paired t-test/ Wilcoxon rank order test, as appropriate. **Results: F group**: Median serum concentration of hsCRP significantly decreased (6.6 to 2.7 mg/dL; $p<0.01$) while there was a significant increase in mean serum concentrations of TIBC (38.1 ± 8.2 to 40.2 ± 8.8 μmol/L; $p=0.05$), fetuin A (0.24 ± 0.06 to 0.28 ± 0.09 g/L; $p=0.05$) and HDL cholesterol (1.1 ± 0.4 to 1.2 ± 0.3 mmol/L; $p<0.05$). There was no change in serum levels of PAPP-A. **D group**: There is a significant increase in serum fetuin A levels (0.26 ± 0.08 to 0.33 ± 0.13 g/L; $p<0.01$). There was no change in any other parameters. **N group**: There was a significant increase in median serum concentrations of hsCRP (4.8 to 5.7 mg/dL; $p=0.05$) and PAPP-A (20 to 22 mU/L; $p=0.05$). **Conclusion:** According to our results, therapeutic intervention in ESRD patients might improve the clinical course of MIAC syndrome, as shown by improved biochemical parameters. On the contrary, in other vice asymptomatic patients the unfavorable course of atherosclerosis and inflammation tends to continue when no intervention is performed.

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: Antiproliferative effects of zinc in colon cancer cell lines

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

Principal Investigator: T. Briatka, S. John

Co-investigators: E. Rudolf

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

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

Summary of 2010 results

Title of the presentation: Diverse sensitivity of cells representing various stages of colon carcinogenesis to increased extracellular zinc

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

Fac. Med., Charles Univ., Hr. Králové: Dept. of Medical Biology and Genetics

The relationship between zinc intake and risk of colon cancer is widely recognized. Despite reported mechanisms of zinc-mediated effects in colonic cells no information is available on whether zinc is capable of inducing cell death of malignant colonocytes. The present study shows that increased external zinc concentrations inhibit cell growth of three different colon cancer cell lines representing different stages of colon cancer: HCT-116, HT-29 and SW620 cells and induce their death. Of the tested cell lines, SW620 cells proved to be the most sensitive to externally added zinc and this sensitivity was at least partly due to increased levels of intracellular free zinc and the inability to overexpress metallothionein. Further studies into the mechanisms of zinc-induced cell injury and cell death revealed oxidative stress as the most important underlying mechanism activating stress kinase-dependent signaling, perturbation of mitochondria and plasma membrane damage. In addition, observed cell death in individual cell populations was cell line-dependent and variable including cells displaying features of apoptosis, necrosis, autophagy and other mixedtypes. In conclusion, presented results for the first time show variability of responses to zinc in colon cancer at different stages as modeled *in vitro* and suggest that zinc-induced cell death despite common underlying mechanism(s) might have a variable nature.

Literature: 1) Jaiswal AS and Narayan S: Zinc stabilizes adenomatous polyposis coli protein levels and induces cell cycle arrest in colon cancer cells. J Cell Biochem 93: 345-357, 2004.

2) Park KS, Lee NG, Lee KH, Seo JT and Choi KY: The ERK pathway involves positive and negative regulations of HT-29 colorectal cancer cell growth by extracellular zinc. Am J Physiol Gastrointest Liver Physiol 285: G1181-G1188, 2003

This study was supported by GAUK No. 132808 and Ministry of Education Research Project No. MSM 0021620820

Address for correspondence: Stanislav John, Dept. of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic, email: johns3ar@lfhk.cuni.cz

Title of the project: Evaluation and development of new perspective antituberculotic drugs and prodrugs active against multidrug resistant strains.

Grant Agency: Ministry of Health ***Project Number:*** NS/10367-3

Principal Investigator: J. Vinšová

Co-investigators: V. Buchta, M. Svobodová, P. Paterová

Starting date: 1.8.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 4596

Summary of 2010 results

Title of the presentation: *In vitro* antimicrobial activity of new synthetic compounds.

Authors: V. Buchta (1), M. Svobodová (1), P. Paterová (1)
Fac. Med., Charles Univ., Hr. Králové: Dept. of Clinical Microbiology (1)

Tuberculosis remains a devasting infection affecting about one third of world population. This is difficult to treat infection especially if caused by MDR and XDR strains of *Mycobacterium tuberculosis* (TB). Hence there is a need new antituberculotic drugs with specific mechanism of action.

Our experiments focused on new analoques of salicylanilide carbamates, benzanilides, chitosan cojugates, and pyrazines which were tested against human pathogenic bacteria, mycobacteria and fungi using broth microdilution CSLI standards. Minimal inhibitory concentration (MIC) and its relationship to *in vitro* antimicrobial effect were evaluated.

The most promising results were obtained after esterification of salicylanilide carbamates with MIC range 2 – 0.5 µmol/L for mycobacteria, including MDR-TB.These esters were efficient also on Gram-positive cocci. The similar *in vitro* effect showed some of pyrazinecarbox-amides. Both of the series had a limited antifungal activity.

Literature: M. Krátký, J. Vinšová, V. Buchta, et al. *Eur. J. Med. Chem*. 45, 6106-6113, 2010. M. Doležal; J. Zitko; Z. Osička, et al. *Molecules*, 15, 8567–8581, 2010.

Project was supported by the Internal Grant Agency of Ministry of Health, No NS/10367-3

Address for correspondence: Vladimír Buchta, Dept. of Clinical Microbiology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Sokolská tř. 581, 500 05 Hradec Králové, Czech Republic; buchta@fnhk.cz

Title of the project: The 7th International Medical Conference

Grant Agency: Charles University ***Project Number:*** 260909

Principal Investigator: M. Červinka

Co-investigators:

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 839

Summary of 2010 results

Title of the presentation: New Frontiers in the research of PhD Students

Authors: M. Červinka

The 7th International Medical Conference took place in Hradec Kralove on November 18-20, 2010. Medical schools across the Europe nominated 42 students of medical doctoral study programmes from 13 European countries (Portugal, Great Britain, Denmark, the Netherlands, Italy, Germany, Austria, Poland, Hungary, Croatia, Georgia, Slovak and Czech Republic). Nine experts from 8 countries (inclusive the President of ORPHEUS (Organisation of PhD Education in Biomedicine and Health Sciences in the European System) and the President of Association of Medical Schools in Europe became the members of International Evaluation Committee. All presentations were published in the conference proceedings. The conference aims, namely, comparing achieved results and levels of PhD programmes at medical schools, presentation of the scientific works, meeting the students and experts from European countries were fulfilled.

Address for correspondence: M. Červinka, Department of Medical Biology and Genetics, cervinka@lfhk.cuni.cz, Faculty of Medicine in Hradec Kralove, Šimkova 870, 500 38 Hradec Králové

Title of the 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:*** 0021620820

Principal Investigator: M. Červinka

Co-investigators: J. Cerman, Z. Červinková, V. Geršl, M. Holeček, J. Martínková, J. Mokrý, L. Sobotka, P. Tomšík, A. Žák

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

Total funds allocated for project - Kč (thousands): 34833

Summary of 2010 results

Title of the presentation: **Experimental and clinical models of metabolic processes, nutrition and therapy.**

Authors: M. Červinka (1), J. Cerman (2), Z. Červinková (3), V. Geršl (4), M. Holeček (3), J. Martínková (4), J. Mokrý (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 University, Prague, 4th Department of Internal Medicine (7)

In the field of nutritional modification of cell proliferation and cell death we have continued in the evaluation of selected microelements (zinc, selenium, and chromium) and other model substances on cell lines derived from colorectal carcinoma, melanoma and gingival fibroblasts. We used advanced cytomorphometric, biochemical and molecular methods for analysis of selected signalling pathways, including DNA-ATM-p53, ERK-p38-JNK, p53-mitochondria and types of cell death.

We have continued our study on effects of L-rhamnose and L-fucose on mouse mammary adenocarcinoma in vivo. We begin analysis of anticancer effects of the alkaloid boldine and steroidal glycoalkaloids from tomatoes (alfa-tomatine), alone and in combination with doxorubicin. We have monitored their effect on tumour growth and mouse survival, expression of transport proteins and induction of apoptosis.

Study of irradiated human T-lymphocytes (MOLT-4) has continued with the aim to find suitable marker of radiation exposure. We have selected protein p53 phosphorylated at serine 15 as a suitable candidate. We have studied antineoplastic properties of vanadocens on MOLT-4 as well. Induction of cell death is very rapid and dependent on activation of caspases followed by p21expression. During study of liver cirrhosis we have analysed effects of extracellular matrix on gene expression in cultivated rat myofibroblasts. Together 92 genes we have analysed by DNA „arrays“, real time RT-PCR and selected protein also by western blotting.

In the study focused on the liver affected by non-alcoholic fatty liver disease (NAFLD) we found that NAFLD significantly increases sensitivity of the liver to toxic injury caused by acetaminophen and tetrachlormethan; liver regeneration after partial hepatectomy is not

altered in the liver with simple steatosis. We also studied changes of gene expression using microarrays in the liver regenerating after partial hepatectomy. We optimized RT-PCR method for quantification of UCP-2 expression in liver and used it for evaluation of the effect of triiodothyronine on UCP-2 mRNA expression. We finished our study with S-adenosylmethionine and we can conclude that liver regeneration was dramatically decreased. Based on the results gained by detailed evaluation of energy metabolism of rat hepatocytes in vitro we conclude that mitochondra are not the primary target of toxic action of model hepatotoxins thioacetamide and D-galactosamine. Nevertheless, mitochondria contribute significantly to the progression of toxic injury.

The study of chronic anthracycline cardiotoxicity continued, namely the role of mitochondria and ubiquitin-proteasome system. Effects of the delayed administration of dexrazoxane in the daunorubicin cardiotoxicity and the toxicity of tyrosine kinase inhibitor were investigated. In L-NAME treated rats, captopril and melatonin prevented LV fibrosis.

We have analysed the role of hyperammonemia in pathogenesis of muscle wasting and amino acid imbalance in patients with liver cirrhosis. We demonstrated that ammonia activates catabolism of branched-chain amino acids (BCAA; Val, Ile, Leu) in skeletal muscle which results in decreased levels of BCAA in the extracellular fluid. We demonstrated that chronic intake of glutamine-enriched diet alters amino acid concentrations in plasma and tissues and significantly impairs the response to starvation.

In collaboration with European project Diogenes (FP6-513946), we finished analysis of fatty acids in adipose tissue of selected patient. We analysed 87 persons after 8 weeks of low-caloric diet, followed by 6 moths of diet with specific glycaemic index. Changes in body mass correlate with the spectrum of specific fatty acids.

We finished study of granulation tissue formation influenced by Hyaluronan-jodide. We continue with study of starvation on compensation of diabetes type I and effects of inflammation on microcirculation in skin of dialysed patients.

In our *in vitro* models of cell differentiation we have obtained original data about properties of mesenchymal stem cells, including their differentiation potential. We correlate the length of telomeres with other biological characteristics. We have isolated 22 new lines of stem cells from dental pulp and 2 new lines from periodontium. We analysed expression of intermediate filaments in regenerated neuromuscular junctions, participation of bone marrow cells on reparation of skeletal muscles, and reparation of subependymal zone in neurodegenerative processes in striatum.

Project was supported by the Ministry of Education Grant MSM 21620820

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

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

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

Principal Investigator: S. Filip

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

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

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

Summary of 2010 results

Title of the presentation: Splenectomy Influences Homing of Transplanted Stem Cells in Marrow Ablated Mice

Authors: S. Filip[1], J. Mokrý [2], J. Vávrová [3], D. Čížková [2], Z. Šinkorová [3], S. Mičuda [4]

[1] Department of Oncology and Radiotherapy, Charles University in Prague, Faculty of Medicine and University Hospital, Hradec Králové;

[2] Department of Histology and Embryology, Charles University in Prague, Faculty of Medicine, Hradec Králové;

[3] Department of Radiobiology, University of Defance Brno, Faculty of Military Health Science Hradec Králové;

[4] Department of Pharmacology, Charles University in Prague, Faculty of Medicine, Hradec Králové;

Cell circulation and *in vivo* homing play important roles in reparation and regeneration processes. To study the role of the spleen on hematopoesis repair in whole body irradiated mice transplanted with $lacZ^+$ marked $lin^-/CD117^+$ bone marrow cells, we compared splenectomized mice (T_S,, with splenectomy performed prior irradiation) to non-splenectomized, irradiated mice (T_N) and to normal (unirradiated) mice. Although a worse hematopoetic reconstitution was observed in T_S mice, their survival was not impaired during the 70-day follow up period after transplantation of the lineage negative, c-kit positive hematopoetic precursors. FACS analysis of endogenous $CD117^+$ cells in the thymus and bone marrow revealed that splenectomy markedly altered the distribution of hematopoetic stem cells. Cell engraftment was shown by histochemical and qRT-PCR analysis of recipient tissues. These methods also confirmed that the transplanted hematopoetic stem cells mobilized to the gastrointestinal tract. The number of donor cells in recipient tissues continued to increase for 30 days after transplantation; the higest numbers were observed in the T_S group. DNA marking analysis led to the conclusion that engrafted cell were not only integrated in recipient tissues but were also capable of performing complex cellular processes, including cell division. Our results indicate the cell-mediated repair can be markedly influenced by the integrity and presence of organs not directly in the repair process, such as the spleen.

Address for correspondence: Stanislav Filip, Department of Oncology and Radiotherapy, Charles University in Prague, Faculty of Medicine and Teaching Hospital, Sokolská 581, 500 05 Hradec Králové, Tel.: +420 495 834 618, e-mail: filip@fnhk.cz

Title of the project: Evaluation of predictive quality factors of health care in oncologic patients.

Grant Agency: Charles University ***Project Number:*** 260904

Principal Investigator: S. Filip

Co-investigators: L. Slováček, J. Kopecký, P. Priester, I. Slánská, J. Dvořák, S. Paulíková, A. Paulík, M. Hodek, J. Grim, H. Kalábová, Z. Mačingová, J. Vaňásková

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1350

Summary of 2010 results

Title of the presentation: Quality indicators of palliative oncologic care assesed at different levels of provided health care.

Authors: Filip, S., Slováček, L., Kopecký, J., Priester, P., Petera, J.
Klinika onkologie a radioterapie LFUK a FN, Hradec Králové

Palliative treatment and related nursing care are currently the major problem in the organization of health care. Outpatient palliative cancer care (OPCC) helps to verify the clinical status of cancer patients and helps to choose the best treatment options and their realization. The OPCC has to be able to provide all treatment possibilities of cancer related symptoms such as cancer pain or symptoms related with cancer therapy. During tracking period in the OPCC there were treated 446 patients - 288 women, mean age 61 years (age range 20-81 years) and 158 men, mean age 56 years (age range 18-96 years). According to the assessment of performance status (PS) there was following stratification: PS-0 (8%), PS-1 (54%), PS-2 (33%) and PS-3 (5%). The OPCC uses a uniform system of medical records, uniform system for assessing the quality and quantity of health care – and determination of "minimal health care." Here we present our results, which reflect the need of cooperation of all, who are involved in health care of cancer patient - collaboration between the general practitioner (GP), oncology departments, including hospice and home care agencies.

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

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

Principal Investigator: F. Gabalec

Co-investigators: J. Čáp, M. Beránek

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

Total funds allocated for project - Kč (thousands): 661

Summary of 2010 results

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

Authors: F.Gabalec (1), J.Čáp (1), M.Beránek (2), D. Netuka (3), V.Masopust (3), J. Náhlovský (4), T.Česák (4)

Fac. Med., Charles Univ. and University Hospital, 2nd Department of Internal Medicine, Hradec Králové (1), Fac. Med., Charles Univ., and University Hospital, Department of Clinical Biochemistry and Diagnostics , Hradec Králové(2), Dep. of Neurosurgery, Central Military Hospital, Prague (3), Dep. Of Neurosurgery, Charles Univ. Hospital in Hradec Králové (4)

Dopamine agonists (DA) are treatment of choice for prolactinomas. In clinically non-functioning pituitary adenomas (CNFAs) the role of DA treatment remain uncertain and results of this treatment in clinical series are conflicting. The aim of this project was to analyze dopamine 2 receptor (D2R) expression in cells of pituitary adenomas indicated for surgical treatment, using quantitative PCR method to measure receptor mRNA. The receptors expression was correlated with clinical and radiological characteristics and with hormone expression, detected by immunocytochemistry. Out of the 87 adenomas investigated, 63 expressed gonadotropins, 7 were silent corticotroph adenomas, 7 were plurihormonal tumors, and only 6 did not express any pituitary hormone on immunohistochemical investigation. All 82 evaluated NFPAs expressed the D2R mRNA. High variability in the D2R mRNA expression was present. The D2R mRNA levels varied from 0.93 to 1.689.137 copies/ 5µl cDNA and from 0.003 to 14.860 after normalization to the GUS gene, respectively. The expression was very low in corticotroph adenomas (relative median quantity after normalization to housekeeping gene 0.01) and lower in plurihormonal tumors (median 0.4) than in gonadotroph (median 1.3) and null-cell adenomas (median 1.9). The difference between corticotroph adenomas and plurihormonal tumors in comparison with other pathological types was statistically significant. The expression of D2R did not depend on the presence or absence of gonadotropins. The positivity of gonadotropins does not predict the D2R quantity. High variability of expression can explain the different response of CNFAs to DA treatment. Thus, the quantitative analysis of D2R expression can help with indication of medical treatment. Project was supported by Charles University Grant Agency Project No.79008.

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Title of the project: Quantitative analysis of somatostatin receptors in pituitary adenomas.

Grant Agency: Ministry of Health ***Project Number:*** NT/11344-4

Principal Investigator: F. Gabalec

Co-investigators: M. Beránek, J. Čáp, J. Marek

Starting date: 1.9.2010 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 2932

Summary of 2010 results

Title of the presentation: Quantitative analysis of somatostatin receptors in pituitary adenomas.

Authors: F. Gabalec (1), J. Čáp (1), M. Beránek (2), D. Netuka (3), V. Masopust (3), T. Česák (4), M. Drastíková (2), J. Marek (5)

Fac. Med., Charles Univ. and University Hospital, 2nd Department of Internal Medicine, Hradec Králové (1), Fac. Med., Charles Univ., and University Hospital, Department of Clinical Biochemistry and Diagnostics , Hradec Králové(2), Dep. of Neurosurgery, Central Military Hospital, Prague (3), Dep. Of Neurosurgery, Charles Univ. Hospital in Hradec Králové (4), 3rd Dep.of Internal Medicine, 1st Faculty of Medicine, Charles Univ. in Prague

The aim of the study is quantitative analysis of somatostatin receptors in pituitary tumours using real-time PCR. This method could help to chose patients profiting from expensive medical treatment with somatostatin analogues and chimeric compounds and preventing residuum tumour growth.

Actually we are in the phase of collecting pituitary tumors from 3 clinics of neurosurgery – from Hradec Králové and from Central Military Hospital in Prague and newly Department of Neurosurgery joined us.. Until now, we have received 32 specimens during 4 months. Pituitary tissue mRNA isolation from stabilization solution – RNAlater by Trizol reagents was successfully performed and PCR reaction was optimalized for somatostatine receptor sst2 and 5. Project is supported by Ministry of Health Project No. NT/11344-4/2010

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

Title of the project: Does non-alcoholic fatty liver disease influence course of liver regeneration after partial hepatectomy?

Grant Agency: Charles University ***Project Number:*** 126109

Principal Investigator: T. Garnol

Co-investigators: Z. Červinková, O. Kučera

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 302

Summary of 2010 results

Title of the presentation: Simple steatosis does not impair early phase of liver regeneration after partial hepatectomy in rats.

Authors: T. Garnol, O. Kučera, P. Staňková, H. Lotková, T. Roušar, Z. Červinková
Fac. Med., Charles Univ., Hradec Králové: Dept of Physiology.

Non-alcoholic fatty liver disease (NAFLD) is a common hepatic disorder which is characterized by fat accumulation in the liver and can progress to fibrosis, cirrhosis or even to liver cancer. Regeneration is a fundamental response of the liver to toxic injury or reduction of liver tissue by partial hepatectomy. There is still lack of data concerning regeneration of the liver affected by NAFLD. To elucidate the ability of steatotic liver to regenerate could have important impact for clinical practice, namely for decisions associated with liver transplantation. The aim of our study was to evaluate whether steatosis affects early phase of liver regeneration after partial hepatectomy (PH) in rats. In concordance with plan, we extended our previous study. Male Sprague-Dawley rats were fed ad libitum a standard pelleted diet (ST-1, 10% of energy from fat) and high-fat gelled diet (HFGD, 71% of energy from fat) for 6 weeks and then 2/3 liver resection was performed. Animals were sacrificed 24, 48 and 72h after PH, control rats were subjected to sham operation (laparotomy) and sacrificed in the same intervals after the operation. In sera activities of ALT, AST, and concentration of bilirubin, glucose, urea, triacylglycerols and cholesterol were measured. Malondialdehyde (MDA) content in the liver (HPLC) and selected tissue cytokines (IL-6, TNF-α, ELISA) were assessed; histopathological samples were prepared (H+E, Sudan III). Respiration of isolated mitochondria was measured by high-resolution respirometry (Oxygraph 2K Oroboros). The extent of regeneration was evaluated by measurement of liver weight, liver DNA content and incorporation of bromodeoxyuridine (BrdU). Each group consisted of 6 animals, the statistical significance was analyzed using one-way ANOVA followed by Tuckey's post hoc test (GraphPad Prism 4.03, USA), $p < 0.05$ was considered as significant. Feeding with HFGD for 6 weeks caused in Sprague-Dawley rats liver steatosis without pronounced inflammatory signs. We found a significant decrease in serum ALT activity in HFGD group in comparison with ST-1 group ($p < 0.05$, 24 h after PH), as well as decrease of urea concentration in all intervals after PH ($p < 0.05$; $p < 0.001$). PH caused significant elevation of liver TNF-α and IL-6 in both control and HFGD groups. This increase was more pronounced in steatotic livers. PH-induced regeneration of the liver with simple steatosis was not significantly affected as documented by liver weight, DNA content in the liver and above all by incorporation of BrdU.

Project was supported by the Charles University Grant Agency No. 126109 and MSM 0021620820.

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Title of the project: Myocardial damage induced by anticancer drugs and ischemia-reperfusion: new possibilities of pharmacological cardioprotection.

Grant Agency: Czech Republic ***Project Number:*** 305/09/0416

Principal Investigator: V. Geršl

Co-investigators: M. Štěrba, O. Popelová, E. Jirkovský, Y. Mazurová, M. Adamcová, T. Šimůnek, A. Vávrová, K. Vávrová, J. Stulík, J. Neckář, F. Kolář, O. Szárszoi

Starting date: 1.1.2009 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6276

Summary of 2010 results

Title of the presentation: Study of mechanisms involved in cardiotoxicity induced by anthracyclines and tyrosine kinase inhibitors.

Authors: Eduard Jirkovský (1), Olga Popelová (1), Martin Štěrba (1), Yvona Mazurová (2), Michaela Adamcová (3), Tomáš Šimůnek (4), Anna Vávrová (4), Petr Nachtigal (5), Kateřina Vávrová (6), Jiří Stulík (7), Jan Neckář (8), František Kolář (8), Vladimír Geršl (1).

Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Histol. Embryol. (2), Dept. of Physiol. (3), Fac. Pharm., Charles Univ., Hr. Králové: Dept. of Biochem. Sci. (4), Dept. of Biol. Med. Sci. (5), Dept. of Inorg. Organic Chem. (6), Fac. Milit. Health Sci., Univ. Defence, Hr. Králové: Dept. of Mol. Pathol. (7), ASCR, Prague: Inst. of Physiol. (8).

In relationship to previous proteomic results, the status of ubiquitin-proteasome system was investigated in the myocardium chronically exposed to daunorubicin (DAU). In line with these findings, only trypsin-like activity was increased due to the treatment and at the same time significant increase in polyubiquitinated proteins was found. Furthermore, an activation of the calpain system was described. We have also investigated potentially immunohistochemically detectable markers of endothelial dysfunction in aortas and femoral arteries of rabbits exposed to different cumulative doses of DAU. No morphological or immunohistochemical abnormalities due to the treatment were detected, which suggests that the arterial endothelium is significantly more resistant to DAU toxicity then the myocardium. A comparison of cardioprotective effects of dexrazoxane (DEX, 60 mg/kg, i.p.) administered with every single dose of DAU and when added to DAU since cumulative dose 300 mg/m^2 was achieved. Both approaches prevented DAU-induced premature mortality (0 vs. 36%), but unlike regular schedule, delayed DEX administration failed to preserve cardiac function and prevent significant troponin T rise. Interestingly, although DEX, sobuzoxane and merbarone were found to protect cardiomyocytes *in vitro* from anthracycline toxicity, they failed to protect the cells from model oxidative stress induced by H_20_2. In addition, investigation of *in vitro* and *in vivo* sunitinib toxicity, synthesis of DEX derivatives and effects of DEX on myocardial infarction size were performed.

The project was supported by the Grant GA CR No. 305/09/0416.

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Title of the project: Neoadjuvant chemoterapy in brest carcinoma

Grant Agency: Ministry of Health ***Project Number:*** NS/10373-3

Principal Investigator: J. Grim

Co-investigators: H. Kalábová, J. Petera, S. Mičuda, E. Brčáková, G. Kolouchová, L. Fuksa, A. Ryška, H. Hornychová, J. Laco, E. Hovorková, P. Jandík, H. Klozová-Urminská, H. Tobková

Starting date: 1.8.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 8000

Summary of 2010 results

Title of the presentation: Neoadjuvant chemoterapy in brest carcinoma

Authors: J. Grim, H. Kalabova, J. Petera, S. Micuda, E. Brcakova, G. Kolouchova, L. Fuksa, A. Ryska, H. Hornychova, J. Laco, E. Hovorkova, P. Jandik, H. Klozova-Urminska, H. Tobkova

Fac. Med. and Hosp., Charles Univ., Hr. Kralove, Dept. of Clinical Oncology and Radiation Therapy

Neoadjuvant chemotherapy is routinely used for operable locally advanced breast cancer. Clinically useful markers predicting response of cancer cells to cytostatics are however lacking. Thus we hypothesized that by using clinical and molecular staging parameters, a novel measure of determining treatment response in patients may be established. Prospective evaluation was performed in 50 patients receiving standard anthracycline/taxane-based neoadjuvant therapy to test for associations with pathological complete response (pCR) as a major predictor of disease-free survival. Expression analysis of hormone receptors (HR) and humand epidermal growth factor receptor 2 (HER2) was performed using immunohistochemistry of core-needle biopsies obtained before treatment. Additionally, molecular data obtained before therapy were compared with those in tumors remaining after chemotherapy. Ten (20 %) of 50 patients reached pCR of the primary tumor after treatment. pCR rates were significantly different between the biology-based tumor types (P = 0.03) with HR-/HER2- and HR+/HER2+ tumors having higher pCR than HR+/HER2- tumors. The comparison of the pretreatment biopsy and the tumor excised after chemotherapy showed increased expression of Topoisomerase IIa in tumors with only partial remission. Our data demonstrate that there is association between expression of selected antigens in initial tumor samples and outcome of the doxorubicin/taxane neoadjuvant regimen.

This study was supported by grant from the Ministry of Health of the Czech Republic No. NS/10373-3/2009.

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Title of the project: 5-fluorouracil dose individualization in patients with colorectal cancer

Grant Agency: Ministry of Health ***Project Number:*** NS/9693-4

Principal Investigator: J. Grim

Co-investigators: J. Martínková, J. Chládek, M. Hroch, O. Slanař

Starting date: 1.7.2008 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 7762

Summary of 2010 results

Title of the presentation: Safety profile of 5-FU exposure a neoadjuvant treatment of colorectal cancer

Authors: M. Hroch[1], J. Grim[2], J. Chládek[1], J. Martínková[1],
1)Faculty of Medicine Hr. Králové, Charles University in Prague: Dept. of Pharmacology
2)Teaching Hospital Hr. Králové: Clinic of Oncology

Introduction: Study focuses on kinetically guided individual dosage prediction based on plasma concentrations. The first step was to develop the analysis of 5-FU in plasma samples, the second was to establish the safety profile reflecting the dosage and drug plazma concentration.

Method: Detail pharmacokinetic profile of 5-FU was evaluated in 17 patients together with safety evaluation to establish the target drug exposure (area under the curve of plazma conctration - AUC and concentration in steady state - Css) during the treatment.

Results: The drug pharmacokinetic is linear over the dosage from 200mg/m2day to 1000mg/m2/day. The safety profile correlated with plasma conctration and drug exposure. Now, it is possible to define the safety target drug exposure during the neoadjuvant (preoperative) chemotherapy.

The next step is to evaluate the safety and efficacy (pathologic complete response – pCR) of individualized 5-FU exposure in two cohorts:

1. patients with standard dosage (i.e. 300mg/m2/day D1-5) with known pharmacogenomic (enzymatic) profile;
2. patients with individualized dosage (i.e. 300mg/m2/day as starting dose) and then the individualization of given 5-FU amount to reach the target AUC.

Address for correspondence: J. Martínková, Dept. of Pharmacology, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, Hradec Králové, Czech Republic

Title of the project: The study of potential importance of epigallocatechin gallate in the prevention and treatment of the liver injury caused by intrahepatic and extrahepatic cholestasis

Grant Agency: Charles University ***Project Number:*** 132309

Principal Investigator: P. Hiršová

Co-investigators: S. Mičuda, G. Kolouchová, E. Brčáková

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 633

Summary of 2010 results

Title of the presentation: Green tea flavonoid epigallocatechin gallate alters hepatic cholesterol and bile acid processing in normal and cholestatic rats

Authors: P. Hirsova (1), G. Kolouchova (1), E. Brcakova (1), L. Fuksa (1), J. Cermanova (1), R. Hyspler (2), S. Micuda (1)

Charles University in Prague, Fac. of Med. in Hr. Kralove: Dept. of Pharmacology (1), Dept. of Gerontology and Metabolic Care (2)

Epigallocatechin gallate (EGCG) was shown to have a cholesterol lowering effect in rats due to increased fecal excretion of cholesterol and bile acids (BAs). However, there is no information on how EGCG affects their biliary excretion (BE). Thus, we evaluated the effect of EGCG on cholesterol and BA homeostasis in normal and ethinylestradiol (EE)-treated rats, in which altered metabolism of cholesterol and BAs has been described. Rats were treated with EGCG (50 mg/kg, 8d), EE (5 mg/kg, 5d), a combination of EE and EGCG, or respective vehicles (controls). At the end of treatment bile was collected to assess BA and cholesterol BE. Relevant proteins were examined by Western blot. EGCG administration to rats increased BAs in plasma, and reduced their BE and bile flow (by 23%, $P<0.05$); while levels of plasma cholesterol were retained, its BE was enhanced by 83% ($P<0.01$). These effects were associated with increased expression of Sr-b1 and decreased levels of Acat-2, Mdr2 and Mrp2 ($P<0.05$). EE treatment diminished bile flow (by 56%, $P<0.001$), BE of BAs, and plasma cholesterol, but maintained cholesterol BE compared to controls. Simultaneously, EE reduced protein levels of Ntcp, Bsep, Mrp2, Mdr2, Oatp1a4 and Sr-b1, and increased Acat-2 and LDL receptor ($P<0.001$). EGCG pretreatment to EE-treated rats resulted in a reduction of plasma cholesterol with unchanged BA plasma concentration, bile flow, and BE of cholesterol and BAs compared to EE group. Therein, the expression of Mrp2 and Acat-2 declined and those of Ntcp and Mrp4 increased ($P<0.05$). A positive correlation was found between BE of BAs and the expression of liver transporters Ntcp, Mrp2, Bsep, Mdr2, Oatp1a1 and Oatp1a4 ($R>0.6$, $P<0.001$). A relationship was also detected between cholesterol BE and Sr-b1, Mdr2 ($R>0.6$, $P<0.001$), and Acat-2 ($R<-0.66$, $P<0.001$). EGCG pretreatment partially prevented EE-induced rise in liver cholesterol content and liver weight, which correlated positively with Acat-2 and LDL receptor expression ($R>0.7$, $P<0.001$) and negatively with Sr-b1 and HMG-CoA reductase expression ($R<-0.68$, $P<0.001$). This study has demonstrated the ability of EGCG to alter liver cholesterol and BA processing. Changes in the expression of relevant enzymes and transporters may imply physiological consequences of using EGCG-rich dietary supplements.

Supported by the Charles University Grant Agency, No 132309.

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Title of the project: MIO vs. ORIF of intraarticular calcaneal fracture treatment.

Grant Agency: Charles University ***Project Number:*** 95708/2008 C

Principal Investigator: T. Holeček

Co-investigators: D. Šimkovič, T. Dědek

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

Total funds allocated for project - Kč (thousands): 317

Summary of 2010 results

Title of the presentation: MIO vs. ORIF of intraarticular calcaneal fracture treatment.

Authors: MUDr. Tomáš Holeček, Doc. MUDr. Dušan Šimkovč, CSc.,
MUDr. Tomáš Dědek, Ph.D.

Aim of study: to confirm the hypothesis, if it is possible to treat intraarticular fractures (fx) of calcaneus using two different method of treatment in accordance of gravity of injury.

Matherial and method: prospective clinical trial; two groups of patients (simple fx, complex fx) according to gravity of injury of calcaneus; grading protokol (morfology of fracture, type of injury of posterior facette of subtalar joint and dimension od bone defect of calcaneal body) assesses the gravity of injury. Minimally invasive osteosynthesis (MIO) shall be used for simple fractures treatment, open reduction and internal fixation (ORIF) for complex fracures treatment. Evaluation of outcomes after 6,12,48 months - check anatomical, clinical, functional and patient reported outcomes.

Outcomes: There was 95 patients (103 fx) included in the study, MIO 85x (82,5%), ORIF 16 (15,5%), indiv. treatment 2 (1,9%); 49 full controlled patiens (51,6%), MIO group 39, ORIF group 8; follow up ∅ 13,2 months. *Anatomical outcomes:* step on the posterior facette of subtalar joint < 2 mm 17 (43,6%) in MIO group, 5 (62,5%) in ORIF group. *Clinical outcomes:* primary malreduction 7x (18%), nonunion 1x (2,6%), reoperations 8x in MIO group. Deep infection 1 with reoperation (12,5%), wound edge necrosis (12,5%) in ORIF group; *Fuctional outcomes:* Deficit of movement: MIO group: eversion Ø 5,2° 10x (25,6%), inversion Ø 7,1° 17x(43,6%); ORIF group: eversion Ø 4° 3x(37,5%), inversion Ø 7,6° 4x (50%).

Patient reported outcomes: Kerr's test: excellent and good 21 (54%) in MIO group; 5 (62,5%) in ORIF group; OAS: excellent and good 19 (48,7%) in MIO group; 4 (50%) in ORIF group.

Conclusion: The aim of study was not realise yet, because there is low rate of completetted outcomes. We expect 70-75% of completted outcomes to deadline of final report. They will be publish in Czech journal with impact factor. In the end of 2011there all outcomes will be completted and publish in foreign language journal.

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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 IV

Grant Agency: Ministry of Health ***Project Number:*** NS/10363-3

***Principal Researcher*:** P. Hůlek

Joint Researchers: A. Krajina, J. Fajfrová, V. Šafka, M. Holeček, S. Mičuda, T. Fejfar, V. Koblížek, L. Hosák, V. Jirkovský, J. Cyrány, M. Hůlková, P. Hlúbik

Starting date: 1.8.2009 ***Duration (years):*** 3

Funds allocated for project - total in Czech crowns: 1311

Summary of 2010 results

Title of the presentation: Hemodynamic, clinical and biochemical monitoring of patients before and after transjugular intrahepatic portosystemic shunt (TIPS), part IV
Ministry of Health, NS/10363-3

Authors: [1]Petr Hůlek, [2]Antonín Krajina, [6]Jana Fajfrová, [3]Václav Šafka, [3]Milan Holeček, [4]Stanislav Mičuda, [1]Tomáš Fejfar, [1]Vladimír Koblížek, [5]Ladislav Hosák, [1]Václav Jirkovský, [1]Jiří Cyrány, [5]Michaela Hůlková, [6]Pavol Hlúbik,
[1]Dept. of Internal Medicine, [2]Dept. of Diagnostic Radiology, [3]Dept. of Physiology, [4]Dept. of Pharmacology, [5]Dept. of Psychiatrics - Charles University in Prague, Faculty of Medicine & University Hospital in Hradec Králové, [5]Dept. of Hygiene, University of Defence, Faculty of Military Health Sciences, 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 three actual problems of patients suffering from liver cirrhosis and portal hypertension: pulmonary dysfunction, hepatic encephalopathy and disruptions in metabolism. It continues with the topic of hepatopulmonary syndorm focusing on its pathogenesis and eventual impact of TIPS. It looks for new factors influencing the precipitation of encephalopathy in connection with portosystemic shunt (TIPS). And it studies the changes of overall metabolism in cirrhosis and its impact on prognosis. Methods consist in catheterization measurement of the parameters of pulmonary and portal circulation and clinical evaluation of respiratory functions, plasmatic levels of selected hormones and mediators in specific compartments of circulation, modern psychometric tests, SPECT of brain, gene olymorphism, investigation of the body composition and metabolic rate, glucose metabolism, tests of liver function and of bacterial intestinal overgrowth..

The year 2010 was the second year of the project. We continued in practice of the extremely complex study protocol, adding further patients to the study. We started laboratory analyses of series of specimens, especially for some mediators (big-ET-1) and arrays of cytokines. We also completed some follow-up data of our patients and successfully published them in foreign prestigious journal. The data of cytokine analysys we have presented at prestigious AASLD Liver Meeting 2010.

Address for correspondence: Petr Hůlek, 2nd Dept. of Internal Medicine, University Hospital, 500 05 Hradec Králové, Czech Republic; hulek@fnhk.cz

Title of the project: Hemodynamic, clinical and biochemical changes before and after transjugular intrahepatic portosystemic shunting (TIPS), part IV

Grant Agency: Charles University ***Project Number:*** 73809

Principal Investigator: M. Hůlková

Co-investigators: L. Hosák, T. Fejfar,

Starting date: 9.4.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 294

Summary of 2010 results

Title of the presentation: Hemodynamic, clinical and biochemical changes before and after transjugular intrahepatic portosystemic shunting (TIPS), part IV

Authors: M. Hulkova, L. Hosak, V. Safka

The aim of the project is to establish how to predict hepatic encephalopathy, which appears after TIPS, using the SPECT (single photon emission computed tomography). Selecting patients at high risk of encephalopathy before TIPS could specify the indications of TIPS. There are commonly used alternatives of TIPS like repeated ascites punctions.

Till now, eighteen patients were included into the study. Two patients died and one patient broke the following - up. In twelve patients there was a hepatic encephalopathy present before TIPS. 66% patients showed new or worsening encephalopathy after TIPS. Hepatic encephalopathy got worse in both of patients who died later on.

To continue sampling data this year is necessary to get sample suitable for statistical analysis.

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Title of the project: Noninvasive monitoring of hepatotoxicity in the course of pharmacokinetically-guided dosing of oral methotrexate and folic acid to psoriasis patients: A two-year prospective study.

Grant Agency: Ministry of Health ***Project Number:*** NS/10364-3

Principal Investigator: J. Chládek

Co-investigators: M. Šimková, J. Vaněčková, P. Hůlek, J. Vávrová, M. Hroch

Starting date: 1.8.2009 ***Duration (years):*** 2,5

Total funds allocated for project - Kč (thousands): 4528

Summary of 2010 results

Title of the presentation: A prospective study evaluating biomarkers of hepatotoxicity in the course of pharmacokinetically-guided dosing of oral methotrexate to psoriasis patients.

Authors: J. Chládek (1, 2), M. Šimková, J. Vaněčková (3), P. Hůlek (4), J. Vávrová (5), M. Hroch (1). Faculty of Medicine, Charles University, Hradec Králové: Dept. of Pharmacology (1), Medical Biochemistry (2), Dermatovenereology (3) Internal Medicine (4) and Clinical Biochemistry and Diagnostics (5)

The major problem of systemic anti-psoriatic therapy with low-dose oral methotrexate (LDMTX) is to attain long-term efficacy with an acceptable toxicity profile. Because of the risk of hepatic fibrosis, frequent evaluation of liver function tests and periodic liver biopsy are recommended during therapy. Non-invasive methods of monitoring the risk of liver fibrosis are being used increasingly. These methods include either biochemical tests (Fibrotest, hyaluronic acid - HA, N-terminal pro-peptide of collagen type III - PIIINP etc.), ultrasound imaging (Fibroscan) or magnetic resonance elastography. A prospective two-year study (Eudra CT 2009-015403-95) involves psoriasis patients randomized into treatment arms A (patients starting LDMTX), B (biologic drugs) and C (long-term users of LDMTX). Dosing of LDMTX to the patients from the arm A is guided pharmacokinetically. Interim results at 6 months are as follows. Erythrocyte MTX of patients in the arm A gradually accumulated until a steady-state concentration of 180±65 nM, while that of patients in the arm C was stable (120±51 nM). Erythrocyte folate levels of patients in all three study arms were comparable. The values of the Fibrotest score were comparable between groups but displayed an increasing trend in the arm A from 0,21±0,05 to 0,28±0,09. Other biomarkers of hepatic fibrosis (HA and PIIINP) showed no elevation and were comparable in all study arms. The simultaneous use of the pharmacokinetic parameters and biochemical tests as biomarkers for the therapeutic and/or adverse effects of LDMTX can improve the results of psoriasis treatment. Monitoring of fibrosis biomarkers may help to increase the number of long-term users of LDMTX. The study requires an increase in its size and future evaluations after higher cumulative doses of MTX. Supported by IGA NS10364-3/2009.

Address for correspondence: Dept. of Pharmacology and Dept. of Medical Biochemistry, Charles University in Prague, Faculty of Medicine and Hospital in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Noninvasive methods for evaluation of exhaled nitric oxide as a biomarker of airway inflammation in children with allergic rhinitis and bronchial asthma.

Grant Agency: Ministry of Health ***Project Number:*** NS/9692-3

Principal Investigator: J. Chládková

Co-investigators: J. Chládek, M. Šenkeřík, T. Chyba

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

Total funds allocated for project - Kč (thousands): 2248

Summary of 2010 results

Title of the presentation: Exhaled nitric oxide (NO) in children with allergy and asthma: modelling aproaches used to quantify bronchial and alveolar outputs of NO.

Authors: J. Chládková (1), M. Šenkeřík (1), I. Krčmová (2), J. Chládek (3,4). Faculty of Medicine and Hospital, Charles University, Hradec Králové: Dept. of Pediatrics (1), Clinical Immunology ans Allergology, Pharmacology (3) and, Medical Biochemistry (4).

In order to estimate flow-independent parameters alveolar concentration (Calv) and bronchial flux of NO (Jaw), models of NO exchange are used to analyze the relationship between the fractional concentration of NO in the exhaled air (FENO) and the expiratory flow. In the present study, two computational approaches were compared and, the optimum range and adequate number of expiratory flows were sugested for the determination of Calv and Jaw, respectively. Duplicate measurements of FENO were performed using a chemiluminiscence analyzer at flow rates of 50, 100, 150, 200, and 250 ml/s in 79 children and adolescents (36 girls, aged 13±3.1 yr) with allergy (n=20) and/or mild to moderate-severe asthma treated with inhaled corticosteroids (n=59). Calv and Jaw were estimated using linear regression analysis of the FENO vs. (1/flow) (method P) and, (flow times FENO) vs. flow (method T) relationships. Similar for both methods, Calv decreased and Jaw increased if expiratory flows moved towards higher values. At the same time, standard errors (SE) of the estimates increased. Using four flows in the optimum range of 100-250 ml/s, the medians (inter-quartile ranges) for Calv (ppb) and Jaw (pl/s) were 3.0 (1.3-4.3) and 1130 (631-1800) for method P and, 2.6 (1.3-4.0) and 1240 (644-1812) for method T, respectively. The median SE of estimates for Calv and Jaw (%) achieved 26.1 and 8.6 (method P) and, 26.2 and 10.9 (method T). Regardless of the method, there was a good agreement between the values obtained using four flows within the range of 100-250 and two flows (100+250), respectively. Both methods showed similar performance. Albeit statistically significant ($P<0.001$), the differences in the estimates for Calv and Jaw are unlikely of clinical importance. The optimum number and range for expiratory flows are four and 100-250 ml/s, respectively. Supported by IGA NS96923/2008.

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

Grant Agency: Ministry of Health ***Project Number:*** NS/10353-3

Principal Investigator: R. Ivančaková

Co-investigators: Z. Broukal

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2568

Summary of 2010 results

Title of the presentation: Alimentary fluoride intake in children aged 4-5 years.

Authors: R. Ivančaková (1), Z. Broukal (2), E. Oganessian (2), E. Lenčová (2)
Fac. Med., Charles Univ.: Dept. of Dentistry, Hradec Králové (1), Institute of Dental Res., GFH, Prague (2).

The aim of the study was the evaluation of circadian alimentary intake of fluoride in 4 – 5 years old children. Circadian alimentary intake of fluoride was estimated in the group of 18 children of the mean age of 4. 75 years and of 20. 69 kg body weight by means of the double plate method in two stages within 6 months. Parents were asked to record the 24 hrs diary and collect duplicated portions of food and beverages taken in this time interval by children. Pooled samples of food and beverages were weighed and samples of solid food were homogenized. Fluoride from food samples was extracted by the micro diffusion hexadecyldisiloxane + perchloric acid facilitated method. The contents of extracted fluoride from solid food and fluoride in beverages was measured potentiometrically by means of fluoride ion sensitive electrode. Fluoride concentrations in mg/l has been calculated according the calibrated values of NaF in concentrations 0.02, 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 mg F/l. The accuracy of the fluoride extraction method was tested on the instant formula Sunar Complex Premium HERO (0.5 mg F in 100 mg powder). The mean circadian fluoride intake in the first stage of study amounted to 0.274 (0.144-1.168) mg/day, in the second stage 0.330 (0.195-1.340) mg/day, which in the recalculation to mg fluoride/kg of bw/day amounted to 0.014 (0.008-0.049), resp. 0.015 (0.010-0.054). The calculated fluoride intake reached the bottom of the optimal fluoride intake threshold 0. 05-0. 07 mg/kg bw/day. When adding the estimate of the possible intake of fluoride from swallowed fluoride tooth paste the total intake has already fully fallen into the rank of the intake threshold. Results substantiate the caution when indicating fluoride supplements and their dosage schedule among pre-school children.

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Title of the project: Proteomic identification of biomarkers of intraamniotic inflammation in amniotic fluid in preterm birth patients

Grant Agency: Ministry of Education ***Project Number:*** ME10025

Principal Investigator: M. Kacerovský

Co-investigators: J. Tošner, J. Lenčo, M. Link, V. Tambor, H. Hornychová

Starting date: 1.1.2010 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2762

Summary of 2010 results

Title of the presentation: Proteomic identification of potential novel biomarkers of intraamnial inflammation in amniotic fluid of preterm birth patients

Authors: M. Kacerovský (1), J. Lenčo (2), V. Tambor (2), M. Link (2)
University Hospital Hradec Králové, Dept. Obstretrics and Gynaecology (1), Univ. Defence, Fac. Military Health Sci. Inst. Molecular Pathology (2)

We have used sophisticated proteomics to identify novel biomarkers of intraamniotic infection and inflammation (IAI) in amniotic fluid of preterm birth patients with intact membranes. These were collected by transvaginal amniocentesis at Perinatal Research Center, Nashville, TN, USA. Totally 64 amniotic fluid samples were classified into two groups according to clinical outcome - patients with confirmed IAI were considered as a positive group, whereas patients with ruled out IAI were taken as a negative control group. In the spring of 2010 we were ready to pursue the first phase of the analysis – the discovery phase. Amniotic fluid samples were assayed in order to determine the total protein content using both BCA and MicroBCA and based on these data, pooled samples of the respective patients groups were created in duplicates. These were subjected to immunoaffinity depletion in order to remove high abundance proteins, which would mask interesting low abundance proteins during analysis. The depleted samples were then digested using trypsin and tagged using iTRAQ labels, which enable simultaneous quantification of individual peptides across samples. The labeling step also enabled combining of the four samples and subsequent processing as a single one. Due to the complex protein composition of amniotic fluid, we decided to employ a novel CysTRAQ approach, which enables peptide fractionation based on presence of cysteine in the peptide sequence, which results in substantial complexity reduction. This in turn leads to a higher number of identified proteins, which are also more precisely quantified. Both cysteinyl and non-cysteinyl peptides were fractionated to 36 fractions using C18 HPLC in basic conditions. These fractions are now ready for nanoHPLC-MALDI-TOF/TOF proteomic analysis.

This project was supported by Ministry of Education, Grant No. ME10025

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Title of the project: Morphological changes of the fetal thymus and adrenal glands on ultrasound as a marker the systemic fetal inflammatory response syndrome

Grant Agency: Czech Republic ***Project Number:*** 304/09/0494

Principal Investigator: M. Kacerovský

Co-investigators: C. Andrýs

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 2169

Summary of 2010 results

Title of the presentation:

Authors: M. Kacerovský

The main goal of our work was to determine sonographically the transverse diameter of the fetal thymus and present nomogram for the transverse diameter of the fetal thymus in uncomplicated singleton pregnancies between 19 and 38 weeks of gestation. The transverse diameter of the fetal thymus was measured by the one experienced examiner in 198 healthy fetuses between 19 and 38 weeks of gestation. The transverse diameters of the fetal thymus were obtained 183 of the 198 subjects. The regression equation was expressed as a function of gestational age: the transverse diameter of the fetal thymus (mm) = 1.001 x gestational age (week) – 0.932 or 0.143 x day – 1.34. Both the correlation coefficients, r = 0.91 for weeks and r = 0.92 for days were found to be highly statistically significant ($p <$ 0.0001). We created normative data (mean, 5^{th} and 95^{th}) for the ultrasound measurements of the transverse diameter of the fetal thymus in healthy singleton pregnancies.

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Title of the project: Identification of biomarkers of intraamniotic inflammation and the systemic fetal response syndrome in amniotic fluid: proteomic approach

Grant Agency: Ministry of Health ***Project Number:*** NS/10382-3

Principal Investigator: M. Kacerovský

Co-investigators: J. Tošner, J. Lenčo, M. Hubálek, V. Tambor, C. Andrýs, M. Drahošová, P. Calda, M. Břešťák, R. Vlk

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 7 064

Summary of 2010 results

Title of the presentation: Proteomic identification of intraamniotic inflammation biomarkers in amniotic fluid: discovery phase

Authors: M. Kacerovský (1), J. Lenčo (2), V. Tambor (2), M. Link (2)
University Hospital Hradec Králové, Dept. Obstretrics and Gynaecology (1), Univ. Defence, Fac. Military Health Sci. Inst. Molecular Pathology (2)

In order to find new potential biomarkers of intraamniotic infection/inflammation we have been collecting amniotic fluid samples by transabdominal amniocentesis from women with preterm premature rupture of the membranes since 2009 at the Dept. Obstretrics and Genaecology, University Hospital Hradec Kralove. In 2010, we reached sufficient number of samples to initiate the discovery phase of the proteomic analysis. This part of the project included 19 positive and 19 negative samples, which were divided into groups based on results from cultivation, PCR, IL-6 assay and histological outcomes. For the discovery proteomic analysis, the samples were pooled and depleted from 14 most abundant proteins. After protein digestion by trypsin, the resulting peptides were labeled by iTRAQ tags allowing relative quantitation of proteins. To get deep into the proteome, we used multilevel-separation strategy, which resulted in fractionation of the peptides into 28 well-separated fractions. Up to date, by analysing 10 fractions we have identified and quantified 682 unique proteins (95% confidence), 109 of which showed significantly dysregulated protein abundance between positive and negative samples. By a quick comparison of our results with publicly available data we observed that the majority of differently abundant proteins has not yet been published, which is very promising in the light of the project aim. On the other hand, we have also identified several proteins previously showed to have elevated concentration in the presence of intraamniotic infection. Hence, these results proved the design of the discovery phase to be correct albeit performed on pooled samples.
This project was supported by Ministry of Health, Grant No. NS/10382 - 3

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Title of the project: Genome Wide Search Leading to Preterm Birth in Visegrad Four and Neighboring Countries

Grant Agency: International Visegrad Found ***Project Number:*** 20920001

Principal Investigator: M. Kacerovský

Co-investigators:

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 140

Summary of 2010 results

Title of the presentation:

Authors: M. Kacerovsky

The main aim of the INTERGENE PTB Network is to increase its research activity in the Visegrad Four (Czech Republic, Hungary, Slovakia and Poland) and also neighboring countries (Croatia, Romania and Ukraine). The partners from Visegrad four summarizing their investigation of existing risks factor linked to preexisting genetic codes. Based on the results the partner will participate in the design of standard treatment protocols for pregnant women are at the risk (secondary intervention). Also contributes to the development of measures for prevention (primary prevention).

The Visegrad partners will implement a clinical research program to make the fundamental for a PhD students. The Visegrad partners agreed that they implement PhD exchange program using the Visegrad Scholarship and Fulbright Foreign Student Program.

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Title of the project: Evaluation of posterior capsule opacification after cataract surgery

Grant Agency: Charles University ***Project Number:*** 103809

Principal Investigator: M. Kalfeřtová

Co-investigators: N. Jirásková

Starting date: 15.5.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 451

Summary of 2010 results

Title of the presentation: AquaLase method and the extent of posterior capsule opacification

Authors: Kalfertova M., Jiraskova N.
Dept. of Ophthalmology, Faculty of Medicine Hradec Kralove, Charles University in Prague and University Hospital Hradec Kralove

Introduction: The aim of this study is to evaluate and compare the extent of posterior capsule opacification (PCO) after torsional phacoemulsification and AquaLase removal of the epithelial cells (right eye) and torsional phacoemulsification without AquaLase using (left eye). For PCO quantification we use two types of software - EPCO 2000 and OSCA.
Methods: In our prospective clinical study we have 50 patients after lens removal for bilateral cataract using torsional phacoemulsification and AquaLase method for cleaning posterior capsule of the right eye. We examine patient 3, 6, 12 and 24 months after surgery, digital retroilumination photographs of the anterior segment, pachymetry, endotelial cells count (ECC) and best visual acuity are obtained. For evaluation of posterior capsule opacification we use EPCO 2000 software (Evaluation of Posterior Capsule Opacification) and OSCA software (Open-Access Systematic Capsule Assessment). The density of PCO by EPCO 2000 software is divided clinically to 4 levels (PCO index), by OSCA system is graded from 1 to 15 (OSCA score).
Results: We have the group of 50 patients. The BCVA is 0.8 and better in the each eye. The ECC and corneal pachymetry results show that the AquaLase method is safe. EPCO results 3, 6 and 12 months after surgery: 0,260 ±0,198; 0,259 ±0,173 and 0,308 ±0,19 (right eye), 0,279 ±0,170; 0,280 ±0,153 and 0,333 ±0,197 (left eye). OSCA results 3, 6 and 12 months after surgery: 0,599 ±0,240; 0,605 ±0,333 and 0,598 ± 0,256 (right eye) and 0,627 ±0,403; 0,635 ±0,360 and 0,629 ±0,328 (left eye).
Conclusions: The AquaLase method is safe for occular tissue. The BCVA is better than 0,8 and improved in all eyes. One year after surgery most cases of PCO is graded as minimal. One patient underwent one year postoperatively Nd-YAG capsulotomy on both eyes.

Supported in part by Charles University Grant Agency No. 103809

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

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

Principal Investigator: G. Kolouchová

Co-investigators: S. Mičuda, E. Brčáková, P. Hiršová

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

Total funds allocated for project - Kč (thousands): 839

Summary of 2010 results

Title of the presentation: Pravastatin modulates differentially iron and cholesterol homeostasis in control and bile duct obstructed rats

Authors: G. Kolouchova (1), E. Brcakova (1), P. Hirsova (1), L. Fuksa (1), J. Cermanova (1), J. Mokry (2), M. Slanarova (3), R. Hyspler (3), S. Micuda (1)
(1) Dept. of Pharmacology, (2) Dept. of Histology and Embryology; Charles Univ., Fac. Med., Hr. Kralove, (3) Dept. of Gerontology and Metabolism, Univ. hospital, Hr. Kralove

Obstructive cholestasis causes changes in the expression of molecules involved in iron homeostasis. Pravastatin may modulate these changes. mRNA level of hepcidin was significantly decreased in BDO (bile duct obstructed) compared with Sh (sham operated) rats but this expression was normalized in BDO-P group (BDO with 1 mg/kg pravastatin administration). Expression of ferritin, ferroportin and transferrin receptor 1 was increased in BDO-P to 180%, 650% and 380% compared with Sh. Hypercholesterolemia is a typical consequence of cholestatic liver diseases. Pravastatin demonstrated ability to attenuate this symptom but detailed information is lacking. We observed a significant increase in liver cholesterol content in BDO rats and in triglyceride liver content in BDO, BDO-P and Sh-P (Sh with pravastatin administration) groups compared with Sh. mRNA levels of LDL receptor increased in BDO and Sh-P groups to 160% and 180% and the expression of Mdr2 increased to 230% and 270% in BDO and BDO-P compared with Sh. Expression of Abcg5/8 were significantly decreased to 10% compared with Sh. Expression of Abca1, Sr-b1and HMG-CoA reductase remained unchanged. Biliary excretion of cholesterol was significantly decreased by cholestasis. In conclusion, we reported the increased hepatic cholesterol levels and reduced biliary excretion of cholesterol associated with reduction of the Abcg5/Abcg8 mRNA levels in BDO group. Pravastatin administration in healthy animals raised biliary excretion of cholesterol, which can be explained with increased expression of Mdr2. Our results demonstrated that pravastatin can attenuate changes caused by chronic cholestasis in rats.

This study was supported by grants from the Grant Agency of Charles University No. 122 408/2008C and from Ministry of Education No. MSM 0021620820 and SVV-2010-260907.
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Title of the project: Mechanisms of antiproliferative and cytotoxic effects of sodium selenite in colorectal carcinoma cells with differing p53 status

Grant Agency: Charles University ***Project Number:*** 129609

Principal Investigator: V. Králová

Co-investigators:

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 291

Summary of 2010 results

Title of the presentation: Selenite-induced cell death in human colon cancer cells

Authors: Věra Králová, Emil Rudolf
Department of Medical Biology and Genetics, Faculty of Medicine in Hradec Králové, Charles University in Prague

Selenium compounds have been shown to play role in chemoprevention of tumours. Sodium selenite was reported to inhibit proliferation and induce different types of cell death in cancer cells in vitro. In our study we tested the effects of sodium selenite on cell proliferation and cell death in human colon cancer cell line HCT 116. Cell proliferation was measured by xCELLigence impedance method. Cell morfology was followed by time-lapse videomicroscopy. Caspase activity and mitochondrial membrane potential were determined by flow cytometry. Activation of DNA damage response was detected using western blotting and immunofluorescence. Sodium selenite at concentration of 10 μM induced cell death in HCT 116 cells characterised by cell detachment and rounding, decrease in mitochondrial membrane potential and activation of caspases. Selenite-induced cell death was prevented with antioxidant MnTMPyP and enhanced by depletion of reduced glutathion with BSO, which suggests role of oxidative stress. The process was accompanied by increase in ATM kinase and histone H2A.X phosphorylation.

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Title of the project: Regulatory subsets Treg, TH17, and TLR receptors as putative biomarkers in immunopathological disorders Sjögren´s syndrome and psoriasis

Grant Agency: Charles University ***Project Number:*** 260906

Principal Investigator: J. Krejsek

Co-investigators: I. Berglová, K. Kondělková, E. Flídrová, G. Onofre Arce

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 588

Summary of 2010 results

Title of the presentation: Regulatory subsets Treg, TH17, and TLR receptors as putative biomarkers in immunopathological disorders Sjögren´s syndrome and psoriasis

Authors: : J. Krejsek, prof., I. Berglova, K. Kondelkova, E. Flidrova, G. Onofre Arce

The study group of Sjögren´s syndrome patients has been established based on careful clinical examination. The panel of representative surface markers on lymphocytes and separately on B cells on monocytes has been selected. Tregs cells are followed using flow cytometry. Samples of serum, plasma, and salivary fluid are stored.
Manuscript of article entitled “B cell Toll-like receptors with respect to the pathogenesis of Sjőgren’s syndrome” has been sent for consideration of publication in Acta Medica.

Selected parameters of inflammation are measured in amniotic fluid of pregnant females with premature rupture of maternal membranes to identify putative biomarkers of intraamniotic infection.
Manuscript of article entitled “The role of innate immunity in the pathogenesis of intraamniotic infections in pregnancies complicated by premature rupture of maternal membranes” is accepted for publication in Cs. Gynekol (in Czech).

The expression of PRR receptors CD163 and TLR2 was investigated in peripheral blood of patients with psoriasis undergoing Goeckerman therapy using 4-colors flow cytometry (manuscript in preparation).
Tregs T cells were investigated in peripheral blood of patients with psoriasis undergoing Goeckerman therapy using 4-colors flow cytometry (manuscript in preparation).

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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. Bartůňková

Co-investigators: J. Krejsek, P. Kuneš, V. Lonský, J. Manďák, M. Kudlová, M. Koláčková, C. Andrýs, K. Jankovičová

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

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

Summary of 2010 results

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

Authors: Jan Krejsek

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 immediatelly 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 differencies between „on-pump“ and „off-pump“ patients. (Acta Med., 2007; 50(3): 187-193).

In cardiac surgical patients the expression of activation marker FcgR1 (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).

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Title of the project: Pre-attentional processing of visual information in man: electrophysiological study

Grant Agency: Czech Republic ***Project Number:*** 309/09/0869

Principal Investigator: J. Kremláček

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

Starting date: 1.1.2009 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 1849

Summary of 2010 results

Title of the presentation: Effect of attention load to visual preattentive processing

Authors: J. Kremláček, J. Langrová, M. Kuba, Z. Kubová, J. Szanyi, F. Vit
Fac. Med., Charles Univ., Hr. Králové: Dept. of Pathological Physiology

An electrophysiological manifestation of automatic change detection independent of attention is called a Mismatch Negativity and it was originally described in the auditory domain. Parallel observation was made in the visual modality (vMMN) later on. We showed that motion stimuli, activating the magnocellular subsystem, can also evoke vMMN, however, no proof of the attentional independence was given. The object of this experiment was to explore the motion evoked vMMN while subject is solving different tasks.

The vMMN was elicited by a change in sequence of expanding/contracting radial motions outside of the central 15 degrees of the visual field while subject visually fixated central part of the display. There were three tasks used to modulate the attention involvement: the continuous performance task of two difficulties and a simple center fixation task. Data of 14 subjects recorded from 64 channels at sampling rate 1024 Hz were processed.

The vMMN was identified within the interval 100 - 160 ms and reached its maximum in parieto-central region. Area under curve (AUC) was evaluated for the aforementioned interval and the region of interest. General linear model for repetitive measurement applied to AUC with two factor design showed significant difference ($F(1,13)=20.710$, $p=0.0005$) between standard and deviant conditions, but no significant effect of the task ($F(1,13)=2.202$, $p=0.817$).

We demonstrated that changing attention focused to the central part of the visual field did not significantly influence generation of the vMMN elicited by moving stimuli in the periphery of the visual field.

Supported by Grant Agency of the Czech Republic 309/09/0869.

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Title of the project: Mutation of K-ras gene in carcinogenesis of endometrial carcinoma

Grant Agency: Charles University ***Project Number:*** 157310

Principal Investigator: E. Křepinská

Co-investigators: M. Chmelařová, V. Palička, J. Špaček

Starting date: 1.5.2010 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 259

Summary of 2010 results

Title of the presentation: Mutation of K-ras gene in carcinogenesis of endometrial carcinoma

Authors: E. Křepinská (1), M. Chmelařová (2), V. Palička (2), J. Špaček (1)
Medical Faculty of Charles University and Faculty Hospital Hradec Králové: (1) Department of Obstetrics and Gynecology, (2) Institute for Clinical Biochemistry and Diagnostics

Two types of endometrial carcinoma are distinquished with respect to biology and clinical course: type I- endometroid and type II- non-endometroid /serous, clear cell/ carcinoma.

Molecular data from multiple studies support the hypothesis of different pathway in the development of type I and type II carcinomas. The most frequent genetic alteration in endometroid carcinoma is PTEN inactivation, microsatellite instability and mutation of K-ras and beta-catenin. Mutation of p53 gene is the most frequent alteration in non-endometroid carcinomas. K-ras protooncogene encodes a membrane GTPase and is related to tumor growth and differentiation. Mutations of K-ras gene are present in about 10-30% of endometroid carcinomas, predominantly found in exon 1 (codons 12 and 13).

In our study, PCR analysis was performed to detect K-ras mutation in a group of patients with I. stage endometroid carcinoma and patients with normal endometrium as a control group.

In the present study, we found K-ras mutation in 23% of specimens with endometroid carcinoma and surprisingly in 15% of the control group. K-ras mutations were more frequent in IA stage and grade 1 of endometroid carcinoma. This founding suggests its role as an early event in carcinogenesis of endometroid carcinoma and we hypothetise that it could have positive predictive value.

Literature: Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: evidence for diverse pathways of tumorogenesis. *Hum Pathol* 1995;26:1268–74.
Caduff RF, Johnston CM, Frank TS. Mutations of the Ki-ras oncogene in carcinoma of the endometrium. *Am J Pathol* 1995;146:182–8.

Project was supported by the Charles University Grant Agency, No. 157310.

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

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

Principal Investigator: M. Kuba

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

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

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

Summary of 2010 results

Title of the presentation: Pattern and motion related visual evoked potentials in Neuroborreliosis - detection of therapy effects

Authors: J. Szanyi, Z. Kubová, J. Kremláček, M. Kuba, J. Langrová, F. Vít, J. Szanyi[1], S. Plíšek[1]

Dept. of Pathophysiology - Electrophysiological Laboratory, Charles University - Faculty of Medicine in Hradec Králové, [1] Dept. of Infectious diseases, University Hospital, Hradec Králové, Czech Republic http://www.lfhk.cuni.cz/ELF

In a follow-up study, visual evoked potentials (VEPs) were examined for objective testing of visual functions during treatment courses of Lyme Neuroborreliosis (LNB) in 30 adult patients.
In these LNB patients VEPs to pattern-reversal or motion-onset stimuli were originally delayed and thus they were repeatedly examined within one to eight years during which one or more antibiotic pulse therapy were applied.
Six patients had Lyme Optic Neuritis (ON). VEP recovery to normal latency values was in three of them. In the group of 24 LNB patients without ON, 14 patients displayed prolonged latencies only to motion stimuli and 10 patients had abnormal latencies both in reversal and motion-onset VEPs. During the follow-up period, 7 patients displayed shortening to normal latencies. In 5 patients VEPs latencies improved only partially and in the remaining 12 patients VEPs did not improve at all.
This study provides objective evidence that in LNB majority of patients without clinically manifesting ON display optic pathway involvement - predominantly magnocellular system/dorsal stream function changes. About half of these cases improved after intravenous antibiotic therapy with a relatively long time course of latency shortening. Recovery to normal VEP latencies was more frequently found after repeated therapy.

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

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

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

Principal Investigator: O. Kučera

Co-investigators:

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

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

Summary of 2010 results

Title of the presentation: Rat liver affected by non-alcoholic steatosis exerts higher sensitivity to acute toxic injury induced by acetaminophen

Authors: O. Kučera (1), P. Staňková (1), T. Roušar (1), Y. Mazurová (2), H. Lotková (1), M. Podhola (3), Z. Červinková (1); Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1), Dept. of Histology and Embryology (2), The Fingerland Department of Pathology (3)

Introduction: Although acetaminophen (APAP) overdose is the most common cause of acute liver failure and non-alcoholic fatty liver disease (NAFLD) is one of the most frequent chronic affections of the liver, there is only little evidence demonstrating enhanced susceptibility of steatotic liver to toxic action of APAP. The aim of our project was to assess whether NAFLD sensitizes rat liver to acute toxic effect of APAP.

Methods: Male Sprague-Dawley rats were fed a standard diet (ST-1, 10% kcal fat) and high-fat gelled diet (HFGD, 71% kcal fat) for 6 weeks and then APAP was applied in a single dose (1g/kg body weight). Animals were sacrificed in 24, 48 and 72 hours after APAP administration and then serum biochemistry and hepatic parameters (activities of mitochondrial complexes, reduced glutathione (GSH), triacylglycerol and cholesterol contents, caspase-3 activity, and concentrations of liver cytokines) were measured and histopathological samples were prepared (H+E).

Results: The degree of liver inflammation and hepatocellular necrosis were significantly higher in HFGD animals after APAP administration, compared to ST-1. Serum markers of liver injury were elevated only in APAP treated HFGD fed animals. Concentration of hepatic GSH was decreased in both ST-1 and HFGD groups at 24 h after APAP application. Liver contents of proinflammarory cytokines were not significantly altered, but hepatic transforming growth factor beta1 was elevated in APAP treated HFGD fed rats. We observed APAP-induced changes neither in activities of respiratory complexes I, II, and IV nor in activity of caspase-3.

Conclusion: Livers from rats fed HFGD are more susceptible to acute toxic effect of APAP, compared to non-steatotic liver.

This work was supported by Grants GAČR 305/08/P184 and MSM 0021620820.

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Title of the project: Influence of rheopheresis therapy on retinal function in age-related macular degeneration

Grant Agency: Ministry of Health ***Project Number:*** NS/9738-4

Principal Investigator: H. Langrová

Co-investigators: M. Bláha, E.Rencová, J. Studnička, D. Solichová, V. Bláha, M. Lánská, P. Štěpánková, E. Mistrík

Starting date: 1.7.2008 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6573

Summary of 2010 results

Title of the presentation: Influence of Haemorheopheresis on Drusenoid Retinal Epithelium Detachment in Nonvascular Age-related Macular Degeneration

Authors: Langrová H., Rencová E., Bláha M., Studnička J., Dvořáková H., Bláha V., Lánská M.

Purpose: To evaluate the influence of haemorheopheresis (RHF) on drusenoid retinal pigment epithelium detachment (RPED) in patients with age-related macular degeneration (AMD).

Methods: At baseline, RPED was found in 12 treated patients (23 eyes) and in 18 controls (24 eyes) with the dry form AMD. Patients were treated with 8 RHF procedures (cascade filtration of 1.5 plasma volume) within 10 weeks. The BCVA was tested using ETDRS charts and the RPED-area was measured in mm^2 by fundusphotography using a software Visupac (Zeiss, Jena, Germany). The examinations were performed at baseline and at 2.5-years follow-up.

Results: Baseline BCVA of treated patients was 0.63 (0.06-1.00) and 0.8 (0.08-1.00) in controls. After 2.5-years, BCVA of treated patients remained unchanged, whereas it decreased insignificantly in the control group to 0.63 (0.06-1.00). In treated patients we noticed reattachment of RPED in 8 eyes, statistically significant reduction of its size in 11 eyes, no change in 2 eyes and nonsignificant RPED-enlargement in 2 eyes. Whereas in the control group, we noticed nonsignificant reduction of RPED-size only in 1 eye, no change in 2 eyes a significant RPED-area enlargement in 15 eyes and moreover we detected progression to the wet form of AMD with development of CNV in 6 eyes (25%).

Conclusions: Improvement of rheological parameters after RHF caused a significant reduction and in 34% of eyes even the reattachment of drusenoid RPED. Haemorheopheresis may slow down or even block the unfavourable progress of the disease.

Supported by Grant IGA MH CR NS/9738-4.

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Title of the project: Sequence-Based Association Study Using Biological Features

Grant Agency: Charles University ***Project Number:*** 134609

Principal Investigator: O. Libiger

Co-investigators: M. Červinka

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 169

Summary of 2010 results

Title of the presentation: Statistical analysis strategies for association studies involving rare variants

Authors: O. Libiger, V. Bansal, A. Torkamani, N. J. Schork

The Scripps Translational Science Institute and Department of Molecular and Experimental Medicine, The Scripps Research Institute; 3344 North Torrey Pines Court, Suite 300, La Jolla, CA 92037, USA

Recent whole genome association studies (GWAS) yielded unequivocal statistical associations between a number of common single nucleotide polymorphisms (SNPs) and a variety of diseases. However, these SNPs only account for a small proportion of the incidence of the associated disease. Possible explanations are that structural variants or rare single nucleotide variants not captured by the variation assayed in the GWAS are important genetic determinants of complex diseases. It could also be the case that many loci contribute to the manifestation of a disease either in isolation or through epistatic interactions. The limitations of genome-wide association (GWA) studies have motivated human geneticists to consider the contribution of collections of rare variants to phenotypic expression. The increasing availability of high-throughput sequencing technologies has enabled studies of rare variants but these methods will not be sufficient for their success as appropriate analytical methods are also needed. We consider data analysis approaches to testing associations between a phenotype and collections of rare variants in a defined genomic region or set of regions. In addition, we explore ways that these approaches can account for known biological features associated with genomic segments (e.g., structural features of proteins) in a way that enables the investigation of variant interactions. We describe the application of a number of statistical methods for testing associations between rare variants in two genes sequenced in their entirety for a study of obesity as well as mitochondrial DNA sequences obtained on individuals with cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL). We consider the relative merits of the different methods as well as important implementation details, such as the leveraging of genomic annotations and determining p-values.

Project was supported by the Charles University Grant Agency, No 134609.

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Title of the project: Pathophysiology of neuropsychiatric disorders, their pathogenesis, prevention and treatment-project 4c:
Early diagnostics and treatment of psychotic disorders in the schizophrenia group, especially with the used of functional metabolic indices and determination of optimal treatment strategy

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

Principal Investigator: J. Libiger

Co-investigators: I. Tůma , J. Masopust, J. Bažant

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

Total funds allocated for project - Kč (thousands): 752

Summary of 2010 results

Is the risk of tromboembolism among psychotic patients linked to treatment or to the disease?

J. Masopust (1), D. Kalnická (1), R. Malý (2), C. Andrýs (3), J. Bazant (1), J. Libiger (1)
Charles University. Faculty of Medicine Hradec Králové, (1) Dept. of Psychiatry, (2) Dept of Hematology, (3) Dept. of Clin. Immunology

The central topic in 2010 was the threat of tromboembolism among patients with psychoses. It complemented the previous findings of an increased prevalence of antipsychotic treatment among patients with tromboembolic events. The markers of the blood coagulation activity were assessed in 25 antipsychotic–naive acute psychotic patients and in matched healthy volunteers. There were evaluated the factors that activate coagulation process and platelets: sP-selectin, soluble selectin, factor VIII and D-dimers. Patients with psychosis had significantly increased levels of sP–selectin and D-dimers compared to healthy subjects matched by age, gender and BMI. There was also a trend to increased factor VIII levels in plasma of patients with psychosis. Because these patients were not exposed to an antipsychotic treatment, it remains for further inquiry to find the reasons for differences in markers associated with the activation of coagulation processes. Speculation about non-specific stress mechanism that activates basic life saving mechanisms during psychotic turmoil needs more studies in acute as well as chronic patients.

Another topic was the survey of tromboembolic risks and their management among patients exposed to physical restraint because of psychotic agitation. Almost half of the group of patients (N= 44) with prolonged (8 hours and more) restraint had an increased score of risk for tromboembolism. The preventative measures were used in 32 % of the patients. The results of the survey led to an adjustment in the guidelines for the evaluation and management of the tromboembolic risk.

Reference: Masopust J, Maly R, Andrys C, Valis M, Bazant J, Hosak L. : Markers of trombogenesis are activated in unmedicated patients with acute psychosis: a matched case control study, BMC Psychiatry 2011,11:2

Address for correspondence: J. Masopust, Psychiatric Clinic and Dept. of Psychiatry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Fakultní nemocnice, Sokolská 581, Hradec Králové, 500 05, Czech Republic

Title of the project: Can statins attenuate a development of cholestatic liver disease?

Grant Agency: Ministry of Health ***Project Number:*** NS/9739-3

Principal Investigator: H. Lotková

Co-investigators: Z. Červinková, L. Kohoutek, P. Staňková

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 2415

Summary of 2010 results

Title of the presentation: The effect of fluvastatin on the progression of cholestatic liver injury induced by bile duct ligation in rats

Authors: H. Lotková (1), Z. Červinková (1), L. Kohoutek (1), P. Staňková (1), S. Mičuda (2), E. Brčáková (2, 3), G. Kolouchová (2)
Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1), Dept. of Pharmacology (2)
Fac. Pharm., Charles Univ., Hr. Králové: Dept. of Biological and Medical Sciences (3)

Extrahepatic biliary obstruction induces variety of changes then leading to liver injury. Intense pro-inflammatory response with increase in the production of cytokines and organ injury develops. Beyond hypocholesterolemic effect, antiinflammatory effect of statins has been reported. Contrary to beneficial effects, statins can increase susceptibility to mitochondrial dysfunction. The aim of our project was to verify the effect of fluvastatin on the development of cholestatic liver injury.

The mechanisms of potential effect including the influence on mitochondrial function, dose and time dependance were studied. Extrahepatic cholestasis was induced by a common bile duct ligation. Fluvastatin (1 and 5 mg/kg) was administered daily by intragastric tube immediately or 48 hours after surgery. Animals were killed 7, 14 and 21 days after surgery. ALT, AST, ALP, GGT and bilirubin were determined in the serum. In the liver, glutathione and cytokines (TNFα, IL-6, TGFß), mitochondrial respiratory activity, histological preparatives and expressions of Mdr1b, Mrp3 and Ugt1a1 mRNA were evaluated.
Deleterious effect of fluvastatin was observed in the cholestatic rat liver. Progression of injury was time and dose dependent. Hepatocellular injury was accompanied with higher production of profibrogenic cytokine TGF beta and sporadic fibrotic changes in the liver histology. Decrease in antioxidative capacity of the liver and mitochondrial dysfunction could contribute to this aggravating effect of fluvastatin as a consequence of the alteration in metabolism and transport of potentially toxic substances.

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Title of the project: The influence of surgery on the local immune response mediated by peritoneal macrophages

Grant Agency: Ministry of Health ***Project Number:*** NS/9649-4

Principal Investigator: L. Sákra

Co-investigators: K. Havlíček, J. Šiller, L. Kohoutek, H. Lotková, Z. Červinková

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 686

Summary of 2010 results

Title of the presentation: The inluence of laparascopic and laparotomic surgery on the activity of peritoneal macrophages

Authors: H. Lotková, M. Pospíšilová, Z. Červinková

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

Peritoneal macrophages play an important role in the immune response after abdominal surgery. However laparoscopic surgery is associated with reduced surgical trauma, literature data support that laparoscopic surgery can lead to the supression of local immune response. The aim of our study was to determine one of the markers of peritoneal macrophage activity - cytokine production 24 and 72 hours after surgery.

Male Wistar rats underwent laparotomic or laparoscopic caecectomy, control animals anaesthesia. 24 and 72 hours after surgery peritoneal lavage was performed. Macrophages were cultured for 24 hours without further stimulation or they were stimulated by lipopolysaccharide (LPS, Escherichia coli, Sigma-Aldrich). Concentration of cytokines TNFα, IL-1 and IL-6 were measured using ELISA kits. Statistical analysis was done using GraphPad Instant 3.06 for Windows (USA).

24 hours after surgery the basal production of pro-inflammatory cytokines TNFα and IL-1 was higher after laparoscopy. IL-6 was undetectable. Similarly, the basal production of pro-inflammatory cytokines by non-stimulated peritoneal macrophages was more expressed 72 hours after laparoscopy. Some authors consider the increase as a manifestation of more pronounced inflammatory response. The increase in the production of cytokines by stimulated macrophages was not so intense after laparoscopy. Whether this attenuation of secretory activity displays a tolerance to noxious stimuli or could be associated with postoperative complications should be verified.

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Title of the project: Assessment of free light chains as auxiliary marker for the evaluation of prognosis and of the response to treatment in patients with multiple myeloma and with monoclonal gammopathy of unknown significance

Grant Agency: Ministry of Health ***Project Number:*** NS/10387-3

Principal Investigator: V. Maisnar

Co-investigators: M. Tichý, J. Vávrová, M. Holečková, R. Hájek, L. Zahradová

Starting date: 1.9.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 3982

Summary of 2010 results

Title of the presentation: Assessment of free light chains as auxiliary marker for the evaluation of prognosis of monoclonal gammopathies and for monitoring of response to therapy of multiple myeloma.

Authors: V. Maisnar (1), M. Tichý (2), J. Radocha (1), J. Vávrová (2), L. Zahradová (3), M. Holečková (2), R. Hájek (3)

2nd Dept. of Medicine - Div. of Clin. Haematology, Faculty Hospital Hradec Králové (1), Institute of Clinical Biochemistry and Diagnostics (2), Hemato-oncological Department., University Hospital Brno (3)

In the realm of research and standardisation activities of the Czech Myeloma Group (CMG), we have been since long investigating the methodical assessment of parameters in multiple myeloma and the examination standardisation in reference centres in the Czech Republic. In the context of international cooperation, we have so far conducted new studies focused on development of novel FLC assay methods. The laboratories of the Institute of Diagnostics and Clinical Biochemistry (UH and FM in Hradec Králové) perform in monoclonal gammopathies FLC assays related to the comparison of the new ELISA method and the method currently used from 2008. The comparison is a part of the submitted project. The evaluation of the free light immunoglobulin chain concentrations are carried out using newly developed diagnostic kits Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA (BioVendor Laboratory Medicine, Inc., the Czech Republic),.immunoturbidimetric assays are performed using analyser Modular P. So far we are collecting samples from patients with monoclonal gammopathies and we are also testing benefit of FLC assessment for monitoring the reponse to treatment in multiple myeloma patients. We collaborate with Registry of Monoclonal Gammopathies databasis of Czech Republic. Currently it is registered 1727 patients with multiple myeloma and 1247 patients with monoclonal gammopathies of undetermined significance. We presume, that we will be able to identify high risk monoclonal gammopathy patients and estabilish better follow-up system.

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Title of the project: Creating of prognostic panel in patients with monoclonal gammopathy of undetermined significance with a target to prevent the risk of malignant transformation.

Grant Agency: Ministry of Health ***Project Number:*** NS/10406-3

Principal Investigator: V. Maisnar

Co-investigators: M. Tichý, R. Hájek, J. Radocha, I. Burešová, J. Vávrová, E. Dementyeva

Starting date: 1.9.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 4093

Summary of 2010 results

Title of the presentation: Flow cytometric analysis in patients with monoclonal gammopathy of undetermined significance and multiple myeloma.

Authors: V. Maisnar (1), K.R. Raja (2), J. Radocha (1), L. Kovářová (2), R. Hájek (2)

2nd Dept. of Medicine - Div. of Clin. Haematology, Faculty Hospital Hradec Králové (1), Hemato-oncological Department., University Hospital Brno (2)

Flow cytometric immunophenotyping is considered an indispensable tool for the diagnosis, classification and monitoring of disease in monoclonal gammopathies. The clinical sensitivity of flow cytometry is comparable with advanced molecular methods. Clinical application of flow cytometry in monoclonal gammopathies has various dimensions, such as differential diagnosis of malignant plasma cell disorder from reactive plasmacytosis, identifying the progression risk in monoclonal gammopathy of undetermined significance (MGUS) and asymptomatic multiple myeloma (MM), and minimal residual disease detection. Flow cytometry-based clonality assessment with immunophenotyping encourages and enables the most stringent method of diagnosis and follow-up. The objective of this review is to update the malignant plasma cells phenotypic profile of MGUS and MM. The most comprehensive antigens, such as CD19, CD27, CD28, CD45, CD56 and CD117, play a significant role in the characterization of normal and malignant plasma cells. Several research groups described the putative phenotype of myeloma cell progenitors, but no remarkable suggestion could be made because of disparity. We want to identify high risk monoclonal gammopathy patients and estabilish better follow-up system. The possible way to this objective is disclosure possible association of malignant phenotypic markers and chromosomal aberrations that identify the specific prognostic features in monoclonal gammopathies. We collaborate with Registry of Monoclonal Gammopathies databasis of Czech Republic. The results of this study will be used in terms of standardisation activities of the Czech Myeloma Group (CMG).

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Title of the project: Projection of new methodological procedures to the clinic work in internal diseases in the year 2010

Grant Agency: Charles University ***Project Number:*** 260902

Principal Investigator: J. Malý

Co-investigators: J. Bureš, J. Horáček, J. Vojáček, J. Petera, R. Malý, R. Pudil, M. Kopáčová

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1800

Summary of 2010 results

Title of the presentation: Projection of new methodological procedures to the clinic work in internal diseases in the year 2010

Authors: Bures Jan, Horacek Jiří, Vojáček, Jan, Petera Jiří, Malý Radovan, Pudil Radek, Kopáčová Marcela,

The survey presents the principal fields targeted by the support from the Specific Science Research in the year 2010.
Establishment of the register of young people with VTE and their risk factors suffering from thromboembolic disease. Examination of new signs in the pathogenesis of venous thrombosis in psychiatric patients.
Examination of the response to pharmacoprevention of arterial thrombosis by monitoring antiaggregation treatment.
Validation of biomarkers of myocardial damage in cytostatic therapy.
Validation of biomarkers of thyroid gland disease in pregnant women.
Hemapheretic operations in neurological and eye diseases.
Electrogastrography – "power analysis" of a model situation (EGG after itopride and volume load). Ex-vivo confocal laser endomicroscopy of gastro- and enteropathy induced by non-steroidal antiphlogistics.
Function changes in the small intestine after short-term administration of high doses of indomethacin with or without the probiotic bacteria *Escherichia coli* Nissle 1917.
Computer-assisted morphometry of the small intestine induced by non-steroidal antiphlogistics.Experimental model of Crohn's disease (induced by dextran sodium sulphate)
Capsule endoscopy of the small intestine in patients with rheumatoid arthritis and osteoarthrosis with microcytic anemia and without anemia.
Advances in brachytherapy of oncological diseases. Evaluation of the quality of life in oncological diseases. Biomarkers of organ damages in oncological therapy.
Markers of myocardial ischemia. Cardiac-specific markers in the course of acute myocardial infarction. Biomarkers of activation of the angiotensin aldosterone system in hypertension.
Publications: 34 papers in peer-reviewed journals and 19 papers in the journals with IF
Address for correspondence: Maly Jaroslav, 2nd Dept. of Medicine, Faculty of Medicine, Charles Univ.

Title of the project: The improvement of the wireless network services in the hostels and the labs of the Charles University in Hradec Kralove

Grant Agency: Ministry of Education ***Project Number:*** 891 A

Principal Investigator: V. Mašín

Co-investigators: J. Andrš

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1749

Summary of 2010 results

Title of the presentation: The improvement of the wireless network services in the hostels and the labs of the Charles University in Hradec Kralove

Authors: Vl. Mašín, J. Andrš

This project was aimed at expansion of the area covered with wireless network (Wi-Fi) and the improvement of quality of service.

We deployed additional 51 Wi-Fi access points in the hostel Na kotli, 8 access points in the hostel Palachova, 5 access point in the labs of the Faculty of Pharmacy and 3 access points in the labs of Faculty of Medicine. These additional access points grant proper Wi-Fi access in all parts of these buildings.

The new wireless controller improved the quality of wireless network service, especially the quality of roaming of the clients moving among different access points.

The quality of service was further improved by deployment of two new servers, which were connected to the disk array, supplied by the Faculty of Pharmacy, to create a high-availability cluster. This server cluster provides the authentication services for the wireless clients in all academic institutions in Hradec Kralove and also runs the management server monitoring health of the network.

We also purchased a notebook computer as a mobile monitoring workstation, which helped us to detect locations with weaker network signal and then to tune up the access points accordingly. This notebook computer is used now as a diagnostic tool in cases of reported disruption of the network service – we can use it to diagnose problems caused by electromagnetic interference, which cannot be easily detected by the built-in management of the access points.

We reached all declared aims of the project and were able to improve the wireless network service in Charles University faculties in Hradec Kralove.

Project was supported by the Ministry of Education Grant Agency, No 891/2010

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Title of the project: A study of mechanisms involved in organ injuries and possibilities of their positive modulation.

Grant Agency: Charles University ***Project Number:*** 260907

Principal Investigator: S. Mičuda

Co-investigators: S. Mičuda, V. Geršl, M. Štěrba, J. Martínková, M. Červinka, E. Rudolf, Z. Červinková, H. Lotková, A. Bezrouk, G. Kolouchová, P. Hiršová, E. Jirkovský, Š. Studená, V. Hubálková, S. Volencová, A. Víšková, V. Králová, D. Rychtrmoc, R. Endlicher, T. Nosek

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1800

Summary of 2010 results

Title of the presentation: A study of mechanisms involved in organ injuries and possibilities of their positive modulation.

Authors: S. Mičuda (1), V. Geršl (1), M. Štěrba (1), J. Martínková (1), M. Červinka (2), E. Rudolf (2), Z. Červinková (3), H. Lotková (3), A. Bezrouk (4), G. Kolouchová (1), P. Hiršová (1), E. Jirkovský (1), Š. Studená (1), V. Hubálková (2), S. Volencová (2), A. Víšková (2), V. Králová (2), D. Rychtrmoc (3), R. Endlicher (3), T. Nosek (4)
Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), De

Administration of numerous xeno- or endobiotics to organism may be associated with serious organ injury. Thus understanding the mechanisms involved in such adverse effects as well as identification of pathways and factors mediating positive compensatory reaction or tissue regeneration may significantly contribute to prevention and attenuation of toxicological events. The aim of the present project was to study regulatory and executive pathways involved in the development, promotion or diminution of organ impairment during various pathophysiological and toxicological situations. We also evaluated a variety of pharmacological approaches targeted to either suppression of specific disease progression or promotion of compensatory and regenerative mechanisms during different tissue injuries. The research was focused on heart, liver, kidney, lung and other organ impairments after administration of drugs (e.g. anthracycline chemotherapy), toxins (e.g. endotoxin of gram-negative bacteria), food components (e.g. high-fat diet) or resulting from accumulation of endogenous compounds (e.g. bile acids during extrahepatic cholestasis). Most salient data from the project contributed to understanding of hepatoprotective effect of statins during cholestatic liver injury, molecular mechanisms of anthracycline cardiotoxicity, capillary leak syndrome and its influence on pharmacokinetics of gentamicin, effect of selen on apoptosis in colorectal cancer cells, silencing RNAs, mitochondrial gene expression impairment in hepatic regeneration during high-fat diet, and characteristics of selected dental materials.

Project was supported by the Charles University project, No SVV-2010-260907.

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Title of the project: Innovation of the subject „Stem Cells and Regenerative Medicine“.

Grant Agency: Ministry of Education ***Project Number:*** 545 F3

Principal Investigator: J. Mokrý

Co-investigators: T. Soukup

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 144

Summary of 2010 results

Title of the presentation: Innovation of the voluntary subject: Stem Cells and Regenerative Medicine

Authors: J. Mokrý, T. Soukup
Fac. Med., Charles Univ., Hr. Králové: Dept. of Histology and Embryology

The aim of the project was to innovate lectures and practical classes of the voluntary subject „Stem Cells and Regenerative Medicine“, prepare study material, provide it to students at the departmental website and stimulate students to active work in lectures and practical classes. All lectures were modified and new relevant findings were included in innovated computer presentations. Simplified versions of most lectures were converted to pdf files and offered to students at http://www.lfhk.cuni.cz/histologie/Histols_web/vyuka/vseobecne/ kmenove_ bunky/anotace.asp. The active approach to the study was stimulated by innovation of practical classes that required students to carry out specific tasks, e.g. to measure precisely tiny volumes of cultivation media by themselves, to measure cell viability and examine the cell phenotype. The copies of new four distinct protocols can be downloaded from the website. To allow microscopic study of tissue microenvironment that hosts tissue-specific stem cells, new histological slides were prepared and stained with haematoxylin-eosin, Luxol blue or peroxidase immunohistochemistry for identification of specific cell types. Histological examination of slides was accompanied by presentation describing a detailed structure of particular niches. A brief survey characterizing the basic properties of stem cells was prepared for placement in a separate site of departmental web. The text was accompanied with schematic drawings, additional information on stem cells and a vocabulary with explanation of the basic terms. This page will be available in internet not only to our students but also to wide public to popularize the area of stem cell research and regenerative medicine with the aim to make understanding to this complex topic easier.
Project was supported by the grant project FRVŠ 545 /2010.

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Title of the project: 4th Morphological Postgraduate Conference

Grant Agency: Charles University ***Project Number:*** 260908

Principal Investigator: J. Mokrý

Co-investigators: D. Slížová, R. Slezák

Starting date: 1.1.2010 ***Duration (years):*** 1

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

Summary of 2010 results

Title of the presentation: Organization of the 4th morphological postgraduate conference

Authors: J. Mokrý (1), D. Slížová (2), R. Slezák (3)
Fac. Med., Charles Univ., Hr. Králové: Dept. of Histology and Embryology (1), Dept. of Anatomy (2), Dept. of Dentistry (3)

The aim of the project was to organize the 4th morphological postgraduate conference and allow students from distinct morphological disciplines to present results of their scientific work. The conference was held at Charles University Medical Faculty in Hradec Králové in November 11, 2010. The scientific programme covered 11 lectures and 16 posters presented by students from six different departments of Medical Faculty and Pharmacological Faculty. Abstracts of the lectures and posters were included in printed books of abstracts with programme that were distributed to all the participants. Texts of selected presentations accompanied with tables and figures were published in the form of full text papers in the proceedings of the conference. The best three lectures and three posters were awarded to motivate them in further scientific activities. The conference was visited by approx. 40 participants who were actively involved in discussions.

Literature: J. Mokrý, D. Slížová (eds.), Proceedings of the 4th morphological postgraduate conference, Libor Dvořák, Hradec Králové, 2010, ISBN 978-80-254-8828-7, 78 pages.

Project was supported by the grant project SVV-2010-260908 from Charles University.

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: Health Care by Biosensor Measurements and Networking

Grant Agency: 6FP EU ***Project Number:*** CARE-MAN

Principal Investigator: V. Palička

Co-investigators: A. Čegan, J. Kovařík

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1540

Summary of 2010 results

Title of the presentation: Validation of the new multi-parameter CARE-MAN device

Authors: A. Čegan (2), V.Palička (1), J.Kovařík (3)

Text:

Fac. Med., Charles Univ., Hr. Králové, Inst. of Clin. Biochemistry and Diagn. (1), Fac. Chem-Technol., University Pardubice, Dept. of Biol. and Biochemical Sci. (2), Reg. Hospital Pardubice, Dept. of Clin. Biochemistry (3)

The main objective of CARE-MAN Project is the development of a point-of-care testing (POCT) system in the application field of 5 different disease areas (cardiovascular, coagulation disorder, inflammation and sepsis, thyroid disease, cancer) with suggested application focus for smaller hospitals and general practitioners. Within the CARE-MAN project biological samples are used for the validation for the new POCT device technology. First tests have been focused on technical parameters using model solutions and control sera. In further steps spiked biological materials from healthy people have been used. Now, real patient samples from ill patients are used to evaluate the device in central laboratory of Med. Faculty University of Tübingen. Our institutes serve to measure serum sample values of different routine laboratory parameters as well as factors which could influence the validation procedure. At the same time samples are tested for infectious agents (HIV, Hepatitis) in the routine laboratory of Med. Faculty University of Tübingen. Study protocol according good laboratory practice was developed, the informed consent and also the application to the ethics committee of Regional Hospital Pardubice was approved. Validation protocol have been prepared, according the criteria used in EU.

Last year 320 different blood, serum and plasma samples have been prepared, at about 3000 routine analysis have been made. The evaluation of the parallel measurement in the "classical" laboratories at Hradec Kralove and Pardubice and newly developed POCT in Germany is under the testing.

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Title of the project: Important health aspect in children, young and elderly

Grant Agency: Charles University ***Project Number:*** 260903

Principal Investigator: V. Palička

Co-investigators: I. Gradošová, S. Hubená, M. Chmelařová, K. Josefová, J. Spáčilová, M. Tichý, P. Živný, H. Živná

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 750

Summary of 2010 results

Title of the presentation: Effects of Amlodipine and Metoprolol on Bone Metabolism in Male Albino Wistar Rats

Authors: V. Palička, I. Gradošová, S. Hubená, M. Chmelařová, K. Josefová, J. Spáčilová, M. Tichý, P. Živný, H. Živná

Antihypertensive and hypolipidemic drugs are wide used agents for the treatment of cardiovascular diseases but the detailed information about their effects on the bone metabolism is missing. The aim of our study was to investigate the effect of amlodipine (AML, dihydropyridine – type calcium channel blockers) and metoprolol (selective β_1 receptor blocker without intrinsic sympathomimetic activity) on the bone metabolism in male albino Wistar rats. Our study was carried out on 24 rats (240±10 g). The rats were randomly divided into 3 groups of 8 animals. The control group (CO) was administered *aqua pro injectione* (0.2 mL/100 g BW; gavage) and the experimental groups amlodipine and metoprolol suspension (AML; 0.3 mg and MET; 0.5 mg in 0.2 mL *aqua pro inj*. /100g BW; gavage) daily for 8 weeks. Bone markers concentrations: the carboxy-terminal collagen I telopeptide (ICTP), osteocalcin (OC), bone alkaline phosphatase (BALP), and the aminoterminal propeptide of procollagen type I (PINP) were measured by enzyme immunoassay method. We investigated the expression bone morphogenetic protein 2 (BMP-2) in proximal tibia using Western blotting. Furthermore, we measured the bone mineral density (BMD) by means of Dual-energy X-ray Absorptiometry (DXA) in lumbar and caudal vertebrae and in femoral areas. Mechanical properties in three-point bending and compression test of femoral neck were measured in right femurs. After 8 weeks AML administration showed statistically significant decrease of serum level of BALP ($p=0.0009$) and ICTP ($p=0.003$) to compared to the controls. Moreover, Western blotting elicited an increased BMP-2 protein level of amlodipine administration. After 8 weeks of MET administration, the expression of BMP-2 was increased. Compression tests of femoral neck maximal load values were significantly lower than CO ($p=0.002$). Our findings suggest that amlodipine shows the influence on the bone metabolism by decreasing bone turnover and probably in consequence increasing expression BMP-2 in rats. Metoprolol has an effect on bone metabolism in rats by reducing maximal load values in femoral neck and increasing expression of BMP-2 in proximal tibia.

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Title of the project: Utilization of cone beam CT for reconstruction of dose distribution delivered in image-guided radiotherapy of prostate carcinoma – bony landmark setup compared to implanted fiducial markers setup

Grant Agency: Charles University ***Project Number:*** 144210

Principal Investigator: P. Paluska

Co-investigators: J. Hanuš, J. Šefrová, L. Rousková, M. Hodek, J. Jansa, L. Kašaová

Starting date: 1.1.2010 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 670

Summary of 2010 results

Title of the presentation: Is there a space for safety margin reduction in prostate IGRT?

Authors: P. Paluska (1), J. Hanuš (2), J. Šefrová (1), L. Rousková (1), M. Hodek (1), J. Jansa (1), L. Kašaová (1)
Dept. of Oncology and Radiotherapy, University Hospital Hradec Králové (1), Dept. of Medical Biophysics, Charles University in Prague, Faculty of Medicine in Hradec Králové (2)

To account for geometric uncertainties during radiotherapy, safety margins are applied. Modern radiotherapy techniques - such as image-guided radiotherapy (IGRT) - give the possibility to apply tighter margins as with conventional RT. This can be beneficial especially in prostate cancer, where the dose to the rectum limits dose escalation.

IGRT systems provide more information than that which is required for simple patient positioning. Utilization of cone-beam CT (CBCT) can provide 3D anatomic information directly in irradiation position. Such information enables reconstruction of a current dose distribution.

We used CBCT to compare two different styles of IGRT - bony landmark vs. implanted fiducial markers setup. We delineated target volumes (prostate, seminal vesicles) and organs at risk (rectum, bladder) on CBCTs acquired directly before the patient's treatment. Then, the dose distribution was reconstructed using the fluence maps from the treatment plan. Fluences from hypothetical alternative plans considered tighter safety margins were also used for the dose reconstruction.

Possibility of margin reduction was evaluated by means of calculated target coverage. Our results indicate that in case of bony landmark setup the safety margin couldn't be reduced, while in case of implanted fiducial markers setup the safety margin could be reduced from 10 mm to 7 mm without compromising target coverage. This creates a potential for dose escalation in the future treatments.

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Title of the project: Intergration of basic mechanical procedures with the orthodontic wire to the practical education of undergraduate study of dentistry

Grant Agency: Ministry of Education ***Project Number:*** 1277 F3

Principal Investigator: A. Mottlová (Pavlicová)

Co-investigators:

Starting date: 1.1.2010 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 89

Summary of 2010 results

Title of the presentation:

Authors: A. Mottlová

Orthodontics is a specialised branch of dentistry concerned with facial growth, with development of the dentition and occlusion, and with the diagnosis, interception and treatment of occlusal anomalies.

The complex issue of orthodontic treatment is significantly simplified for the practical daily use by future dentists and focuses mainly on the early diagnostics of occlusal anomalies. However, general dentists are often concerned with complaints of orthodontic origin, with patient's need to relieve discomfort or even pain because of a damaged fixed orthodontic appliance. This causes difficulties to those dentists who lack basic orthodontic knowledge and thus delay patient's relief for several days. Therefore our main research objectives focus on improvement of undergraduate orthodontic education. We emphasize increasing manual dexterity of our students to learn the basic work procedures with orthodontic wires. Our project is focused on dentistry students of 3rd and 4th grade (theoretical and practical training on models) and students of 5th grade (theoretical and practical training) in subjects of clinical dentistry and orthodontics.

The implementation of this issue increases expertise on one hand and manual dexterity of graduate students on the other. We consider this very important as our graduate students immediately after passing the state exam are fully qualified to work independently.

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Title of the project: Genetic prediction of late toxicity of radiotherapy for cervical cancer

Grant Agency: Ministry of Health ***Project Number:*** NT/11334-4

Principal Investigator: J. Petera

Co-investigators: M. Beránek, S. Brokešová, I. Sirák, V. Palička, M. Vošmik, M. Chmelařová

Starting date: 1.9.2010 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3638

Summary of 2010 results

Title of the presentation: ATM polymorphism in radiosensitive patients with cervical carcinoma – pilot results.

Authors: Petera J, Beránek M, Sirák I, Brokešová S, Palička V., Vošmik M, Chmelařová M.

The aim of the study is examination of ATM and TGF β1 polymorphism and their correlation with late toxicity in patients with cervical carcinoma treated by chemoradiotherapy.
Blood samples were achieved in 34 patients, pilot results were evaluated in 20 patients. Positive control samples carrying the homozygote 5557A genotype in the *ATM* gene were successfully constructed. A method of PCR-restriction fragment length polymorphism analysis (PCR-RFLP) for ATM and TGF β1 was validated.
Three of twenty women (16 %) in the study were heterozygotes for the 5557A allelic variant. None of these patients had gastrointestinal or urological toxicity of grade III/IV.
Continuation of the project will allow to evaluate value of ATM and TGF β1 polymorphism for risk of late complications after chemoradiotherapy for cervical cancer.

Address for correspondence: Prof. MUDr. Jiří Petera, Ph.D., Dept. of Oncology and Radiotherapy, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: The importance of hepcidin for the diagnosis and treatment of anemia in children

Grant Agency: Ministry of Health ***Project Number:*** NS/9951-4

Principal Investigator: D. Pospíšilová

Co-investigators: P. Džubák, M. Hajdúch, M. Kollareddy, O. Pozler

Starting date: 1.1.2008 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6501

Summary of 2010 results

Title of the presentation: QUANTIFICATION OF HEPCIDIN IN BLOOD AND URINE OF CHILDREN WITH INFLAMMATORY BOWEL DISEASES BY ELISA METHOD AND MASS SPECTROMETRY

Authors: J.Houda1, P. Džubák2, B.Ludíková1, O. Pozler3, D.Pospíšilová1,
1Department of Pediatrics, Faculty of Medicine of Palacky University and Teaching Hospital in Olomouc
2Laboratory of Experimental medicine, Department of Pediatrics in Olomouc
3Department of Pediatrics, Faculty of Medicine Charles University and Teaching Hospital in Hradec Králové

The goal of the study is to create a method to determine the hepcidin and pro-hepcidin level in blood and urine in children with inflammatory bowel disease, then to define the relationship between hepcidin and pro-hepcidin with the type of anemia and with the activity of inflammatory disease. We collected blood and urine samples from patients with Crohn's disease or ulcerative colitis. Creating of the database helps to follow up the basic laboratory results (hemoglobin level, RBC, MCV, MCH, levels of iron, ferritin and sTfR) and clinical findings (physical examination, physical activity evaluated by question-form, number of transfusions). Quantification of the peptides is performed by ELISA method and by mass spectrometry. We collected 81 patients (50 males, 31 females) in our database; the age is 6-18 years. We found anemia (Hb < 120 g/l) in 61.7% of patients and serious anemia (Hb < 100 g/l) in 12.3%. We described microcytic anemia in 48.8% and hypochromic anemia in 56.6% of the patients. The iron level was decreased in 66.2%, ferritin was decreased in 51.5% and increased sTfR level was found in 49.2%. In 2 patients it was necessary to administrate transfusions of erythrocytes due to severity of anemia. Physical activity was affected in 7.4%. We performed ELISA test on 13 patients. The range of hepcidin level in blood was 12.7 – 87.3 ng/ml. 5 of tested children had anemia and 3 of them had increased hepcidin level over the normal range. Anemia was present in 61.7% of patients with inflammatory bowel disease. Its occurence influenced not only the clinical symptoms of inflammatory disease but also the therapeutic approach. Laboratory findings showed the abnormal iron metabolism.We found 3 of 5 patients having anemia and increased hepcidin level.

Study supported by grant IGA MZ ČR NS9951-4/2008

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Title of the project: Role of echocardiography at biventricular pacemaker optimisation

Grant Agency: Charles University ***Project Number:*** 66809

Principal Investigator: R. Praus

Co-investigators: P. Pařízek, M. Tauchman, V. Bláha, J. Popelka

Starting date: 4.5.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 450

Summary of 2010 results

Title of the presentation: The effect of cardiac resynchronization therapy on systolic function of right ventricle.

Authors: P. Pařízek (1), M. Tauchman (1), L. Haman (1), V. Bláha (2)
University Hospital, Hr. Králové, First Department of Internal Medicine (1), University of Defence, Faculty of Military Health Science, Hr. Králové (2).

Aim: To assess effect of cardiac resynchronization therapy (CRT) on systolic function of right ventricle (RV) in responders and non-responders in a prospective study.
Group of patients and methods: In 58 patients with heart failure NYHA class II-IV (average QRS duration 193 ± 33 ms) a biventricular system was implanted between 7/2005 and 5/2008. At baseline, 3 and 15 months after the implantation the following parameters were determined: NYHA, quality of life, 6-min walk test (6MWT), echocardiography including assesment of systolic function of RV by TAPSE (tricuspid annular plane systolic excursion) and ventricular dyssynchrony. A responder was defined as patient who improves in quality of life, NYHA class and/or 6MWT more than 10 %.
Results: 15 months after CRT we found 38 responders (66 %) and 19 non-responders (33 %). In the group of responders we found statistically significant improvement of the systolic function of RV and also significant decrease of RV size after 15 months of CRT (TAPSE resp. before CRT 17,8 ± 4,0 mm, 15 months after CRT 19,4 ± 3,7 mm, $p < 0,05$, RV size before CRT 29,3 ± 5,0 mm, 15 months after CRT 27,8 ± 4,2 mm, $p < 0,05$). In the group of nonresponders these changes were not observed, on the contrary, after 15 months we observed significant progression of tricuspid regurgitation.
Conclusion: 15 months after CRT we found a statistical significant improvement of systolic function of RV and a significant reduction of right ventricular size in CRT responders.

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

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

Principal Investigator: P. Widimský

Co-investigators: P. Gregor, M. Aschermann, R. Pudil, R. Rokyta, Z. Straka

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

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

Summary of 2010 results

Title of the presentation: Acute heart failure: prognostic significance of vena cava inferior diameter

Authors: R. Pudil, M. Tichy, V. Blaha, J. Vojacek
Charles University Prague, Medical Faculty Hradec Kralove, Department of Medicine, and Institute of Clinical Biochemistry and Diagnostics

Echocardiography is mandatory for confirmation of heart failure diagnosis and should be performed shortly following suspicion of acute heart failure (AHF). Some of echocardiographic parameters are known to be independent outcome predictors of AHF. But little is known about the prognostic significance of inferior vena cava diameter (IVCD) in AHF. The prognostic significance of IVCD, so far is not well documented. Irregardless, it is a known fact that is plays a prime role in determination of right atrial pressure, right and left ventricular dysfunction even in the emergency settings. In our pilot study, we measured IVCD and analyzed mortality data in patients admitted to emergency department with AHF. Study population consisted of 92 pts (65 males; 62.3 ±13.9 yrs), with severe dyspnea caused by AHF. During 1-year follow-up, 32 pts died. Mean IVCD of all the patients was significantly increased compare to control group (22.2±2.1 mm, vs.15.4±1.9 mm, $p<0.01$); ejection fraction (EF) was significantly decreased (37±15%, vs. 64±5%, $p<0.01$). Left ventricular mass index (LVMI) was increased (165±40 g/m^2, vs. 121±12 g/m^2, $p<0.01$). In the survivors, the IVCD was lower (21.3 ±1.7 mm, vs.24.4±2.1 mm, $p<0.05$). Left ventricular ejection fraction was inversely associated with IVCD ($r = - 0.35$, $p<0.01$, n92). After adjustment for age, gender, diabetes mellitus, and history of coronary artery disease, IVCD above the median (20mm) emerged as a strong predictor of short- and long-term mortality: Hazard ratio for 7 day mortality was 2.2 (95 % CI: 1.2-7.5), for 28 days mortality 1.8 (95% CI: 1.1-6.1), and for 1-year mortality 4.1 (95% CI: 1.3-12.6). Our study results demonstrated that IVCD is an easily accessible measurable parameter in acute settings and its measurement can serve as a useful prognostic tool in patients with AHF.

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Title of the project: Characteristic of resistance of adult stem cells to genotoxic stress

Grant Agency: Czech Republic ***Project Number:*** 304/09/1568

Principal Investigator: M. Řezáčová

Co-investigators: J. Mokrý, J. Vávrová, D. Muthná, T. Soukup

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 3564

Summary of 2010 results

Title of the presentation: Reaction of human mesenchymal stem cells isolated from bone marrow to ionizing radiation

Authors: D. Muthna1, T. Soukup2, J.Vavrova3, J. Mokry2, J. Cmielova1, B. Visek2, A. Jiroutova1, R. Havelek1, J. Suchanek4, and M. Rezacova1

Fac. Med., Charles Univ., Hr. Králové: Dept. of Med. Biochem.(1), Dept. of Histol. Embryol. (2), Dept. of Dent. (4); Fac. Milit. Health Sci., Univ. Defence, Dept. of Radiobiol (3)

We isolated mesenchymal stem cells from bone marrow of patients undergoing hip joint replacement. We prove that ionizing radiation decreases proliferation of mesenchymal stem cells isolated from bone marrow in dose-dependent manners. Even the highest dose tested (20 Gy) does not significantly activate caspases and does not significantly decrease cell viability. After irradiation with the doses 6 and 20 Gy the cells were accumulated in G2 phase of the cell cycle. Mesenchymal stem cells isolated from bone marrow respond to ionizing radiation induced stress by premature senescence (detected as beta-galactosidase positivity) from third day after irradiation. 24 h after irradiation we detected accumulation of p53 and its phosphorylated forms and induction of p21. Increase in protein p16 was observed from 3rd day after irradiation with maximum at the end of experiment (13th day). We can conclude that mesenchymal stem cells isolated from bone marrow respond to ionizing radiation-induced damage by permanent cell cycle arrest in G2 phases and by stress-induced premature senescence.

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Title of the project: Noninvasive detection of proinflamatory markers of oxidative stress in irradiated as an indicator of received dose of radiation. Protective effect of acetyl-L-carnitine (RONSDOZ)

Grant Agency: Ministry of Defense ***Project Number:*** OVUOFVZ2008

Principal Investigator: M. Řezáčová

Co-investigators: J. Vávrová, J. Osterreicher, A. Tichý, J. Pejchal, Z. Vilasová, J. Chládek, M. Hroch, A. Babicová, L. Mervartová

Starting date: 1.11.2008 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3402

Summary of 2010 results

Title of the presentation: Markers of oxidative and nitrossative stress in whole-body irradiated rats

Authors: J. Chládek (2,3), A. Babicová (3), J. Vávrová, J. (1), Osterreicher (1), A. Tichý (1), J. Pejchal (1), Z. Vilasová (1), M. Hroch (2), M. Řezáčová (3)
Fac. Milit. Health Sci., Univ. Defence, Dept. of Radiobiol.(1), Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (2), Dept. of Medical Biochemistry (3)

Ionizing radiation damages the cells either directly or indirectly through production of reactive oxygen species (ROS). Nitric oxide is another reactive species induced by irradiation. Some theories propose that ROS and reactive nitrogen species (RNS) are involved in activation of particular cytosolic signal transduction pathways in response to ionizing radiation. Our study compared the changes in production of ROS and RNS after whole-body irradiation of rats to those after intraperitoneal administration of 10 mg/kg bacterial lipopolysaccharide (LPS). The results indicate that within the first 24 h after irradiation the amount of exhaled NO remains unchanged, ionizing radiation does not affect expression of iNOS protein in the lung homogenate, which contrasts with the dose-dependent increase of plasma NOx observed in this study. The changes in arginine-nitric oxide metabolome which occur during the first 24 h following whole-body gamma irradiation of rats with sublethal to lethal doses are marginal compared to the effects of LPS. Irradiation causes a dose-dependent increase of plasma NOx at 6 h. Expression of iNOS in the lung tissue, NOx in the lung homogenate and arginine in the plasma and BAL remain unchanged. The levels of NOx in BAL fluid increase moderately but show no relationship to the dose.

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Title of the project: Mechanism of liver regeneration termination after partial hepatectomy

Grant Agency: Charles University ***Project Number:*** 94509

Principal Investigator: D. Rychtrmoc

Co-investigators: M. Pospíšilová, L. Hubálková, A. Víšková, F. Kunc, Z. Červinková

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 510

Summary of 2010 results

Title of the presentation: Liver regeneration termination: an aim to far?

Authors: D. Rychtrmoc (1,3), A. Libra (3), L. Hubálková (2,3), Z. Červinková (1)
Fac. Med., Charles Univ., Hr. Králové, Dept. of Physiology (1), Dept. of Med. Biology and Genetics (2); GENERI BIOTECH s.r.o., Hradec Králové (3)

The aim of our project is to collect and analyze comprehensive data on gene expression changes during liver regeneration induced in rats by 2/3 partial hepatectomy. Thus we performed 2/3 partial hepatectomies (PH) after Higgins and Anderson in 62 male Wistar rats aged 7-8 weeks, another 18 rats were used as controls. After recovery period ranging from 1 to 26 days, we acquired plasma samples and samples of regenerating liver tissue. Plasma samples were used to assess ALT, AST, TAG, bilirubin and malondialdehyde levels in order to characterize progress of liver damage and recovery. Besides that we compared weights of liver on the day of animal sacrifice to the presurgery values to evaluate completeness of liver mass restoration. Finally samples of regenerating liver tissue were used for DNA microarray gene expression analysis, which was the first time we used this advanced method for a time series experiment at our departments. After initial optimalization we decided for two partially overlapping experiments. The first included samples from postoperative days 1, 2, 3, 5, 7, 9, 11 and 14 and the other focused on days 1, 2, 3, 5, 7, 9 and 10. The former scheme reflected our effort to find out more about mechanisms of liver regeneration termination, which have not been clearly defined so far. However this turned out to be a substantial challenge, because neither gene expression data nor weights and biochemical measurements lead us to clear conclusion about regeneration termination timing. That is why in the latter experimental setting we focused on time intervals within the first ten days after PH. Thanks to the design of both series resulting data may be analysed together and provide insights about molecular events going on during the liver regeneration process. Microarray data analysis was definitely the most demanding part of our work.

Project was supported by the Charles University Grant Agency, No. 94509/2009.

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Title of the project: Selected topics in oncologic and cardiac pathology

Grant Agency: Charles University ***Project Number:*** 260901

Principal Investigator: A. Ryška

Co-investigators: T. Rozkoš, J. Laco, E. Hovorková, F. Sobande

Starting date: 1.1.2010 ***Duration (years):*** 1

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

Summary of 2010 results

Title of the presentation: Presence of Her-2/neu receptor in gastric carcinoma and importance of its detection for biological treatment

Authors: Rozkos T., Laco J., Ryska A.
The Fingerland Department of Pathology, Charles University, Faculty of Medicine
and University Hospital in Hradec Kralove

Part of metastatic gastric carcinomas is Her-2/neu (HER2) positive. Nowadays there is possibility to use biological treatment against HER2(trastuzumab), therefore HER2 status should be examined.

Aim of our study is1) To introduce dual colour in situ hybridisation (ISH) method to evaluate amplification of HER2 gene 2) To determine the proportion of amplified and immunohistochemically (IHC) HER2 positive cases. 3) To compare our results with relevant clinicopathological data. Our study was designed as retrospective on the series of 97 advanced gastric carcinomas diagnosed at our department in years 2005 – 2009. IHC detection of HER2, E-cadherin and EGFR was performed. All IHC HER2 + and a control group of IHC – cases (together 50) were examnied by dual colour ISH. Evaluable cases number is 91 (53 intestinal,19 diffuse, 5 mucinous, 14 mixed). IHC of HER2: intestinal –8/53 weak positivity (2+), 13/53 strong positivity (3+); diffuse – 2/19 3+, mixed and mucinous – all negative. E-cadherin positivity: 79 (86,8%) cases (52/53 intestinal, 13/19 diffuse, 3/5 mucinous, 11/14 mixed), EGFR positivity: 46 (50,5%) cases (26/53 intestinal, 11/19 diffuse, 1/5 mucinous, 8/14 mixed). HER2 amplified by dual colour ISH: 0/30 of IHC negative (0+, 1+), 2/8 of IHC 2+ (both cases showing borderline amplification), 12/13 of IHC 3+. Overexpression of HER2 differs significantly in subtypes of gastric carcinoma. Expression in each single case is also markedly heterogenous. No relationship between HER2 and EGFR expression was found. IHC HER2 3+ expression closely correlates with amplification detected by dual colour ISH, except of one case where chromosome 17 polysomy was present.

Address for correspondence: T. Rozkoš, The Fingerland Department of Pathology, Charles University, Faculty of Medicine and University Hospital in Hradec Kralove, Sokolská 581 Hradec Králové 500 05, Czech Republic

Title of the project: Optimising extracorporeal elimination strategy with regard to the calcium and phosphate metabolism disturbances (CKD-MBD) in patients with chronic kidney failure

Grant Agency: Ministry of Health ***Project Number:*** NT/11355-4

Principal Investigator: R. Šafránek

Co-investigators: S. Dusilová-Sulková, M. Kubišová, E. Mistrík, L. Sobotka

Starting date: 1.9.2010 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 2836

Summary of 2010 results

Title of the presentation: *S*kin microcirculation during hemodialysis, preliminary results

Authors: R. Šafránek (1), E. Mistrík (1), S. Dusilová-Sulková (2), M. Kubišová (1), P. Moučka (1)

1 Dept. of Metabolic Care and Gerontology, University Hospital Hradec Králové, Czech Republic

2 Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Introduction: In long-term dialysis patients it is of upmost importance to remove retained fluid without hemodynamic instability for protection of cardiovascular system. In clinical settings, hemodynamic stability is mainly evaluated using macrohemodynamic parameters such as blood pressure or pulse rate in everyday clinical practise. Only little or no attention is paid to microcirculation of peripheral organs.

Methods: All measurements were done in hemodynamically stable patients in maintenance hemodialysis. Skin perfusion was evaluated using laser Doppler line scanner. Both hands and feet of every patient were measured before and 30, 90 and 215 minutes after the start of hemodialysis and 20 minutes after its end. Hemodialysis proceeded standardly (low flux steam sterilized polysulphone dialyzer; two needles; blood flow 300 ml/min; dialysate flow 500 ml/min; isothermic hemodialysis. We also measured basic laboratory parameters including markers of calcium and phosphate metabolism, we assessed hydration status.

Results and conclusion: The skin perfusion on toes as well as instep and hand significantly decreased during hemodialysis. Higher post-dialysis hydration was associated with lower decrease in skin perfusion during hemodialysis. Patients with poorly compensated hyperparathyroidism had more severe decrease in skin perfusion.

Project was supported by the Internal Grant Agency of the Ministry of Health of the Czech Republic, No. NT/11355-4.

Address for correspondence: R. Šafránek, Dept. of Metabolic Care and Gerontology, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Changes in Chemoresistance/Chemosensitivity of Ovarian Cancer Cells

Grant Agency: Ministry of Health ***Project Number:*** NS/9737-3

Principal Investigator: I. Sedláková

Co-investigators: M. Červinka, J. Tošner, J. Laco, A. Řezáč, J. Špaček

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

Total funds allocated for project - Kč (thousands): 2613

Summary of 2010 results

Title of the presentation: Chemoresistance/chemosensitivity in ovarian cancer patients
Authors: Iva Sedláková (1), Miroslav Červinka (2), Jindřich Tošner (1), Kateřina Brigulová (2), Jan Laco (3), Adam Řezáč (1), Jiří Špaček (1), Peter Škapinec (1), E.Čermáková (4),
Dept. of Gynecology and Obstetrics University Hospital and Fac. Med. Hradec Králové (1)
Dept. of Medical Biology and Genetics Medical Faculty Charles University (2)
Dept. of Pathology University Hospital (3)
Dept. of Medical Biophysics Medical Faculty Charles University (4)
Objective: To compare the chemoresistance/chemosensitivity in solid tumors and ascitic fluid in ovarian cancer patients using MTT assay.To determine resistance proteins MRP, LRP, Pgp. To evaluate the correlation of drug resistance and histological subtype, stage and grade.
Methods: MTT – (3-(4,5 – Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) chemosensitivity assay was performed in 62 samples of ovarian cancer tissue and 43 samples of ascitic fluid in ovarian cancer patients. We studied the in vitro drug resistance to cisplatin, carboplatin, paclitaxel, topotecan, gemcitabin, etoposid.*Results:* The highest incidence of drug resistance in vitro had gemcitabin and carboplatin and the lowest incidence of drug resistance had cisplatin and topotecan. Cisplatin had lower incidence of drug resistance in vitro than carboplatin. Grade and stage of epithelial ovarian cancer did not correlate to the drug resistance/sensitivity in vitro in ovarian cancer patients. The histological subtype of epithelial ovarian cancer correlated to the resistance and sensitivity to chemotherapeutic agents in vitro. Ovarian cancer patients with primary drug resistance to paclitaxel and carboplatin in vitro had shorter progression free interval and worse prognosis of the disease.*Conclusion:* The lowest incidence of drug resistance in vitro had cisplatin. Ovarian cancer patients with in vitro resistance to paclitaxel and carboplatin had significantly higher risk for progression of disease when treated with standard platinum-paclitaxel based regimens. The resistance/sensitivity assay would contribute to the targeted treatment and better prognosis of ovarian cancer patients.

Project was supported by the Ministry of Health Grant Agency, No 9737-3

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Title of the project: Photosensitizers in Dentistry

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

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

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

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

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

Summary of 2010 results

Photosensitizers in Dentistry 2006 – 2010

R. Slezák[(1)], M. Karásková[(2)], L. Ryšková[(1)], O. Krs[(1)], V. Buchta[(1)], D. Slížová[(1)], J. Černý[(2)], J. Rakušan[(2)], R. Ivančaková[(1)], A. Šimůnek[(1)], M. Červinka[(1)], Faculty of Medicine in Hradec Králové[(1)], Research Institute for Organic Syntheses, Rybitví[(2)]

The aim of the project was to develop new phthalocyanine derivates (PCs) with significant antimicrobial properties potentially useful in human medicine, especially in dentistry. Original laboratory devices and methods had to be developed. Totally kationic and anionic PCs of various structure were synthetized. Chemical and physical properties were tested with the emphasis to the ability of FCs´s production of singlet oxygen $^1O_2$ initialized by monochromatic light 760 nm of wavelength. In vitro, the photo/cytotoxicity of FCs was detected using tumorous and non-tumorous cell cultures (Hep2, human gingival fibroblasts) in modified test of metabolic activity. The toxicity differed according to the irradiation and concentration of PCs but without any association to the cell linie. The antimicrobial effect of FCs to various types of G+ and G- bacteria including multiresistant strains, yeasts of *Candida* sp., and herpes simplex virus was evaluated. The most effective agents were PCs No. 1074/352 and No. 1074/283. The efficacy of FCs was higher in bacteria then in yeasts. Antiviral effect was only transient. The binding of FCs to various types of nanoparticles decreased their antimicrobial activity. A serie of nanomaterials was selected for the fixation of FCs with the significant antimicrobial activity. Their retention in gels and colloidal mixtures as nanoparticle carriers on tested surfaces of various natural tissues (enamel, dentin and cementum, human gingiva) and artificial structures (filling and impression materials, dental ceramics and alloys, titanium alloys of various surface types) was evaluated in SEM and stereomicroscope. The retention of FCs was associated with the viscosity of carriers but not with the type of the surface. All additional mechanical procedures (e. g. water spray, toothbrushing) negatively influenced the retention of nanocarriers. Results of the research has been published particulary and a patent No. PV 2010-628 has been issued. Clinical testing of FCs with relevant antimicrobial effect will follow.

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Title of the project: Neuropsychiatric Aspects of Neurodegenerative Diseases

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

Principal Investigator: E. Růžička

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

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

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

Summary of 2010 results

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

Authors: P. Smolik

Sleep disorders in neurodegenerative disorders had been proofed well in many scientific publications. Our study should contribute to the knowledge of the mutual influence of both psychopathology and sleep disorders and, pharmacological interventions in patients with different neurodegenerative disorders, first of all in those with dementia of Alzheimer type or mixed Alzheimer and vascular dementia and, various psychiatric disorders with the presumption of neurodegenerative processes according to recent scientific studies. The next steps in our research have elaborated two basic themes:

1. The relationship between NREM sleep and consolidation of long-term memory.
   Metaanalysis of scientific studies had been provided and was presented at the VII. Slovak-Czech congress of sleep medicine in Bratislava. The abstract was published in the Book of Congress Abstracts.
2. The role of melatonin in the treatment of sleep disorders.
   The opened clinical study of 55 patients treated with melatonin PR (prolonged release) had been realized and the results were presented at the 24. Czech and Slovak neurological congress in Hradec Kralove. The abstract was published in the Journal of Czech and Slovak Neurology 2010;73/106 (suppl 2), p. 79.

   The collection of dates in psychiatric patients with sleep disorders was provided in the year 2010 in concord with the main schedule of the common studies of Sleep disorders in neurodegenerative disorders.

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Title of the project: PurStem – Revolutionising the large-scale production of high quality adult stem cells

Grant Agency: 7FP EU	***Project Number:*** PurStem

Principal Investigator: T. Soukup

Co-investigators: F. Barry, M. Murphy, C. Coleman, R. Cancedda, Ch. Gentili, D. McGongale, E. Jones, T. Rowan, J. Thornton, S. Elliman, C. Clissmann

Starting date: 1.11.2008	***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 10575

Summary of 2010 results

Title of the presentation: PurStem – Revolutionising the large-scale production of high quality adult stem cells

Authors: Soukup T. (1), Barry F. (2), Murphy M. (2), Coleman C. (2), Cancedda R. (3), Gentili Ch. (3), McGongale D. (4), Jones E. (4), Rowan T. (5), Thornton J. (2), Elliman S. (6), Clissmann C. (7)

Charles University in Prague, Medical Faculty in Hradec Kralove (1), National University of Ireland, Galway, Ireland (2), University of Genoa, Italy (3), University of Leeds, Great Britain (4), Ovagen, Great Britain (5), ProCure Laboratories, Ireland (6), Pintail, IrelandCell (7)

Upon successfully isolating MSCs using the PurStem unified procedures developed in WP 1 of the project, MSC were then isolated and cultured using commercially available serum free media. Thereafter, an investigation of the levels of bFGF in media and released by MSC during culture and subculture was undertaken. The effects of PDGF-BB, EGF, bFGF, TGFB1 and GDNF as serum free supplements were also examined. MSC seeded on fibronectin coated plates proliferated in a serum free media that contained a combination of growth factors. Using the data obtained in WP3 and WP2, CUNI rationally selected growth factors with potential for use in serum-free culture as supplements to maintain MSC growth. Using CFU assays and xCELLigence technology CUNI started to test several combinations of growth factors / supplements and to perform high throughput analysis of proliferation and viability. The key results are: - increased proliferation capacity of cells cultivated in 0.5% FCS media supplemented with 0.125% HSA and growth factors (PDGF-BB and EGF); - increased number of CFU / cells attached following inoculation in media supplemented with ITS. For better comparison of results we defined surface markers and list of biomarkers specific for the optimal MSCs cultured in low-serum and serum-free conditions: - gender, age, CFU assay, PEf, viability; - proliferation (PD, DT), phenotype (P0, P1, P3); - telomere length (qPCR); - ROS - individual variability, H2O2 induction.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2013 under grant agreement no. 223298.

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Title of the project: The role of mitochondria in toxic liver injury and in liver regeneration

Grant Agency: Czech Republic ***Project Number:*** 305/09/P145

Principal Investigator: P. Staňková

Co-investigators:

Starting date: 1.1.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1446

Summary of 2010 results

Title of the presentation: The toxic effect of thioacetamide and galactosamine on rat liver in vivo

Authors: P. Staňková , O. Kučera , T. Roušar , H. Lotková , Z. Červinková
Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology

Mitochondria serve as a common target for hepatotoxic drugs. Studies of effective protection require knowledge of the mechanisms leading to liver damage. Thioacetamide (TAA) and D-Galactosamine (GalN) are widely used model hepatotoxins, but precise mechanism of their action has not been elucidated yet.
TAA (100 mg/kg) or GalN (400 mg/kg) were applied intraperitoneally to male Sprague-Dawley rats in one dose. Animals were sacrificed in 24, 48 and 72 hours after toxins administration and serum activities of ALT and AST, total bilirubin, total cholesterol, urea and TAG were measured. Part of the liver was removed for ATP content analysis, the other part was used for mitochondria isolation and for preparation of liver homogenate. Histopathological samples were also prepared. Various combinations of respiratory substrates were used for mitochondrial respiration (Oxygraph Oroboros-2k), mitochondrial membrane potentilal (TMRM accumulation, Aminco-Bowman), mitochondrial ROS production (CM-DCFDA, Tecan Infinite M200) and mitochondrial swelling measurements. Caspase -3 activity was estimated. Malondialdehyde production (TBARS, HPLC) and GSH/GSSG (HPLC) served as markers of the redox state.
We observed maximal liver injury 24 hours after TAA administration and 48 hours after GalN application. The signs of regenerative response were observed after 48 hours (TAA) and after 72 hours (TAA, GalN) respectively. Our results indicate that both hepatotoxins induce oxidative stress and inhibit to a greater extent the oxidation of NADH-dependent respiratory substrates than oxidation of flavoprotein-dependent substrates. It seems that modulation of substrate specificity could be interesting strategy to supply energy demand in regenerating liver and results are in agreement with experiments performed *in vitro* conditions.
This work was supported by research grants MSM 0021620820 and GAČR 305/09/P145

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Title of the project: The efficiency of colonic capsule endoscopy in detection of colorectal polyps and cancers comparing to colonoscopy: multicenter, prospective crosses over study

Grant Agency: Ministry of Health ***Project Number:*** NT/11524-5

Principal Investigator: Š. Suchánek

Co-investigators: I. Tachecí, P. Drastich, M. Beneš

Starting date: 1.10.2010 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 1682

Summary of 2010 results

Title of the presentation: The efficiency of colonic capsule endoscopy in detection of colorectal polyps and cancers comparing to colonoscopy: multicenter, prospective crosses over study

Authors: I.Tachecí[1], Š.Suchánek[2], P.Drastich[3], M.Beneš[3]

[1] Second Dpt. of Internal Medicine, University Hospital, Hradec Králové

[2] Department of Internal Medicine, Central Military Hospital, Praha

[3] Department of Hepatogastroenterology, IKEM, Praha

The Czech Republic belongs to the countries with high colorectal cancer (CRC) incidence and mortality (7828, resp. 4138 individuals in 2006). Most of the CRC rises from adenomatous polyps due to inherited or acquired genetic defects. Screening (early diagnosis in asymptomatic individuals aged over 50) is an important part of secondary prevention and the main aim is diagnosis and polypectomy of adenomas, diagnosis of early stages cancers and lowering the need of adjuvant therapy.

Our multicenter prospective study is focused on comparing efficiency of colonic capsule endoscopy and colonoscopy in detection of colorectal polyps and cancers in individuals with average risk of colorectal cancer. In years 2010 – 2014, 232 healthy people (asymptomatic individuals aged $\geq$ 50) will be examined, first by colonic capsule and afterwards by conventional colonoscopy. Besides the total number of detected polyps and cancers, the sensitivity and specificity in certain subgroups (size and characteristic) of polyps will be observed and the bowel preparation and acceptability of the examinations will be evaluated. One of the goals of the study is to verify whether capsule colonoscopy could be useful in colorectal cancer screening in population with high prevalence of this disease.

Our project started in October 2010. We developed new methodology and protocol of the capsule colonoscopy, organized 2 investigators and co-investigators meetings (including endoscopy nurses) and organized the successful pilot investigation.

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Title of the project: New therapeutic procedures during cardiopulmonary resuscitation in an animal model of ventricular fibrillation

Grant Agency: Ministry of Health ***Project Number:*** NS/10383-2

Principal Investigator: A. Truhlář

Co-investigators: V. Černý, Z. Turek

Starting date: 1.8.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 903

Summary of 2010 results

Title of the presentation: Terlipressin/adrenaline versus adrenaline alone in a time-realistic porcine model of prolonged ventricular fibrillation: a randomized controlled study

Authors: A. Truhlář (1,2), Z. Turek (1), V. Černý (1,3), D. Kodejšková (1), J. Suchánková (1)
Dept. of Anaesthesiology and Intensive Care Medicine, Charles University in Prague, Fac. Med. in Hradec Králové, University Hospital Hradec Králové (1); Hradec Králové Region Emergency Medical Services, Hradec Králové (2); Department of Anesthesia, Dalhousie University, Halifax, Nova Scotia, Canada (3)

Introduction: Recent data show that terlipressin, a vasopressin analogue, may be advantageous to restore blood pressure in asphyxial and prolonged cardiac arrests but its role in ventricular fibrillation (VF) remains unknown. The aim of this study was to compare haemodynamic effects of terlipressin/adrenaline versus placebo/adrenaline in a time-realistic model of VF. *Methods:* Fourteen domestic pigs (30-35 kg) were randomly assigned into group A (n = 7) and B (n = 7). After 5 min of untreated VF, compression-only resuscitation was applied for 10 min, followed by advanced life support for 45 min. At a time of 19 min, terlipressin in a single-dose of 30 μg•kg-1 was added to the first dose of adrenaline in group A, while placebo was given in group B. After that, adrenaline was administered every 3 min. Coronary (CorPP) and cerebral (CPP) perfusion pressures were calculated in all animals. Data were analyzed using repeated measurements ANOVA and a Fisher´s protected LSD post hoc test. *Results:* Adjunct terlipressin to adrenaline maintained CorPP above 10 mmHg for 17.7 min longer than adrenaline alone (P = 0.003) that was unable to prevent severe refractory hypotension. CorPP (mean ± S.D.) measured at 35, 45, and 55 min after the onset of VF was 12 ± 4, 11 ± 6, and 10 ± 5 mmHg in the terlipressin group A; and 6 ± 4, 1 ± 5, and -1 ± 4 mmHg in placebo group B (P = 0.03, < 0.001, and < 0.001). CPP measured at the same times was 23 ± 7, 20 ± 7, and 23 ± 7 mmHg in group A; and 13 ± 7, 6 ± 5, and 6 ± 7 mmHg in group B (P = 0.01, < 0.001, and < 0.001). *Conclusion:* The study showed that a single dose of terlipressin, when added to adrenaline in a time-realistic porcine model of VF, is effective for achievement of higher vital organ perfusion pressures compared to adrenaline alone. *The study was supported by grant IGA MH CZ NS10383-2/2009 and research project MZO 00179906.*

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Title of the project: Effects of intravenous anesthetic agents on hepatosplanchnic microcirculation in rats

Grant Agency: Charles University ***Project Number:*** 135809

Principal Investigator: Z. Turek

Co-investigators: M. Kaška, V. Černý

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 120

Summary of 2010 results

Title of the presentation: Effect of intravenous anesthetics on hepatosplanchnic microcirculation in rat – Sidestream dark-field (SDF) imaging study

Authors: Z. Turek (1), M. Kaška (2), V. Černý (1)

Fac. Med., Charles Univ., Hr. Kralove: Dept. of Anesthesia and Intensive Care (1), Dept. of Surgery (2).

Effect of intravenous anesthetic agents on macrohemodynamics has been in details described frequently. Immediate effects of these agents in hepatosplanchnic region at microcirculatory level are subject of intensive research. The goal of this study was to evaluate the microcirculatory alterations in hepatosplanchnic region in rat after induction dose and during continuous sedative dose of selected iv anesthetics when using Sidestream Dark-field (SDF). Male Wistar rats (n = 30) were anesthetized intravenously either with propofol (n=6), ketamine (n=6), midazolam (n=6) or thiopental (n=6) after preceding initial maximal dose of intraperitoneal pentobarbital (60mg/kg) to ensure 90 minutes of surgical anesthesia, also in control group (n=6), where only normal saline of corresponding volume instead of additional anesthetic was given. Microcirculatory parameters of the intestinal wall (functional capillary density - FCD of the longitudinal and circular muscle layer), of the liver (functional sinusoidal density - FSD and postsinusoidal venular velocity - PSVV) and of the renal cortex (FCD) were assessed by SDF imaging and LDF at the baseline, just after induction dose and after 30 and 60 minutes of sedation using an appropriate anesthetic agent. Macrohemodynamic data were monitored throughout the study.

When compared to baseline, statistically significant increase of both FSD ($p<0.01$, +25%), PSVV ($p<0.05$, +20%) and intestinal FCD ($p<0.05$, +15%) was observed in propofol group after induction dose, the same increase was confirmed when compared to control group. Statistically significant decrease of intestinal longitudinal ($p<0.05$,-18%) and circular FCD ($p<0.05$,-22%) was observed in ketamine group after induction dose. The midazolam group has shown statistically significant decrease in FSD and ileal FCD after induction dose and during sedation, thiopental group has shown no significant changes in microcirculatory parameters throughout the study. Project was supported by the Charles University Grant Agency, No 247/01

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Title of the project: Patient´s conception of illness - a topic for enrichment of the nursing model, nursing diagnostics and intervention

Grant Agency: Ministry of Health ***Project Number:*** NS/10348-3

Principal Investigator: E. Vachková

Co-investigators: J. Mareš, M. Schneiderová, M. Votroubková, H. Ulrychová

Starting date: 1.1.2009 ***Duration (years):*** 3

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

Summary of 2010 results

Title of the presentation: Patient´s conception of illness II.

Authors: Jiří Mareš (1), Eva Vachková (2), Michaela Schneiderová (2), Michaela Votroubková (2), Hana Ulrychová (2) Fac. Med. Charles Univ., Hradec Králové: Dept. of Social medicine (1), Division of Nursing (2)

The general objective of our research was, in selected health facilities, to study the patient's conception of diseases typical for the Czech population and to show possibilities of using the patient´s conception of illness in the individualization of nursing care. In 2010, we selected and translated three questionnaires with the permission of their authors. The IPQ-R, Revised Illness Perception Questionnaire (Moss-Morrisová, Weinman, Petrie, 2002) was translated, tried on 350 Czech patients, and standardized in a Czech version. Within the framework of a pilot study, the CIPQ-Children´s Illness Perception Questionnaire (Walker, Papadopoulos, Lipton et al., 2006) was tried on 40 patients, and finally, we translated and adapted the ICQ-Illness Cognition Questionnaire (Evers, Kraimaat, Van Lankveld et al., 1998). We also tried qualitative methods – the individual interview, which allows for a deeper analysis of patients´ views of their illness. Relying on the results, three types of studies were prepared for the monograph „Patient´s conception of illness II." (Mareš, Vachková et al., 2010): Theoretical level is represented by three review articles. They deal with the patient´s conception of health, social representation of health and disease, and the place of patient´s conception of illness in the targeted patient´s education by nurses. Methodological problems are represented by articles that examined the use and application of selected questionnaire methods for finding the patients´ approach to illness and validated these methods for Czech conditions. An overview of qualitative methods used abroad to a deeper understanding of patient´s conception of illness was worked out. The patients´ views of their condition obtained by interviews were analyzed as well. Terminology related to patients´ conception of their disease is represented by the second glossary of terms (Mareš, 2010).

Literature: J.Gaab IPQ-R (German Version): Causal Illness Attributions. Zürich: Universität Zürich, Klinische Psychologie and Psychotherapie, 2004, 6 p.

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Title of the project: Morphological Changes of Pulmonary Vascular Bed after Pulmonary Embolism (PE)

Grant Agency: Ministry of Health ***Project Number:*** NS/9691-4

Principal Investigator: Z. Vavera

Co-investigators: J. Vojáček, J. Malý, R. Pudil, P. Eliáš

Starting date: 1.1.2009 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 5944

Summary of 2010 results

Title of the presentation: Morphological Changes of Pulmonary Vascular Bed after PE

Authors: Zdeněk Vavera (1), Jan Vojáček (1), Jaroslav Malý (2), Radek Pudil (1), Pavel Eliáš (3)

University Hospital Hradec Kralove: 1st. Dept. of Medicine (1), 2nd Dept. of Medicine (2), Dept. Diagnostic Radiology (3)

The aim of our prospective study is to describe a relationship between simply and routinely aviable data (morphologic, lab, clinic, anamnestic) and risk of chronic thromboembolic pulmonary hypertension (CTEPH), as a complication of acute pulmonary embolism (PE). CTEPH has a strong negative impact on patient`s quality of life and life prognosis.

Despite it`s rather rare occurrence signifacance of CTEPH increases with possibility of aviable and succesful causal therapy (pulmonary endarterectomy).

This project also tries to answer the question of CTEPH incidence in PE patients population. We performed entry pulmonary artery CT angiography, echocardiography and lab (incl. troponin-T and NT-proBNP) examinations at day 0., control troponin-T and NT-proBNP assesment (if pathologic at day 0.) after 7-10 days. Cardiac ultrasound was repeated before discharge and after 6, 12 and 24 months. 6-months visit includes also control pulmonary artery CT angiography and D-dimer level assessment (when there is not known reason for its elevation). Patient recriutment finished in 2010 to ensure 24 month follow-up for all patients till the end of trial. Of about 160 involved patients 106 underwent 6-months visit, 92 patients 12-month visit and 54 finished follow-up with 24-months visit. 71% of patients had intraluminal abnormalities on control CT pulmonary angiography, 9 patients had pulmonary hypertension, 3 of them chronic thromboembolic, 6 of them of other causes. 1 of CTEPH patients is symptomatic, suitable for pulmonary endarterectomy, but refused by surgeon and recives specific farmacotherapy. Statistic evaluation of CTEPH risk factors will be done after data collection finishing.

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Title of the project: Catheterization treatment of patients with severe aortic stenosis

Grant Agency: Ministry of Health ***Project Number:*** NS/9741-3

Principal Investigator: J. Vojáček

Co-investigators: J. Šťásek, J. Harrer, J. Bis, J. Vojáček, M. Brtko, P. Polanský, M. Vejběra, J. Kovalský

Starting date: 1.1.2009 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands):

Summary of 2010 results

Title of the presentation: Catheterization treatment of patients with severe aortic stenosis

Authors: J. Vojáček

Altogether 38 patients (19 males, 19 females, age 62-87, mean 78.8±5.6 years) with significant aortic stenosis and very high risk for surgical aortic valve replacement underwent percutaneous aortic valve implantation (TAVI) at the Dept Interventional Cardiology, Dept Medicine I, Hradec Kralove in the time period from January 2009 through December 2010. Transfemoral implantation was performed in 28 patients and transapical in 10 subjects. TAVI Heart Team was created with two leading cardiac surgeons and two leading interventional cardiologists and this Team made final decisions on the mode of treatment of each high risk patient with aortic stenosis. Edwards-Sapien and later Edwards-Sapien XT valves were implanted, 23 mm valve in 19 and 26 mm valve in 19 patients. The aortic gradient decreased from 46.9±15.2 mm Hg to 13.8.±1.4 mm Hg and 10 patients had 1/4 and 5 patients 2/4 degree of aortic regurgitations with no severe aortic regurgitation. There was no mortality during the procedure and during the first 30 days. One patient with transapical approach had low position of implant and therefore immediately second valve to correct position had to be implanted. Two patients had hemopericardium with successful drainage, one patient had lower extremity ischemia with successful vascular surgery and two patients had stentgraft implanted to the femoral artery. Two patients suffered impairment of renal functions with hemodialysis and 4 patients with transfemoral and 2 with transapical approach required transfusions.

In conclusion, TAVI is a new and very effective and save alternative of treatment for patients with significant aortic stenosis and very high risk for classical surgical aortic valve replacement.

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Title of the project: Cytotoxicity of Dental Materials

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

Principal Investigator: L.Vavřičková

Co-investigators: T. Dostálová, J. Ulrichová

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

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

Summary of 2010 results

Title of the presentation: Cytotoxicity of Dental Materials

Authors: L. Vavřičková (1), T. Dostálová (2), J. Ulrichová (3)

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Department of Medical Chemistry and Biochemistry, Faculty of Medicine and Dentistry, Palacky University, Olomouc (3)

The corrosion and cytotoxicity of dental materials are more related to metallic materials than non-metallic ones. In comparison with some dental alloys the ceramic materials are considered biocompatible with maximum material safety. The aim of this study was to determine the cytotoxic influence of various dental materials.

Altogether 11 types of ceramic materials and 6 dental alloys have been chosen for the analysis. The test monitored the cytotoxic influence of the solid sample on the cell line of mouse fibroblasts NIH 3T3 in the cell culture. The test of direct contact and the extract test were monitored for testing of the cytotoxicity in vitro.

All materials were considered non-toxic in the direct contact test. Lithium disilicate ceramics IPS e.max Press was considered slightly toxic in the extract test. Biocompatible materials must be as neutral as possible and not cause adverse allergic reactions or inadequate response of the immune system. Although dental ceramics is considered biocompatible, biocompatibility depends on the type and composition of the materials. All tested dental materials can be considered non-toxic in the direct contact test. Lithium disilicate ceramic was little toxic in the extract test, probably caused by presence of lithium ions in the material. The cytoxicity of this type of ceramic material was worse in comparison with Ni-Cr dental alloys in study tested which however slightly contradicted with the data gained from literature where the chrome-nickel dental alloys are considered cytotoxic.

Project was supported by Charles University Grant Agency No. 81508.

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Title of the project: Corrosion of Prosthetic Dental Materials

Grant Agency: Ministry of Health ***Project Number:*** NS/9744-3

Principal Investigator: : L. Vavřičková

Co-investigators: D. Dufková, T. Dostálová, A. Krejčová, J. Šrámková,

Starting date: 18.5.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 1270

Summary of 2010 results

Title of the presentation: Corrosion of Prosthetic Dental Materials

Authors: L. Vavřičková (1), D. Dufková (1), T. Dostálová (2), A. Krejčová (3), J. Šrámková (3)

Department of Dentistry, Charles University in Prague, Faculty of Medicine in Hradec Králové (1), Department of Paediatric Stomatology, Charles University, 2nd Medical Faculty, Praha (2), Department of Analytic Chemistry, Faculty of Chemical Technology, University of Pardubice (3)

The aim of this study was to determine the level of corrosion and verify the release of elements from the saliva of selected dental alloys and ceramic materials by GF-AAS and ICP-MS.

In total, 11 types of ceramic materials and 6 dental alloys have been chosen for the analysis.

The composition of ceramic materials was qualitatively evaluated by X-ray spectrometry. For evaluation of ceramic materials´ corrosive behaviour, leaching under extreme conditions that does not occur in real usage was carried out (3 ml 0.1 mol.l^{-1}, HCl, 120 hours, 37 ± 1 °C) and analysed by mean ICP- OES. The saliva was analyzed by GF-AAS after delivering simulated denture (cast from dental alloy) for the corrosion behaviour of the materials.

In extracts of ceramic materials, measurable amounts of followed elements were found: sodium (0.065 – 1.1), magnesium (0.012 – 0.15), iron (0.32 – 1.2), manganese (0.0042 – 0.096), zinc (0.014 – 2.7), silicon (up to 0.63), aluminium (up to 0.97), yttrium (up to 0.21) and titanium (up to 2.6, all in mg.l^{-1}). Zirconium and gold were found in leachates. Chromium release in dental alloys depends on the dental alloy composition and the time after delivering simulated denture. The nickel release depends on the delivering time only.

The presence of released ions in the extracts was confirmed for all dental materials tested. None of the known dental materials including ceramics can preserve absolute resistance against all corrosion forms.

Project was supported by the Internal Grant Agency of Ministry of Health No. NS 9744-3

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

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

Principal Investigator: J. Vojáček

Co-investigators: H. Ševčíková, R. Ševčík, M. Pecka, J. Malý, I. Fátorová

Starting date: 1.1.2007 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 0

Summary of 2010 results

The antiplatelet effect of acetylsalicylic acid (ASA) and clopidogrel (CLO) varies among individual patients. We assessed the short-term (STR) and long-term reproducibility (LTR) of light transmission aggregometry (LTA) in patients treated by ASA and MULTIPLATE analyser assessment after high sensitivity adenosindiphosphate (HS ADP) stimulation in those on CLO. We also prospectively evaluated the correlation between inadequate platelet aggregation response to ASA and MMP-2, MMP-3, MMP-9, TIMP-2, MCP-1, sCD40L, hsCRP and endoglin plasma levels. Moreover, the relationship between TF and TFPI plasma level and the platelet aggregability assessed by LTA was analyzed in patients treated by ASA.**Methods:** Residual platelet reactivity (RPR) was measured twice using LTA in group of 207 consecutive patients (56 females, mean age 67±9 years) on ASA therapy in 10 ± 6 month interval and MULTIPLATE analyser assessment was performed in 93 subjects (males 69, females 24, age 66.9 ±9.9 (40- 85) years on CLO in the interval 6±4 months. STR was assessed in 15 patients (6 females, mean age 61 ± 7 years) with 10 measurements on 2 consecutive days. Light transmition aggregometry was performed to assess residual platelet activity (RPA) in 64 patients (12 females, 52 males, age 43-81, mean 62.52 $\pm$ 8.48 years), in whom also TF, TFPI, matrix metalloproteinasis-2 (MMP-2), matrix metalloproteinasis-3 (MMP-3), matrix metalloproteinasis-9 (MMP-9), tissue inhibitor of metalloproteinasis-2 (TIMP-2), monocyte chemoattractant protein-1 (MCP-1), soluble CD40 ligand (sCD40L), high sensitivity CRP (hsCRP) and endoglin plasma levels were measured.

Results: There was no correlation between both measurements in the long-term part of the study and also Bland Altman plot showed typical diverging pattern in LTA assessment of the effect of ASA therapy and MULTIPLATE analyser assessment of CLO. However, LTA and MULTIPLATE analyser STR was very good with correlation coefficient 0.800 and 0.793, respectively ($p<0.05$), confirmed by Bland Altman plot. No association was found between high RPR and MMP-2, MMP-3, MMP-9, TIMP-2, MCP-1, sCD40L, hsCRP and endoglin plasma levels. The proportion of patients with high RPR was significantly increased in those with plasma level of TF > 190 ng/mL (14.3% vs 4.3 %) ($p<0.05$) as well as in those with TFPI plasma level < 20 pg/mL (17.4% vs 3.6%) ($p<0.001$). Increase of TF (204.7$\pm$35.2 ng/mL vs 174.9$\pm$69.2 ng/mL, $p<0.05$) as well as decrease of TFPI (20.2$\pm$10.0 pg/mL vs 26.3$\pm$9.2 pg/mL, $p<0.05$) were observed in patients with high RPR.**Conclusions:** Whereas short term intraindividual reproducibility of the assessment of platelet reactivity is very good, in the long term perspective the antiplatelet ASA effectivity may be influenced by additional variables and repeated measurements are warranted. Increased RPR in patients treated with ASA is associated with increased TF and decreased TFPI plasma levels.

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Title of the project: Research and development of the new isolation technique of conventional and unheard-of amaranth grain components for industrial utilization and nutritional products fortification

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

Principal Investigator: Z. Zadák

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

Starting date: 1.7.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 625

Summary of 2010 results

Title of the presentation:

The evaluation of Amaranth and yeast protein quality on the growth curves of rats

Authors: Z. Zadák, A. Tichá, R. Hyšpler, M. Slanařová, D. Solichová, I. Svobodová, P. Žďánský, J. Krejcarová, M. Vacková, S. Janáčková

Dept. of Metabolic Care and Gerontology

The Aim of study: The biological value of proteins is the decisive factor in the effect of supplemented proteins on nitrogen balance. Grain amaranth protein contains around 5% lysine, which is limiting amino acid in other grain. The purpose of our study was to prepare a protein mixture with high biological value (mixture amaranth and yeast of autolysate 8:11), evaluate it on the growth and nitrogen metabolism in rats and compared it with casein and only yeast diet.

Methods: 24 male Wistar rats were divided into 3 diet groups of 8 animal each. Mixture of amaranth and yeast vs. casein and vs. yeast diets were tested. Daily intake of the diets and body weight of rats were investigated during 35 days. The biochemical parameters of nitrogen metabolism in the blood and urine and total nitrogen in faces and stercus of the terminal small intestine were determined once a week. The nitrogen balance and digestibility were calculated.

Results: Results are presented as mean ± SD. Growth rate (%/day) was found for casein group (CG) 2.78% ±0,33; amaranth-yeast group (A-YG) 3,34% ±0,58 and yeast group (YG) 2,89%±0,0,58. The significant differences were found in nitrogen balance (CG – 0,81±0,04; A-YG - 0,78±0,035; YG -0,92±0,1; P 0,002), plasmatic total protein (CG – 65±3,5 g/l; A-YG - 59±1,4 g/l; YG -57,6±1,2 g/l; P 0,001), albumine (CG – 41,2±1,5 g/l; A-YG - 39,5±0,9 g/l; YG -36,8±3 g/l; P 0,003), total cholesterol (CG – 2,2±0,3 mmol/l; A-YG - 1,5±0,2 mmol/l; YG -1,5±0,2 mmol/l; P 0,001) and digestibility (CG – 84,2±0,5, A-Y group – 85,8±1,5; P 0,05) .

Conclusions: The diet prepared with amaranth and yeast of autolysate had a very good influence on the growth and nitrogen metabolism of rats. This protein mixture as a valuable source of high-quality protein of non-animal origin.

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

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

Principal Investigator: Z. Zadák

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

Starting date: 1.8.2008 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 280

Summary of 2010 results

Title of the presentation: The effect of Imunocomplex© formulation on inflammation marker neopterin in elderly patients – a clinical trial

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

The rationale: β-glucans are long-chain polysaccharides composed of glucose units. These compounds are considered a non-specific immunomodulants stimulating macrophages, T-lymphocytes, NK-cells or activators of complement system. These effects are mediated by Tall-like receptors 2 (TLR 2). This dietary supplement was produced by C2P company (Czech Republic).
Methods: Fifty institucionalized patients (age 60 – 99 years) were randomly divided into two groups, treated group (A) and placebo group (B). Group A was supplied with one capsule of Imunokomplex three-times a day for thirty days. The clinical examination and urine sampling for neopterin/creatinine ratio (a marker of immune system activation) was performed in days 0, 3, 7, 30 and 60. Data are presented as median (interquartile range) of dimensionless value.
Results: The significant changes in clinical status were not found. The neopterin/creatinine ratio changed from 0.336 (0.262, 0.462) to 0.296 (0.189, 0.443) and from 0.355 (0.252, 0.626) to 0.339 (0.247, 0.448) in group A and B, respectively. The change was not significant in either group, although group A showed slight decrease in neopterin/creatinine ratio.
Conclusions: Evaluated dietary suplement was well tolerated by the patiens. No significant adverse effects were found. The insignifance of neopterin/creatinine ratio results is probably owing to a large interindividual variability of this test and more specific laboratory tests should be used.

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

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

Principal Investigator: Z. Zadák

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

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

Total funds allocated for project - Kč (thousands): 26853

Summary of 2010 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. Bureš (2), V. Černý (3), A. Ferko (4), P. Hůlek (5), R. Hyšpler (6), N. Jirásková (7), J. Malý (8), B. Melichar (9), V. Palička (10), J. Petera (9), A. Ryška (11), V. Tošnerová (12), J. Vojáček (13)

Faculty of Medicine and Teaching Hospital, Hradec Králové:

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

Dr. Hyšpler – The team working in the area of the impairment and reparation of DNA have completed its methodological basis, and in the form of pilot experiments have applied it to two principal fields: 1) use of the method of impairment and reparation of DNA as a significant indicator of the action of cytostatics in the treatment of pulmonary tumours; 2) application and reparation of DNA in patients at risk exposed to numerous toxic medicaments including antimycotic agents and the effects of higher concentrations of oxygen in ventilation with a high degree of FiO_2. A methodological model of the selection of patients and the use of SCGE (employing the study of DNA impairment in separated lymphocytes) has been prepared in this field. By means of research projects a unique laboratory has been established for the study of impairment and reparation of DNA, the employment of which has an important role for the future. The team working in the field of clinical physiology of nutrition and indirect calorimetry have completed another part of the study, which was published the journal *Nutrition International* and which elaborated a corrected Benedict-Harris equation for pregnant women. Among other things, an important result is the verified technology Epicream (PN OT – 11-2010) for the manufacture of the product. It includes the protocol concerning the test determining skin sensitivity No. 291110 (a single-use closure test using 15 volunteers aged from 26 to 59 years) and the protocol concerning the microbiological test No. 3432/10, sample number 7742 (testing laboratory 1082 accredited by ČIA). For this verified technology the functional design Epicream (PN OT – 11-2010) has been manufactured, which is also a valuable result.

Prof. Malý – A considerable contribution is the result obtained in the treatment of macular degeneration by means of haemorheopheresis. Similarly a very valuable result is the

employment of the new biomarker for the detection of cardial toxicity in the course of transplantation of the bone marrow in hematological malignities. The subgroup working in the field of oncology have significantly contributed to the field of high-dosed brachytherapy. The cardiological subgroup have very significantly developed the field of the study of myocardial impairment in radiofrequency catheter ablation and also the endocrinological aspects of diagnostics of primary aldosteronism.

Prof. Bureš – A multicentric epidemiological study of the prevalence of infection by *Helicobacter pylori* and dyspepsia in the Czech Republic. Bacteriocinogenia in an experimental model of entero- and colopathy due to non-steroidal antiphlogistics. Ex-vivo confocal laser endomicroscopy of gastro- and enteropathy due to non-steroidal antiphlogistics. Computer-assisted morphometry of the small intestine after non-steroidal antiphlogistics. Capsule endoscopy of the small intestine in patients suffering from rheumatoid arthritis and osteoarthrosis with microcytic anaemia and without anaemia.

Prof. Černý – Evaluation of different volume liquid regimens of cooling at the stage after the arrest of circulation on the parameters of macrocirculation. Evaluation of the efficiency of the mechanical support of the heart (Heart Mate) on microcirculation. Effects of the nature of the insult and age on the rapidity of convalescence of organ functions on patients at risk.

Prof. Palička – Extensive research activities (with pertinent publications) have been devoted to the metabolism of the bone marrow and the effects of various substances on it (ezetimibe, high-protein diet and diet with increased glutamine content, blockers of the calcium channel). A great attention has been paid to the improvement of the diagnostics of cardiovascular diseases with the use of new biomarkers and protein biochips. There has been a great effect of the publications based on many years lasting studies focused on the genotoxic and apoptotic effects of Goekerman therapy in patients suffering from psoriasis, as well as the examination of urine mutagenicity and genotoxic risk in children with psoriasis, exposed to polycyclic aromatic hydrocarbons and UV radiation. The subgroup of ophthalmologists have been devoted to the research of the artificial cornea, keratoprosthesis AlphaCor, and have priority in this activity in the Czech Republic. The subgroup of pathologists have studied inflammatory infiltrates in chronic sclerotizing sialoadenitis using immunohistochemical analysis, primarily the changes in tumour tissues. It included both the quantification of the inflammatory response in the carcinoma of the esophagus and immunohistochemical analysis of mismatch repair proteins in colorectal tumours. The oncological subgroup have continued to examine the effects of chemotherapy and targeted treatment on the systemic inflammatory response and retinol and α-tocopherol levels. A number of presentations of tumour diseases have been published, interesting from the scientific standpoint and clinically unusual, e.g. isolated liver metastases of renal carcinoma or duplicate tumour of the kidney in a patient with metastazing colorectal carcinoma. The team further elaborates the determination of cell cytotoxicity in patients with immunodeficits.

Assoc. Prof. Tošnerová – The team have been devoted to the practical use of their results in industry, namely the development of two utility designs and one functional design of the training device “Dragofly” which is intended to improve the manipulation with and rehabilitation of patients with impaired mobility.

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