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

XVI. VĚDECKÁ KONFERENCE

P R O G R A M

25. ledna 2012

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

XVI. vědecká konference Lékařské fakulty Univerzity Karlovy v Hradci Králové a Fakultní nemocnice Hradec Králové 25. ledna 2012

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. Milan Holeček, DrSc.**

09.15 - 09.30 Sledování opacit zadního pouzdra po operaci katarakty
MUDr. Marie Kalfeřtová
GA UK 103809 (LF)

09.30 - 09.45 Studium potenciálního významu epigalokatechingalátu v prevenci a terapii jaterního poškození podmíněného intrahepatální a extrahepatální cholestázou
Mgr. Petra Hiršová
GA UK 132309 (LF)

09.45 - 10.00 Úloha echokardiografie při optimalizaci biventrikulárního stimulátoru
MUDr. Rudolf Praus
GA UK 66809 (LF)

10.00 - 10.15 Účinky seleničitanu sodného na proliferaci a buněčnou smrt buněk kolorektálního karcinomu s odlišným p53 genotypem
Mgr. Soňa Benešová (RNDr. Věra Králová, Ph.D.)
GA UK 129609 (LF)

10.15 – 10.30 Využití "cone beam" CT pro rekonstrukci dávkové distribuce obrazem řízené radioterapie ca prostaty - porovnání zaměření na kosti vs. zaměření na implantované markery
Ing. Petr Paluska
GA UK 144210 (LF)

10.30 – 11.00 *Přestávka – občerstvení*

Sekce II Předsedající: **prof. MUDr. Vladimír Geršl, CSc.**

11.00 - 11.15 Identifikace biomarkerů intraamniální infekce a syndromu zánětlivé odpovědi plodu v plodové vodě: proteomický přístup
MUDr. Marian Kacerovský
IGA MZ NS/10382-3/2009 (FN)

11.15 - 11.30 Morfologické změny thymu a nadledvinek v ultrazvukovém obraze plodu jako marker syndromu fetální zánětlivé odpovědi.
MUDr. Marian Kacerovský
GA ČR 304/09/0494 (LF)

11.30 - 11.45 Charakteristika odolnosti dospělých kmenových buněk vůči genotoxickému stresu.
doc. MUDr. Martina Řezáčová, Ph.D.
GA ČR 304/09/1568 (LF)

11.45 - 12.00 Pacientovo pojetí nemoci - téma obohacující ošetřovatelské modely, ošetřovatelskou diagnostiku a intervenci
Mgr. Eva Vachková
IGA MZ NS/10348-3/2009 (LF)

12.00 - 13.30 *Přestávka na oběd*

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

13.30 - 13.45 Vliv systémové léčby rheoferézou na funkční stav sítnice u věkem podmíněné makulární degenerace
doc. MUDr. Hana Langrová, Ph.D.
IGA MZ NS/9738-4/2008 (LF)

13.45 - 14.00 Pilotní studie neinvazivního monitorování hepatotoxicity v průběhu dlouhodobé farmakokineticky řízené léčby psoriázy metotrexátem a kyselinou listovou
doc. Ing. Jaroslav Chládek, Ph.D.
IGA MZ NS/10364-3/2009 (LF)

14.00 - 14.15 Orální zdraví předškolních dětí, příjem fluoridů a postoje a chování rodičů v prevenci zubního kazu dočasného chrupu
doc. MUDr. Romana Ivančaková, CSc.
IGA MZ NS/10353-3/2009 (LF)

14.15 - 14.30 Hemodynamické, klinické a biochemické sledování nemocných před a po transjugulárním intrahepatálním portosystémovém zkratu (TIPS), část IV
prof. MUDr. Petr Hůlek, CSc.
IGA MZ NS/10363-3/2009 (LF)

14.30 - 14.45 Patogeneze malnutrice při renálním selhání a vliv léčebné intervence
prof. MUDr. Vladimír Bláha, CSc.
IGA MZ NS/9743-4/2008 (LF)

14.45 – 15.15 *Přestávka – občerstvení*

Sekce IV Předsedající**: prof. MUDr. Radek Pudil, Ph.D.**

15.15 – 15.30 Předoperační regionální chemoterapie v léčbě karcinomu tlustého střeva
prof. MUDr. Alexander Ferko, CSc.
IGA MZ NS/9690-4/2009 (FN)

15.30 – 15.45 Morfologické změny plicního řečiště po plicní embolii
MUDr. Zdeněk Vavera
IGA MZ NS/9691-4/2009 (FN)

15.45 – 16.00 Neoadjuvantní léčba karcinomu prsu
MUDr. Jiří Grim, Ph.D.
IGA MZ NS/10373-3/2009 (FN)

16.00 – 16.15 Individuální predikce dávkového režimu 5-fluorouracilu v léčbě kolorektálního karcinomu
MUDr. Jiří Grim, Ph.D.
IGA MZ NS/9693-4/2009 (FN)

16.15 – 16.30 Sledování kvality tkáňové perfuze během kardiochirurgické operace s použitím miniinvazivního mimotělního oběhu pomocí přímé tkáňové oxymetrie
doc. MUDr. Jiří Manďák, Ph.D. (MUDr. Vladimír Svitek, Ph.D.)
IGA MZ NS/10376-3/2009 (FN)

16.30 – 17.00 *Přestávka – občerstvení*

Sekce V Předsedající: **prof. MUDr. Jaroslav Mokrý, Ph.D.**

17.00 – 17.15 Stanovení volných lehkých řetězců jako pomocného markeru v hodnocení prognozy a odpovědi na léčbu u nemocných s mnohočetným myelomem a monoklonální gamapatií nejasného významu
doc. MUDr. Vladimír Maisnar, Ph.D.
IGA MZ NS/10387-3/2009 (FN)

17.15 – 17.30 Utilisation of the mesenchymal stem cell receptome for rational development of uniform, serum-free culture conditions and tools for cell characterization.
MUDr. Tomáš Soukup
FP7 PurStem **(LF)**

17.30 – 17.45 Chronická onemocnění vznikající na podkladě nepřiměřené reaktivity imunitního systému, jejich patogeneze a možnosti včasné diagnostiky a léčby
prof. RNDr. Jan Krejsek, CSc.
(odp. řešitel: prof. MUDr. Jiřina Bartůňková, DrSc. - 2.LF UK)
MSM 0021620812 **(LF)**

17.45 – 18.00 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
prof. MUDr. Radek Pudil, Ph.D.

SOUHRNY VÝZKUMNÝCH ÚKOLŮ

ŘEŠENÝCH NA LF UK A VE FN V HRADCI KRÁLOVÉ

(ABECEDNĚ)

Title of the project: Diffuse large B cell lymphoma and follicular lymphoma - analysis of prognostic factors and treatment guideliness to patient´s outcome; lymphoma project of Czech Republic

Grant Agency: Ministry of Health ***Project Number:*** NT/12193-5

Principal Investigator: M. Trněný

Co-investigators: D. Belada, L. Boudová, A. Janíková, M. Jankovská, T. Papajik, K. Kubáčková, M. Matuska, M. Lysý

Starting date: 1.6.2011 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 7026

Summary of 2011 results

Title of the presentation: Diffuse large B cell lymphoma and follicular lymphoma - analysis of prognostic factors and treatment guideliness to patient´s outcome; lymphoma project of Czech Republic

Authors: M. Trněný (1), D.Belada (2), L.Boudova (3), A.Janíková (4), M.Jankovská (5), T.Papajik (6), K.Kubáčková (7), M.Matuska(8), M.Lysý (9)

General Hospital Prague, Dept.of Internal Medicine (1), Clinic of Internal Medicine, Charles University and Teaching Hospital, Haematology Dept., Hradec Kralove (2), Fac.Hospital Plzen (3), Fac.Hospital Brno (4), Fac.Hospital Olomouc (6), Fac.Hosp.Kralovske VInohrady, Prague (5), Faculty Hosp.Motol, Prague (7), Fac.Hosp.Ostrava (8), Hospital Ústí Nad Labem (9)

Czech Lymphoma Study Group (CLSG) consist of the majority of University Hospitals in Czech Republic (CR) which are focused on lymphoma diagnosis and management as well as of the number of regional hematology and oncology centers. The proposed multicentric project is based on the current level of collaboration in this field in Czech Republic and is focused on analysis of selected subgroups of Non-Hodgkin´s lymphomas (Diffuse large B cell lymphoma and follicular lymphoma) registered in CLSG registry and to describe the situation in Czech republic - clinical features, therapy, outcome. The number of patients in our databasis is estimated about 6000. The importance of proposed project is seen in the addressing of unanswered question of description of "real-life" situation in lymphoma field in Czech Republic and the analysis of different lymphoma subgroups and the description of clinical features, therapeutic approaches and the outcome of patients in Czech Republic. During 2011 we analysed cohort of patients with relapsed follicular lymphoma - retrospective analysis on subgroup of patients has been performed. In our clinic about 60 newly diagnosed patients with diffuse large B cell lymphoma has been diagnosed, and about 15 newly diagnosed patients with follicular lymphoma. All these patients were included into analysis. Results has been presented and discussed on local meetings in Czech Republic as well as on international meeting of American Society of Haematology in December 2011 and International meeting in Lugano.

Project was supported by Internal Grant Agency of Ministry of Health NT/12193-5.

Address for correspondence: Czech Lymphoma Study Group -David Belada, Clinic of Internal Medicine, Charles University and Teaching Hospital, Haematology Dept., Sokolska street 581, Hradec Kralove 5, 50005, Czech Republic.

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: S. Benešová (2011), V. Králová (2009-2011)

Co-investigators: E. Rudolf

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

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

Summary of 2011 results

Title of the presentation: Selenite-induced apoptosis and autophagy in human colon cancer cells in vitro.

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

Selenium compounds are known to inhibit proliferation of malignant cells and induce various types of cell death. In colon cancer cells, sodium selenite (Se) activates several signaling pathways whose interactions and ultimate endpoints may vary in individual study models. In our study we showed that Se induced cell cycle arrest, inhibited proliferation and activated apoptosis in human colon cancer cells in vitro. We also found differences in sensitivity to Se-dependent growth inhibition and apoptosis in colon cancer cells with functional (HCT 116) and deleted (HCT 116-p53KO) p53 gene. Moreover, detailed morphological and biochemical analyses revealed the presence of autophagy in Se-treated cells. We showed that apoptosis is accompanied by autophagy in Se-treated HCT 116 cells and that the absence of p53 in these malignant colonocytes changes patterns of response to Se-induced stress including respective roles of apoptosis and autophagy.

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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):*** 3,5

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

Summary of 2011 results

***Title of the presentation*: S**kin perfusion (SP) during hemodialysis (HD) and nutritional status relate to later occurrence of ischemic skin defects and cardiovascular (CV) death

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

Objectives: to analyze whether HD patients with critical skin perfusion may suffer from ischemic skin defects, cardiovascular events and mortality more frequently and to find the optimal balance between efficacy and safety of hemodialysis in each individual patient. **Methods:** Peripheral skin blood flow was measured using a laser Doppler line scanner (LDLS®, Moor, Devon, UK) in 10 different areas of the dorsal part of the instep and the toes of each foot before and during HD with ultrafiltration (897 ± 465 mL/procedure) in 31 HD patients (10 female, 21 male; age 36-79 y, BMI = 28 ± 5.0). No skin defects or apparent acute disease or infection were detected in any patient at the time of LDLS measurement. The feet of the patients were clinically re-examined carefully over the next 18 months. Kaplan Meier logrank analysis was used to analyze possible effects of skin perfusion on incidence of new skin defects and wounds, cardiovascular events and survival of patients. **Results:** SP before and during HD correlated with S-albumin (r=0,36, p=0,05; r=0,47, p=0,007), respectively. Skin perfusion significantly related to CV mortality, as shown in Table:

Mortality		Survivals, n=25	Non-survivals, n=6	Pvalue(t-test)
Skin perfusion before HD	Toes	96 (65; 131)	69 (68; 73)	0.003
	Instep	102 (90; 117)	84 (69; 91)	0.08
Skin perfusion during HD	Toes	91 (38; 156)	62 (43; 88)	0.32
	Instep	65 (40; 84)	46 (25; 50)	0.12

Conclusions: We have shown that decrease in skin microcirculation may predict future development of skin defects, cardiovascular events and mortality, i.e. that even hemodynamically stable and therefore "safe" HD procedure can have silent long-term consequences.

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: The role of insulin resistance in the pathogenesis of cardiometabolic risk in diabetes mellitus

Grant Agency: Ministry of Health ***Project Number:*** NT/12287-5

Principal Investigator: V. Bláha

Co-investigators: F. Musil, A. Šmahelová, R. Hyšpler, J. Lesná, J. Víšek, M. Bláha, L. Sobotka, D. Solichová

Starting date: 1.7.2011 ***Duration (years):*** 4,5

Total funds allocated for project - Kč (thousands): 6907

Summary of 2011 results

***Title of the presentation*:** Comparison of the impact of a low-glycemic index diet and a commonly used diabetic diet – a randomized crossover study.

Authors: V. Bláha , J. Víšek, S. Lacigová*, D. Čechurová*, Z. Rušavý*, F. Musil, A. Šmahelová, R. Hyšpler, J.Lesná, M.Bláha, L. Sobotka, D. Solichová. Department of Gerontology and Metabolism, University Hospital in Hradec Kralove, *Department of Medicine I, University Hospital and Medical Faculty in Pilsen, Czech Republic.

Background: Most studies on low-GI diet usually compare diets with low and high GI which does not necessarily take into account the common dietary recommendations for patients with diabetes.

Objective: The aim of this study was to compare the impact effectiveness of a diet with a low glycemic index versus a common diabetic diet in selected metabolic and anthropometric parameters.

Design: 20 volunteers with the type 2 diabetes treated only with metformin were randomly split into two groups. Each group was advised to follow a common diabetic diet (DD) or a diet with a low glycemic index (GI) for a period of 3 months in a crossover design. The effectiveness of the two diets was evaluated according to the selected metabolic and anthropometric parameters using a hyperinsulinemic euglycemic clamp with endogenous glucose production measurement, indirect calorimetry and bioimpedance analysis.

Results: Body weight after 3 months following DD was 93kg (83-104) vs. GI 92kg (85-104) $p<0.05$, BMI DD 31.3 kg/m^2 (27.5-35.9) vs. GI 30.7 kg/m^2 (27-35.3) $p<0.05$, body fat DD 28% (25.5-43) vs. GI 27% (23-43) $p<0.05$ (data are presented as a median and interquartile range). The diets did not differ in effects on glycosylated hemoglobin, fasting glucose, lipid profile, insulin sensitivity or hepatic glucose production.

Conclusions: In comparison with a common diabetic diet, the diet with low GI leads to a slight weight loss, as well as the BMI and body fat reduction. The diet with low GI can be used as an additional tool for weight reduction in diabetic patients.

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: Evaluation and development of new perspective antimycobacterial 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, P. Paterová, M. Vejsová

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

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

Summary of 2011 results

Title of the presentation: Evaluation of in vitro antimycobacterial activity newly designed pyrazine derivatives

Authors: V. Buchta (1)*, P. Paterová (1), M. Vejsová (1), J. Vinšová (2), M. Doležal (3)
Dept. of Clinical Microbiology, University Hospital Hradec Králové (1), Dept. of Pharmaceutical Chemistry and Drug Control (2) and Dept. of Inorganic and Organic Chemistry (3), Faculty of Pharmacy, Charles University, Hradec Králové

In the period 2009 to 2011, 130 new pyrazine carboxylic acid derivatives were tested for in vitro antimycobacterial activity. Most of them represented substituted chloropyrazines prepared with aminodehalogenated microwave-assisted reactions using appropriately substituted amines (aryl-, alkyl-, benzyl-) or aromatic hydrazines. The structure of most pyrazine compounds was confirmed by NMR, IR and elemental analysis.

Testing in vitro antimycobacterial activities was carried out at the Department of Clinical Microbiology, University Hospital in Hradec Kralove against M. tuberculosis H37Rv, M. tuberculosis CNCTC My 152/73, M. kansasii CNCTC My 235/80, M. avium CNCTC My 80/72 M. avium CNCTC My 152/73 by means of modified microdilution broth method. Tests were performed on Šula medium (pH 5.5) and strains were incubated at 37°C. Results, minimum inhibitory concentration (MIC, mg/L), were read after 10 to 14 days.

N-(3-iodo-4-methylphenyl)pyrazine-2-carboxamide was the most efficient among pyrazine-carboxamide derivatives with MIC < 2.0 mg/L [1], of alkylaminopyrazines showed the best effect 5-methyl-6-(octylamino)pyrazine-2,3-dicarbonitril (MIC = 8.0 mg/L against M. tuberculosis H37Rv) and, in addition, against MOTT (Mycobacteria Other Than Tuberculosis) [2]. In the case of aryl-and alkylaminopyrazines there was a direct correlation between antimycobacterial effect and molecular weight of the compounds and their lipophilicity.

1. Doležal, M. et al. Molecules, 2010,15(12):8567-81.
2. Doležal, M. et al. Book of Abstracts: The 18th EuroQSAR Symposium, Discovery Informatics & Drug Design, Greece, Rhodes, September 19-24, 2010, p. 335 (II-95).

Address for correspondence: *Dept. of Clinical Microbiology, University Hospital Hradec Králové, Sokolská tř. 581, 500 05 Hradec Kralové; buchta@fnhk.cz

Title of the project: The 8th International Medical Postgraduate Conference

Grant Agency: Charles University ***Project Number:*** 262905

Principal Investigator: M. Červinka

Co-investigators:

Starting date: 1.1.2011 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 710

Summary of 2011 results

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

Authors: M. Červinka

The 8th International Medical Postgraduate Conference in Hradec Králové took place in Hradec Kralove on November 10-12, 2011. 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

We consider the meetings of postgraduate students in biomedicine very important for at least two reasons. One of the reasons is the opportunity to compare achieved results, the possibility to present one's own success and learn from others. Surely there are enough similar opportunities at specialized scientific congresses. Nevertheless, our meeting is different because it has an interdisciplinary character. The main aim is not to prove your "being the best" in your own field but to persuade your colleagues that your field is interesting, can be beneficial and deserves to be introduced to the others. Take advantage of this occasion not only to learn the news in other medical fields but also to think about the bits of knowledge in other medical areas, which can be valuable for you and your postgraduate work. Though there is only "one medicine", the mutual overlapping of its disciplines can result in great benefits for all.

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 experimental and clinical model 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, M. Červinka, 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): 35358

Summary of 2011 results

Title of the presentation: Experimental and clinical models ifor advacemtn of knoledge and clinical practice

Authors: J. Cerman, Z. Červinková, M. Červinka, V. Geršl, M. Holeček, J. Martínková, J. Mokrý, L. Sobotka, P. Tomšík

Effects of ionizing radiation and drugs on leukaemia cell line MOLT-4 were studied: DNA damage and cell death after fractionated irradiation were significantly accumulated in control healthy cells, but in rapidly proliferating tumorous cells the lack of sensitivity to dose fractionation degree was found. Valproate decreased the reparation capacity of irradiated cells. Inhibition of ATM kinase pathway by caffeine increased the post-irradiation apoptosis and delayed mitoxantrone-induced cell death.

In our models of colon and cervical cancer, we have carried out studies concerning the effect of nutritional elements (zinc, selenite and sulforaphane) on molecular pathways governing cellular stress leading to senescence and apoptosis, with special emphasis on selected compartments such as mitochondria and lysosomes. Also, we have continued in exploration of stress mechanisms underpinning toxicity of heavy metals on skin fibroblasts. Using advanced cytometric as well as molecular techniques we were able to establish several molecular links of these processes including activities of metallothioneins, p53 and Bid.

We have continued our studies on non-alcoholic fatty liver disease (NAFLD) in vivo and started research of NAFLD in in vitro conditions. We examined the acute toxic effect of acetaminophen on steatotic rat hepatocytes in primary culture. Biochemical, functional and morphological evaluation clearly document that acetaminophen exerts higher toxicity on live
Genes involved in the liver connective tissue metabolism was studied in liver myofibroblasts, the cultivation of which in collagen gel affected expression of genes involved in extracellular matrix remodelling and rapid collagen turnover.

We focused on functional and molecular changes induced by chronic anthracycline treatment and their further progression in the 10-week post-treatment follow up. In particular, we analyzed oxidative stress, mitochondrial dysfunction and the response of Nrf2-pathway controlling antioxidant/cytoprotective response and PGC-1α-regulated mitochondrial biogenesis pathway. Although we found increased markers of oxidative stress in the treated myocardium, our data do not suggest its direct executive involvement in the development of chronic anthracycline cardiotoxicity and its progression into the heart failure in the post-treatment period. Furthermore, we found no induction of Nrf2 pathway. Moreover, although we have described mitochondrial dysfunction and damage, no activation of mitochondrial biogenesis pathway was noted. Instead, the pathway was suppressed, which might be connected to development of heart failure in the post-treatment follow up.

We studied how chronic intake of a high-protein (casein-enriched) diet affects the protein and amino acid homeostasis and the response of the body to starvation. We found that chronic intake of a high-protein diet has not positive effect on protein balance in any tissue and results in marked alterations in aminoacidemia both in intracellular and extracellular fluid. It was demonstrated that there is an adaptive response of the organism to enhanced intake of protein which may result in unexpected response to different physiological and pathological conditions, such as starvation.

Human mesenchymal stem cells have been isolated not only from the bone marrow but also from periodontal ligaments and dental pulp. Dental pulp stem cells (DPSCs) were able to be propagated in vitro over 60 passages, they expressed mesenchymal markers CD29, CD44, CD73, CD90, CD166, STRO-1, vimentin aa well as stem cell markers (Sox2, nucleostemin, CXCR4, nestin) and only low levels of haemopoietic and endothelial markers. DPSCs revealed a multipotent differentiation potential into osteogenic, adipogenic and chondrogenic cell lineages. However, an excessive in vitro expansion resulted in shortening of the telomere length of DPSCs as documented by measurement of TRF length and quantitative PCR. Experiments performed in whole body irradiated animals, in which a haemopoiesis repair was induced by transplantation of GFP+ bone marrow cells, confirmed a high degree of long-lasting cellular chimaerism not only in the bone marrow but also in non-haemopoietic tissues incl. thymus, liver, spleen and small intestines. The transplanted cells assumed phenotypes of cells of a mesodermal origin and in recipient tissues they were able to perform such complex cellular processes like a mitotic division as documented by co-expression of proliferative markers.

The bioavailability N-(omega)-hydroxy-nor-L-arginine (nor-NOHA, the inhibitor of arginases) was evaluated in rats after a single-dose administration (10-90 mg/kg) using a new HPLC-MS/MS method. Nor-arginine was identified as a principal metabolite.

Pravastatin administration in rats with cholestasis induced by bile duct ligation for 7 days demonstrated dose-dependent protective effect on the development of liver injury. The responsible molecular mechanism was the down regulation of transcription of genes encoding crucial proteins for cholesterol, bilirubin and bile acid synthesis. Moreover, we have developed a model of early sepsis in rat for studies focused on PK/PD of drugs in pathological covariates induced by endotoxemia. In man, amplitude of the visual evoked attenuates in time. Correlation analysis revealed an increase of variability in shape and latency of a single response. The main effect was caused by drop the single response amplitude.

We finished assessment of hyaluronate iodine complex on healing of skin autotransplants in patients with varicose ulcers. Good effect of preparation of tissue bed before transplantation was found, however, the application of the complex after transplantation did not show clinically relevant effect.

A new model of impaired cutaneous wound repair with type II diabetes mellitus and obesity was postulated in the Zucker type of rats.The model of diabetic rat with delayed wound healing useful for study of wound healing in diabetic subjects was finished. The results we published in published this year. In preliminary results we demonstrated positive effect of hyaluronan iodine complex. In common study with Institute of Endocrinology of Academy of science, we shoved stabile thyroid function in spite of increased urinary excretion in patients who were treated by iodine based wound dressing. This indicates that iodine based wound dressing is safe even if given for long time.

Address for correspondence: M. Červinka, Dep. of Medical Biology and Genetics, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Department of Biology, Simkova 870, 500 38 Hradec Kralove, Czech Republic

Title of the project: Preoperative regional intraarterial chemotherapy in the treatment of the large bowel carcinoma

Grant Agency: Ministry of Health ***Project Number:*** NS/9690-4

Principal Investigator: A. Ferko

Co-investigators: Č. Neoral, B. Melichar, A. Krajina, M. Chobola, Z. Šubrt, E. Kubala, M. Kocher, K. Vysloužil, P. Skalický

Starting date: 25.9.2009 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 5982

Summary of 2011 results

Title of the presentation: Preoperative regional arterial chemotherapy

Authors: A.Ferko (1), A.Krajina (2), M.Chobola (1), Z.Šubrt (1), E.Kubala (3)
Teaching Hospit. Hr. Králové: Dept.of Surgery (1), Dept.of Radiology, Dept.of Oncology (3)

The aim of the study is to find out whether the preoperative intraarterial regional chemotherapy may improve overall survival of the patients with the large bowel carcinoma (primary goal). Secondary goal of the study is to analyze whether the preoperative intraarterial regional chemotherapy can decrease the incidence of metachronous liver metastasis of the large bowel carcinoma. The study included patients with microscopically verified large bowel carcinoma stage II (T3-4N0M0) or stage III (T0-4N1-2M0). Patients after previous therapy of malignant disease, patients with severe organ dysfunctions, patients with stenosing tumors, and patients with peroperatively found liver metastasis or peritoneal disseminatin were excluded from the study. Results in two groups of patients were compared. Group A included patients with preoperative regional chemotherapy (50 mg of oxaliplatin and 1000 mg 5-fluorouracil) seven days prior to surgical procedure. The surgical procedure included complete removal of the tumor together with regional lymph nodes. All the patients received adjuvant chemotherapy. Control group (group B) was historical group of patients analyzed retrospectively with comparable characteristics; the patients underwent removal of the tumor together with regional lymph nodes followed by adjuvant chemotherapy. The follow-up of the patients was 36 months. We evaluated disease-free survival, the liver metastasis-free survival and overall survival in both groups. The patients were enrolled in the group A since September 2009. Relatively low number of the patients is due to the strict criteria of the study, intolerance of the chemotherapy in several patients, and several patients did not agree with enrolment into the study. Preoperative regional chemotherapy, the surgical procedure and the postoperative course were uncomlicated in all the patients. All the patients received adjuvant chemotherapy as planned. None of the patients in the follow-up has signs of dissemination or recurence of the disease. Follow-up of the patients continues according to the planned method of the study and will be eveluated after the period of 36 months.

Address for correspondence: Prof. A. Ferko, MD, PhD., Head of Department of Surgery, Charles University, Medical Faculty, Teaching Hospital Hradec Králové, Czech Republic

Title of the project: Quality of life and cost of the treatment in patients with pancreatic carcinoma. Multicentric study

Grant Agency: Ministry of Health ***Project Number:*** NS/9998-3

Principal Investigator: M. Ryska

Co-investigators: A. Ferko, B. Jon, R. Repák, F. Čečka

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

Total funds allocated for project - Kč (thousands): 5752

Summary of 2011 results

Title of the presentation: Current trends in the therapy of the pancreatic carcinoma

Authors: A. Ferko (1), B. Jon (1), R. Repák(2), F. Čečka (1)
Department of Surgery (1), Second Department of Internal Medicine (2), Medical Faculty and University Hospital Hradec Králové, Czech Republic

Pancreatic carcinoma is one of the most devastating malignant diseases. Its poor prognosis is caused by aggressive biological behavior, late presentation and low rate of resecability. The only potential curative method of the treatment is the surgical resection. If the tumor is located in the head of the pancreas, partial pancreaticoduodenectomy is performed. If the tumor is located in the body or tail of the pancreas, we perform distal pancreatectomy. Those surgical procedures have high morbidity, and the mortality should be under 5%.

The aim of the study was to analyze the quality of life of the patients after the radical resection. This study was multicentric and included 5 pancreatic centres in the Czech Republic. The patients, who underwent the radical resection, were given the questionnare SF-36 analyzing the quality of life before the operation and every three months in the follow-up. The quality of life and the survival were compared with the patients who did not have radical resection.

During the period of the duration of the study we performed 45 radical resections, 42 palliative surgical procedures, and 7 explorative laparotomies in our department. Survival, quality of life, and morbidity were analyzed in all the patients.

In conlusion, radical pancreatic resection is the only potentially curative method of treatment of the pancreatic cancer. It significantly prolongs the survival. The quality of life is not inferior compared to the patients who receive palliative surgical bypass, other palliative form of the treatment, or no treatment.

Address for correspondence: A. Ferko, Department of Surgery, Medical Faculty and University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

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 2011 results

Title of the presentation: Quantitative analysis of somatostatin receptors in pituitary adenomas – continuous report for Faculty of Medicine

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 Dept. of Internal Medicine, 1st Faculty of Medicine, Charles Univ. in Prague

The aim of the study is quantitative analysis of somatostatin receptors (sst) in pituitary tumors using real-time RT-PCR and correlation with immunohistochemical profile. This method could help to choose patients profiting from expensive medical treatment with somatostatin analogues and chimeric compounds and preventing residuum tumor growth. Up to now we continue in collecting pituitary tumors from 3 Dept. of Neurosurgery. Quantitative analysis was performed in 78 clinically non-functioning pituitary adenomas for sst_2, sst_3 and sst_5. We are optimizing reactions for sst_1 and sst_4. All adenomas expressed sst_2 and sst_3. Sst_5 was expressed in 42 % of adenomas. High variability of expression for particular type was present. Sst_2 mRNA was expressed from 1174.8 to 146 680.8 copies/5µl cDNA, sst3 62.9–46 914.3 and sst5 mRNA 0–43 776.6 copies/5µl cDNA. Sst_2 and sst_3 expression was not statistically different in regard to histological type of adenoma. Sst_5 was highly expressed in silent corticotroph adenomas. A very heterogeneous level of SSTR expression may be the reason why experimental use of dopamine and somatostatine analogs and „dopastatins“ is not clinically effective in the majority of CNFAs.

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: 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 2011 results

Title of the presentation: Study of mechanisms involved in the post-treatment period of the chronic anthracycline cardiotoxicity.

Authors: E. Jirkovský (1), O. Popelová (1), M. Štěrba (1), Y. Mazurová (2), M. Adamcová (3), T. Šimůnek (4), A. Vávrová (4), K. Vávrová (5), J. Stulík (6), A. Boudíková (7), P. Mandíková (7), J. Neckář (7), F. Kolář (7), V. 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 Inorg. Organic Chem. (5), Fac. Milit. Health Sci., Univ. Defence, Hr. Králové: Dept. of Mol. Pathol. (6), ASCR, Prague: Inst. of Physiol. (7).

Investigation of functional and molecular changes induced by chronic daunorubicin (DAU) treatment and their further progression during the 10 week post-treatment period was performed. Chronic anthracycline cardiotoxicity was induced in rabbits with DAU (3 mg/kg, *i.v.*, once weekly, 10 weeks). A significant decrease in the left ventricular (LV) systolic function during the DAU treatment further progressed to congestive heart failure and LV dilation in the post-treatment period. Analysis of oxidative damage to the LV myocardium revealed only a loose relationship with the development and parameters of heart failure (e.g., unchanged GSSG/GSH ratio, no activation of Nrf2 or majority of its gene targets). Thus, the question of pivotal and direct role of the oxidative stress in the development of anthracycline-induced heart failure deserves further investigation. In addition, possibilities of pharmacological protection of anthracycline cardiotoxicity were further studied and new dexrazoxane (DEX) analogues were synthesized and tested. Moreover, activity of DEX (in a narrow dose range) to suppress arrhythmias in isolated rat hearts subjected to ischemia/reperfusion was shown, whilst a higher dose of the drug was needed to limit myocardial infarct size in open-chest rats.

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

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

Title of the project: 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 2011 results

Title of the presentation: Safety profile of 5-FU exposure in 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)**University Hospital in Hradec Králové: Department of Oncologyand Radiotherapy***

Introduction: Study focuses on kinetically guided individual dosage prediction based on 5-FU plasma concentrations. The first step was to develop the analysis of 5-FU and chief metabolite dihydrofluorouracil (5-FUH2) in plasma samples, the second was to establish the safety profile reflecting the dosage and drug plazma concentration. The third step was to define the effective 5-FU exposure to reach pathological complete response (pCR, i.e. ypT0, ypN0) after the preoperative (neoadjuvant) chemoradiotherapy.

Method: Detail pharmacokinetic profiles of 5-FU (i.e. AUC - area under the curve of 5-FU plazma concentrations, concentratrion of 5-FU in steady steate – Css, and the rate of 5-FU metabolism, i.e. the ratio between concentrations of 5-FU chief metabolite dihydro-fluorouracil 5-FUH2 to plazma 5-FU concetrations) were evaluated in 39 patients together with efficacy and safety evaluation to establish the target drug exposure during the preoperative treatment.

Results: The drug pharmacokinetic parameters were linear over the dosage from 200mg/m2/hours to 1000mg/m2/24hours. The efficacy and safety profile correlated with plasma 5-FU concentrations (i.e. with the drug exposure), reflecting the rate of 5-FU metabolism. The effective and safe cummulative expossure (AUC) to reach pCR during the neoadjuvant chemoradiotherapy is 90-100 mmol.h/L, i.e. 5-FU Css about 100-120 µmol/L for the whole 5 weeks. The initial dose should start with 400mg/m2/24hours for 2 weeks (the recommended dose rate for now is 200mg/m2/24hours) and than corrected reflecting the 5-FU plazma concentrations in steady state (Css) and the rate of 5-FU metabolism (i.e. the ratio between 5-FUH2 and 5-FU plazma concentration in steady state during the first 2 weeks of treatment).

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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 2011 results

Title of the presentation: Expression of ER, HER2, KI67, and NQO-1 predicts pathological complete response to neoadjuvant chemotherapy in breast cancer.

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 (NCT) is considered the standard therapy in primary breast cancer (BC). However, interindividual variability in efficacy of routinely used cytostatic regimens leads to intensive sought after prognostic markers affording prediction of NCT outcome. The aim of this study was to evaluate several biological markers as predictors of pathological complete response (pCR) in patients with BC. Sixty one patients with BC who received NCT with doxorubicine, cyclofosfamide, and docetaxel were enrolled in this prospective study. Protein expression of antigens in tumor samples from core needle biopsies obtained before NCT and in tumor remnants after NCT was evaluated by immunohistochemistry (IHC). The mRNA expression of genes was analyzed by quantitative RT-PCR in tumor samples obtained by mammary gland resection after NCT. Results: The pCR rate was 24%. HR- (Hormone estrogen and progesterone receptor negative), and HR-/HER2- (Human epidermal growth factor receptor 2) tumors had highest pCR rates. The pCR rate was also higher in Ki-67+ and NQO1-negative tumors. Post-operative evaluation showed that NQO1 expression was significantly increased, while Ki67 and HER2 decreased, in the residual tissue after NCT. In conclusion, our data suggest that measurement of NQO1 expression may be a novel diagnostic biomarker for the prediction of positive response to preoperative NCT.

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

Address for correspondence: J. Grim, Dept. of Clinical Oncology and Radiation Therapy, Charles University Hospital and Faculty of Medicine in Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Creation of E-learning courses on topographical anatomy

Grant Agency: Ministry of Education ***Project Number:*** 1073 F3

Principal Investigator: P. Hájek

Co-investigators:

Starting date: 1.1.2011 ***Duration (years):*** 1

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

Summary of 2011 results

Title of the presentation: Creation of E-learning courses on topographical anatomy

Authors: P. Hájek

The abstract refers to E-learning courses Topografická anatomie horní končetiny (Topographical anatomy of upper limb) and Topografická anatomie hlavy a krku (Topographical anatomy of head and neck), both in Czech language. These courses are available on Moodle portal of Faculty of Medicine in Hradec Kralove under link http://moodle.lfhk.cuni.cz/moodle/course/category.php?id=11.

Topographical anatomy is more practical then systematic anatomy and is closer to perception of clinical medicine. Unfortunately, time stress makes students to focus on systematic anatomy and to sidetrack the topographical anatomy. That is why we decided to compose serial of e-learning courses.

Each lesson of the courses contains study material (text, pictures, schemes, photos, videos), quizzes and questionnaires. Authorial approach should guarantee an absence of any extraneous copyrighted materials. Feedback components are built not only for students but also for the creators, so the courses can be improved and completed continuously. The courses aspire to complete current contents of practical classes suitably. However, they have a task to clear blind spots not only in syllabi, but better to say, in motivation of students.

The materials are aimed to the undergraduate study of General Medicine, but they may serve for study programs of Dentistry or Physiotherapy as well. The courses are opened not only for students of the 1st year studying the anatomy by their schedule but also for students of highest years to remind topographic relations in various regions of the body.

References: Hájek P. Topografická anatomie dolní končetiny. Multimediální podpora výuky klinických a zdravotnických oborů - Portál Lékařské fakulty v Hradci Králové [online 10.5.2011] URL http://mefanet.lfhk.cuni.cz/clanky.php?aid=27 ISSN 1803-280X.

Address for correspondence: P. Hájek, Charles University, Faculty of Medicine in Hradec Kralove, Department of Anatomy, Šimkova 870, 500 38 Hradec Kralove, 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. Doleželová

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

Total funds allocated for project - Kč (thousands): 964

Summary of 2011 results

Title of the presentation: Epigallocatechin gallate enhances cholesterol biliary excretion in control rats and lowers plasma and liver cholesterol in ethinylestradiol-treated rats

Authors: P. Hirsova, G. Kolouchova, H. Lastuvkova, E. Dolezelova, J. Cermanova, S.Micuda
Fac. Med., Charles Univ., Hr. Kralove: Dept. of Pharmacology

Green tea flavonoid epigallocatechin gallate (EGCG) was shown to have a cholesterol-lowering effect in rodents due to increased fecal excretion of cholesterol. We evaluated effect of EGCG on cholesterol homeostasis in the liver of control and ethinylestradiol (EE)-treated rats. Rats were treated with EGCG (50 mg/kg, 8d), EE (5 mg/kg, 5d), a combination of EE and EGCG, or respective vehicles (controls). Protein and gene expression was examined by Western blot and qRT-PCR. EGCG administration to rats almost doubled biliary excretion of cholesterol while its plasma levels were retained. These effects were associated with an increase in cholesterol transporter Abcg5/8, scavenger receptor b1 (Sr-b1) and decrease in acyl-CoA:cholesterol acyltransferase 2 (Acat2) expression. EE treatment significantly reduced plasma cholesterol, but its biliary excretion was maintained compared to controls. Simultaneously, EE reduced Sr-b1 and markedly increased Acat2 and LDL receptor. EGCG pretreatment in EE-treated rats resulted in a further reduction of plasma cholesterol levels with unchanged biliary excretion of cholesterol. Therein, the expression of Acat2 and LDL receptor declined compared to EE group. EGCG pretreatment partially prevented EE-induced rise in liver cholesterol content and liver weight. Plasma cholesterol levels correlated positively with gene expression of transcription factor Srebp-2 (sterol regulatory element-binding protein) and negatively with a nuclear receptor small heterodimer partner (Shp). Liver cholesterol content correlated positively with gene expression of Srebp-1 and Shp, and negatively with Srebp-2. These results demonstrated ability of EGCG to alter hepatic cholesterol homeostasis that may contribute to the hypocholesterolemic effect of EGCG.
Supported by the Charles University Grant Agency, No 132309.

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Title of the 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 Investigator: P. Hůlek

Co-investigators: A. Krajina, J. Fajfrová, V. Šafka, M. Holeček, S. Mičuda, T. Fejfar, V. Koblížek, L. Hosák, J. Štefánková, V. Jirkovský, J. Cyrany, M. Hůlková

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

Total funds allocated for project - Kč (thousands): 5401

Summary of 2011 results

Title of the presentation:

Authors: P. Hůlek, A. Krajina, J. Fajfrová, V. Šafka, T. Fejfar, V. Koblížek, V. Jirkovský, S. Mičuda, E. Doleželová, L. Hosák, M. Hůlková, M. Beránek, M. Holeček, J. Cyrany, L. Šišpera, R. Jelínková, I. Bartošková, J. Tomandl, E. Malířová, J. Doležal, J. Chládek, M. Solař, R. Praus, J. Brožík, J. Štefánková. University Hospital in Hradec Králové; Charles University in Prague – Faculty of Medicine in Hradec Králové; University of Defence – Faculty of Military Health Science; Masaryk University in Brno – Faculty of Medicine.

TIPS treats some serious complications of portal hypertension (PH), but it also threatens some patients by encephalopathy or unexpected death. Our complex study aimed to discover pathophysiological links for complications of PH as well as the prognostic factors for TIPS.

The study covered 20 cirrhotic patients coming for TIPS. In them, central hemodynamics, blood gases, cardiac USG, pulmonary function tests, CT of lungs, pulmonary scintigraphy, brain SPECT, psychological tests and psychiatric investigation, BDNF gene type, nutrition and metabolic rate, liver function tests, intestinal bacterial overgrowth, glucose metabolism, many biochemical parameters and cytokines were investigated and followed up for 6 months.

The most interesting findings are: 1) key role of some hemodynamic parameters for the short-term survival prognosis, 2) high prevalence of minor psychiatric disturbances after TIPS affecting virtually all patients, 3) high prevalence of pulmonary functional disturbances in this kind of patients disproportional to general population, 4) mostly splanchnic origin of cytokines altering the pulmonary vascular functions, 5) significant role of both nutrition and metabolic rate for the outcome and favourable effect of TIPS in this field.

Conclusion: 1) Cardiovascular deterioration should be thoroughly investigated before TIPS to prevent short-term mortality. 2) Hypothesis of liver origin of cytokines altering the pulmonary vasculature in portal hypertension was not confirmed. 3) Short term deterioration of mental functions after TIPS should be anticipated in vast majority of patients.

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

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

Total funds allocated for project - Kč (thousands): 4532

Summary of 2011 results

Title of the presentation: Longitudinal evaluation of biomarkers of hepatotoxicity in patients with chronic plaque psoriasis on pharmacokinetically-guided therapy with oral methotrexate.

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)

Low-dose oral methotrexate (LDMTX) is an effective immunosuppressive drug. However, its long-term use in psoriasis patients is hampered by the risk of liver fibrosis. Either direct biomarkers for fibrosis (hyaluronic acid [HA], N-terminal propeptide of collagen type III [PIIINP]) or indirect multi-test algorithms (Fibrotest, Hepascore) could replace nonspecific liver enzyme tests and reduce the need for periodic liver biopsies. A prospective study (Eudra CT 2009-015403-95) evaluated serum fibrosis biomarkers, folates and erythrocyte MTX polyglutamates (MTXPG) in adult patients with psoriasis who A/ initiated LDMTX therapy, B/ took LDMTX > 2 years and, C) were treated with biologics.The outcome of individualized therapy in the group A was excellent: after 6 months, the mean improvement in the psoriasis area and severity index (PASI) was 80% (SD 20%) and 79% of patients reached PASI75. The ratio of MTXPG/blood folates was a strong predictor of outcome. Except for Fibrotest, the baseline levels of fibrosis markers of the group A were positively correlated with PASI. Moreover, the values of HA and Hepascore at 6 months were decreased by 40 and 24% ($p<0.05$), reflecting the improvement in PASI. No such changes were observed in the groups B and C with a stable PASI. In the group A, the ALT and AST activities transiently increased at month 3 without corresponding elevations in fibrosis markers under the study. During the first 12 months of therapy, the Fibrotest values were similar in all groups, showed no changes and corresponded to minimal or absent fibrosis. 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.

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: Oral health of pre-school children, fluoride intake, parental attitudes and behavior towards prevention of dental caries of 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): 2586

Summary of 2011 results

Title of the presentation: Caries increment in primary dentition and some correlations of the 3 years longitudinal study.

Authors: R. Ivančaková (1), Z. Broukal (2), E. Lenčová (2) Fac.Med., Charles Univ., Hradec Králové, Dept. of Dentistry (1). Institute of Dental Res., GFH, Prague (2). Caries experience, dental status, oral hygiene and salivary levels of cariogenic streptococci were examined in the three years prospective study among preschool children in relation to the dynamics of caries increment. An examination of dental status has been conducted in a cohort of 3 to 5 yr-old children in Prague (P) and Hradec Kralove (HK). The criteria for including the child in the study were an informed consent of parents and no systemic disease of the child. Following parameters were calculated: % caries free, dt, dmft, ri, sci and salivary level of SM. The results were statistically evaluated. The total count of examined children was 276 (153 boys, 123 girls) at the beginning of the study, 264 (142+122) 2. examination and 249 (135+114) 3. examination. The mean count of dt of both cohorts was 1.40 (1. exam.), 1.29 (2. exam) and 1.47 (3. exam.) No statistical difference between HK a P. The mean dmft amounted to 1.75 vs. 2.44 vs. 2.57. The caries increment between 1. and 2. examination was 0,68 dmft/child, between 2. and 3. examination 0,26 dmft/child. The caries increment was significantly higher for both genders between 1. and 2. examination. The mean value of salivary SM was 1.40 vs. 1.29 vs. 1.47. The level of salivary SM was significantly higher in boys between 1. and 2. examination. No differences in both boys and girls between 1. and 3. examination. The level of salivary SM was significantly lower between 1. and 2. examination. There was the positive correlation between both cariology indices (dt, dmft) and salivary SM levels but the correlation between caries increment and salivary SM levels was not significant. The evaluation of SM seems to be a negative predictor of caries risk in children.

Supported by the grant of Ministry of Health No. NS/10353-3, Czech Republic.

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Title of the project: Comparison of the efficiency of the autologous serum – eye drops and the umbilical blood serum – eye drops in the patients with severe dry eyes

Grant Agency: Ministry of Health ***Project Number:*** NT/12376-4

Principal Investigator: I. Fales

Co-investigators: N. Jirásková

Starting date: 1.11.2011 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3727

Summary of 2011 results

Title of the presentation:

Authors: We are the co-investigators of the project NT 12376 on the comparison of the efficiency of the autologous serum – eye drops and the umbilical blood serum – eye drops in the patients with severe dry eyes that have no improvement after treatment with artificial tears or lubricants. This project has started in November 2011. Up to now we have solved the technical matters on eye drops production and we have started to select patients eligible for treatment.

Address for correspondence: jirasnad@fnhk.cz

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. Lenčo, J. Tošner, M. Link, V. Tambor, H. Hornychová

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

Total funds allocated for project - Kč (thousands): 2763

Summary of 2011 results

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

Authors: M. Kacerovský (1), J. Lenčo (2), V. Tambor (3)
University Hospital Hradec Králové, Dept. Obstretrics and Gynaecology (1), Univ. Defence, Fac. Military Health Sci. Inst. Molecular Pathology (2), University Hospital Hradec Králové, Biomedical Research Center (3)

We have been using advanced proteomics to identify novel potential biomarkers of intraamniotic infection in amniotic fluid from preterm birth patients with intact membranes. All samples were collected during active labor by transvaginal amniocentesis at the Perinatal Research Center, Nashville, TN, USA. In total, 64 amniotic fluid samples from caucasian women were classified into two groups according to the clinical outcome - women with proven intraamniotic infection with subsequent histological chorioamnionitis were considered a positive group, whereas patients with ruled out intraamniotic infection and no signs of inflammation of the placenta were taken as a negative control group.
We have successfully finished the proteomic discovery phase of the project on pooled representative sample from each group. Using two-dimensional separation employing reversed phase chromatography with two different pH conditions and MALDI-TOF/TOF mass spectrometry, we successfully identified 846 different protein molecules. The quantitative information obtained owing to the iTRAQ labeling revealed 28 proteins that showed significantly different abundance ($p \leq 0.05$) in positive group as compared to negative control group.
Based on the statistical and clinical criteria we selected 15 proteins that would deserve further validation. We therefore prepared 50 samples for verification of the iTRAQ results using advanced SRM (Selected Reaction Monitoring) proteomic technology. This verification phase will be carried out during 2012. Moreover, some of the most promising findings will be confirmed using ELISA assays by the US project partner.

This project was supported by Ministry of Education, Grant No. ME10025
Address for correspondence: Marian Kacerovsky, University Hospital Hradec Králové, Dept. Obstetrics and Gynecology, Sokolská 581, 500 05 Hradec Králové

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): 2196

Summary of 2011 results

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

Authors: M. Kacerovsky, C. Andrys
Charles University in Prague, Medical Faculty Hradec Kralove. Faculty Hospital in Hradec Kralove.

The main aim of the project was to evaluate the changes of transverse diameter of fetal thymus and transverse diameter of adrenal gland in pregnancies complicated by preterm prelabor rupture of membranes and whether there are changes when either histological chorioamnionitis or fetal inflammatory response syndrome are present.
Totally, 246 women with preterm prelabor rupture of membrane were recruited within years 2009-2011. The transverse diameter of fetal thymus was measured three times and the mean was used for the analyses. A lower median of transverse diameter of fetal thymus was found when fetal inflammatory response syndrome was present (with: median 20.0, IQR 8.4 vs. without: median 27.4, IQR 8.9; $p < 0.0001$) in crude analysis, as well as after the adjustment for gestational age ($p = 0.005$). The transverse diameter of fetal adrenal glands was measured three times and the mean was used. A lower median of transverse diameter of fetal adrenal glands was found when fetal inflammatory response syndrome was present (with: median 21.9, IQR 4.4 vs. without: median 23.0, IQR 4.5; $p < 0.0001$) in crude analysis, but not after the adjustment for gestational age ($p = 0.25$).
Moreover, we revealed that there is association between type of flow pattern in splenic vein and the presence of systemic fetal inflammatory response syndrome. The flow pattern of splenic vein is not pulsatile under normal conditions.We found a relationship between pulsation in the splenic vein and histological chorioamnionitis (LR 13.2) as well as systemic fetal inflammatory response syndrome (LR 5.7). Ultrasound evaluation of splenic vein could be a non-invasive tool for the prediction of these inflammatory complications.
This project was supported by Czech Science Foundation No. 304-09-0494.

Address for correspondence: Marian Kacerovsky, University Hospital Hradec Králové, Dept. Obstetrics and Gynecology, Sokolská 581, 500 05 Hradec Králové

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. Link, 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): 7064

Summary of 2011 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 (3)

University Hospital Hradec Králové, Dept. Obstetrics and Gynaecology (1), Univ. Defence, Fac. Military Health Sci. Inst. Molecular Pathology (2), University Hospital Hradec Králové, Biomedical Research Center (3). In order to find new potential biomarkers of intraamniotic infection/inflammation we have been collecting amniotic fluid samples by transabdominal amniocentesis and umbilical cord blood samples after delivery of neonates from women with preterm premature rupture of the membranes. Totally, 251 women were recruited during 2009-2011. The project was divided into three parts. The first one was aimed at proteomic discovery of novel potential markers of intraamniotic infection/inflammation. This part included analysis of 19 positive and 19 negative samples, which were divided into groups based on the results of cultivation, PCR, IL-6 assay and histological outcomes. The samples were pooled and depleted from 14 most abundant proteins. After 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 which led to identification of 851 distinct proteins. Relative quantity of 133 proteins showed significant change between positive and negative group. Three proteins attracted our attention most (sCD163, cathelicidin and myeloperoxidase). These molecules were determined in larger cohorts of at least 110 patients. The results pointed out on the benefit of employing advanced proteomic techniques into fishing of potential protein markers of intraamniotic infection. The second aim was to describe the relationship between intraamniotic infection/inflammation and levels of amniotic fluid MMP-9 and IL-8. Women with either histological chorioamnionitis or microbial invasion of the amniotic cavity had higher levels of both MMP-9 and IL-8 than women without these complications. Last aim was to assess changes in umbilical cord blood cortisol, DHEA-S, and their ratio in relationship to the presence and absence of FIRS. Only umbilical cord blood DHEA-S level was higher when FIRS was diagnosed.

This project was supported by Ministry of Health, Grant No. NS/10382 - 3

Address for correspondence: Marian Kacerovsky, University Hospital Hradec Králové, Dept. Obstetrics and Gynecology, Sokolská 581, 500 05 Hradec Králové

Title of the project: Analysis of selected prognostic and predictive markers of oropharyngeal and laryngeal tumours

Grant Agency: Charles University ***Project Number:*** 444311

Principal Investigator: D. Kalfeřt

Co-investigators: J. Vokurka

Starting date: 29.3.2011 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 242

Summary of 2011 results

Title of the presentation: The assessment of p16INK4a (p16) expression in glottic laryngeal cancer

Authors: Kalfeřt D.[1], Laco J.[2], Čelakovský P.[1], Vokurka J.[3]

Dept. of Otorhinolaryngology and Head and Neck Surgery, University Hospital, Faculty of Medicine in Hradec Kralove, Charles University in Prague (1), The Fingerland Dept. of Pathology, University Hospital, Faculty of Medicine in Hradec Kralove, Charles University in Prague (2), Dept. of Otorhinolaryngology, Head and Neck Surgery, University Hospital Motol, First Faculty of Medicine, Charles University in Prague (3)

Purpose: Squamous cell carcinomas of the larynx are the most frequent tumours of the head and neck. The etiologic role of high-risk human papilomavirus (HPV; in particular type 16 and 18) has recently been studied in head and neck squamous cell carcinoma (HNSCC), mainly in oropharyngeal cancer. The literature data suggests that overexpression of p16INK4a is strongly related to the presence of HPV16/18. Association of HPV infection with laryngeal cancer has not been so far well documented.

Objective: To assess the significance of p16 expression in glottic laryngeal cancer.

Material and Methods: Fifty eight patients after primary surgery of the glottic squamous laryngeal cancer were enrolled in the retrospective study. The p16 expression was immunohistochemically detected in tumour tissue. The results were statistically correlated with clinical-pathological parameters.

Results: Protein p16 was expressed in laryngeal cancer of 15 patients (26%). Statistically significant higher p16 expression was proven in non-smokers in comparison with smokers (75% versus 18%; p=0.003). Recurrent cancer was diagnosed in 9 patients (15,5%), and all these tumours were p16 negative. No statistically significant correlation of p16 expression in cancer with and without recurrence was detected (p=0.094).

Conclusions: Preliminary results revealed the potential association of HPV infection with glottic laryngeal cancer, especially in non-smokers. Expression of p16 in laryngeal carcinoma seems to be an appropriate predictor of favourable prognosis. Anyway the pathobiology of this tumour as well as predictive role of p16 expression in laryngeal cancer still remains to be better elucidated.

This work was supported by Charles University Grant Agency (Grant No. 444311)

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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): 629

Summary of 2011 results

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

Authors: Kalfeřtová M., Jirásková N.
Dept. of Ophthalmology, Faculty of Medicine in Hradec Kralove, Charles University in Prague, University Hospital Hradec Kralove

Purpose: Posterior capsule opacification (PCO) is still one of the most common complication following cataract surgery with IOL implantation. We evaluated the extent of PCO after cataract surgery - torsional phacoemulsification and liquifaction method (AquaLase) removal of the epithelial cells (right eye) and torsional phacoemulsification (left eye). For PCO quantification we used two types of software.

Methods: In our prospective clinical study we examined patients 3, 6, 12 and 24 months after surgery, digital retroillumination photographs of the anterior segment, pachymetry, endothelial cell count (ECC) and best corrected visual acuity (BCVA) were obtained. In our study we had 56 patients (17 men, 39 women). For evaluation of PCO we used EPCO 2000 software (Evaluation of Posterior Capsule Opacification) and OSCA software (Open-Access Systematic Capsule Assessment). The density of PCO is graded by EPCO software to 4 levels (total PCO index) and by OSCA system to 15 grades OSCA score.

Results: The BCVA two years postoperatively is 0.892 ± 0.13 (right eye), 0.890 ± 0.151 (left eye). Nd:YAG laser capsulotomy one year after surgery underwent one patient (both eyes) and one patient two years postoperatively (right eye).

EPCO results (3M; 6M; 12M; 24M) - right eye: 0.289 ± 0.223; 0.276 ± 0.176; 0.309 ± 0.185; 0.418 ± 0.253, left eye: 0.302 ± 0.191; 0.301 ± 0.168; 0.355 ± 0.206; 0.468 ± 0.309.

OSCA new analysis results (3M; 6M; 12M; 24M) - right eye: 0.612 ± 0.279; 0.603 ± 0.339; 0.559 ± 0.265; 0.642 ± 0.401, left eye: 0.630 ± 0.398; 0.629 ± 0.366; 0.535 ± 0.331; 0.574 ± 0.340.

Conclusion: The AquaLase liquifaction method is save for occular tissue. BCVA improved in all eyes. Two years after surgery most cases of PCO is graded as minimal, there is no statistical diference between right eye and left eye. Two patients underwent Nd:YAG laser capsulotomy, one patient one year after surgery (both eyes), one patient two years postoperatively (right eye).

This work was supported by Charles University Grant Agency (Grant No. 103809)

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Title of the project: Development of preoperative preparation for a more effective surgical therapy

Grant Agency: Charles University ***Project Number:*** 262904

Principal Investigator: M. Kaška

Co-investigators: A. Ferko, J. Harrer, M. Broďák, P. Šponer, S. Řehák, J. Tošner, P. Rozsíval, V. Chrobok, R. Slezák, V. Černý, D. Šimkovič, J. Manďák, J. Vojáček, P. Žáček, M. Kanta, P. Dostál, J. Špaček, H. Langrová, N. Jirásková, A. Šimůnek, R. Ivančaková, and postgradual students of surgical departments of the Medical Faculty and the Teaching Hospital in Hradec Králové

Starting date: 1.1.2011 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 910

Summary of 2011 results

Title of the presentation: Actual management of surgical patients in preparation for operation

Authors: Kaška M. et al.

Introduction. Surgeons of all paricipated departments use in a patient preparation to surgical operations some actual methodologies with reduction of an operative trauma. An essential methodology is "fast track" or ERAS (enhancement of recovery after surgery). Patient is preparated according to his/her individual physiological condition with a minimal negative influence of surgery on psychosomatic condition of all organism (examination of nutrition condition and subsequent preoperative nutritive care as it is important, no-fasting before operation - no longer as two hours preoperatively, no-preparation of gastrointestinal tract by enema and others for bowels used "washing out" methods, peripheral - not general anaesthesia, lower dosis of antalgetics and opioids, intensive prophyllaxis of paralytic ileus, thromboembolic disease, and next perioperative risks as there are healing disorders etc.).

Clinical results. At surgical departments e.g. the hospital stay was abbreviated from 12 days to 8 days on the average with keeping most of above-mentioned methods during last 4 years. Active investigation of potential risks during perioperative time period in all special surgical domains is succeful as in a field of general surgery, vascular surgery, neurosurgery so as in gynecology and obstetrics, ophthalmology, dentistry etc. Ophthalmologists are performing management of patients in one day surgery mode with good effect and very low level of perioperative complications (postoperative infection). Gynecologists are using new methods in prediction of endometrial cancer as mutation K-ras is in cancerogenesis and ultrasonic examination in prenatal disgnostics of a fetal pathology. Dentists passed succeful experiments with stem cells for better healing of bone deffects and a clinical trials relating to implantology.

Conclusions. Introducing of fast track and next preparative methods for surgery into clinical practice has positive effect on patient conditions, therapy results, and economy.

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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, J. Manďák, M. Koláčková, C. Andrýs, K. Jankovičová, D. Vlášková, Z. Holubcová, V. Vroblová, D. Holmannová

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

Total funds allocated for project - Kč (thousands): 1564

Summary of 2011 results

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

Authors: Jan Krejsek, et al.

Research project aimed cardiac surgery as a clinical model of inflammatory response. Patients undergoing coronary artery bypass grafting either on the beating heart ("off-pump") or using cardiopulmonary bypass ("on-pump") or modified "miniinvasive" cardiopulmonary bypass was enrolled to our project. This project was approved by the local Ethics Commitee and written consent was obtained from each participant. Peripheral blood samples were collected before surgery and after surgery up to 5th postoperative day. Humoral parameters were determined by ELISA. Cell-mediated immunity was followed by flow cytometry.

Results

Our research enabled us to describe the substantial differences in the development of inflammatory response between "off-pump" and "on-pump" surgery being more pronounced in latter one. However, more pronounced proinflammatory activities in "on-pump" patients are counterbalanced by the elevation of both humoral (IL10), and cellular (CD163 receptor) antiinflammatory mechanisms in these patients. We found, that the only presence of methylprednisolone in cardiopulmonary bypass fluid was able induce significantly the serum level of homeostatic IL-10 cytokine. The dynamics of PTX3, a new member of pentraxin family, in cardiac surgical patients was for the time described in our study. New and in essence original data addressing the mechanisms of apoptosis, the expression of CD200/CD200R regulatory molecules, and RANK/RANKL expression, receptors for interferon gamma, and dynamics in the level of proinflammatory cytokines in cardiac surgical patients were effectively published by us.

In conclusion, our research contributed to the better understanding of the pathophysiology of the inflammatory response induced by cardiac surgery.

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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 2011 results

Title of the presentation: Latency jittering correction in motion-onset VEP amplitude decay during prolonged visual stimulation

Authors:

J. Kremláček, M. Kuba, Z. Kubová, J. Szany, J. Langrová, F. Vít
Fac. Med., Charles Univ., Hradec Králové: Depth. of Phatological Physiology

Visual evoked potentials to motion-onset stimulation (M-VEPs) gradually attenuate in amplitude during examination. To estimate the origins of the suppression of M-VEPs during stimuli repetition we used correlation technique to minimalize latency jittering of single sweeps and evaluated the effect of such correction on the amplitudes of three M-VEP dominant peaks P1, N2 and P3.

During prolonged visual motion stimulation, the variability of corrective latency shifts in the occipital region increased (r = 0.35 : 0.44) and the number of single responses corresponding to the average curve declined in occipital and parietal derivations (r = -0.48 : -0.62).

While the P1 peak amplitude did not exhibit any time-specific behaviour, the N2 amplitude exhibited a significant decay of 29.4 % that was partially reduced to 16.6 % in the central occipital derivation by the latency jitter and non-correspondence corrections. The strongest attenuation (32.7 %) was observed in the P3 amplitude and was less sensitive to the corrections, dropping only to 27.9 %.

The main part of the response suppression to repeated motion stimulation was caused by amplitude drop and represents non-stationary process that likely correspond to a fatigue model. Using selective averaging and latency jitter correction, the effect of response suppression was partially removed.

Project was supported by the Grant Agency of Czech Republic, No 306/09/0869.

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

Grant Agency: Charles Univeristy ***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): 605

Summary of 2011 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. 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, molecular biological analysis was performed to detect K-ras mutation in a group of 60 patients with I. stage endometroid carcinoma and 20 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 in 15% of the control group. Data support the importance of K-ras mutation in pathogenesis of endometroid carcinoma, but our suggestion, that K-ras mutation could have a positive predictive value, wasn´t established. There was no statistical significant correlation between presence of K-ras mutation, stage and grade of endometroid carcinoma.

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 2005 - 2011 results

Title of the presentation: Electrophysiological diagnostics of neuro-psychiatric disorders

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

The results of new diagnostic tools implementation (motion-onset and cognitive visual evoked potentials –VEPs) were described in the following articles (accessible "in extenso" on the above specified web page):

KUBOVÁ, Zuzana, SZANYI, Jana, LANGROVÁ, Jana, KREMLÁČEK, Jan, KUBA, Miroslav, HONEGR, Karel: Motion-onset and pattern-reversal VEPs in diagnostics of Neuroborreliosis. **J. Clin. Neurophysiol.**, 2006, vol. 23 (5), p. 416-420.

KUBA, Miroslav, KUBOVÁ, Zuzana, KREMLÁČEK, Jan, LANGROVÁ, Jana: Motion-onset VEPs: characteristics, methods, and diagnostic use**. Vision Research**, 2007, vol. 47 (2), p. 189-202.

KREMLÁČEK, Jan, KUBA, Miroslav, KUBOVÁ, Zuzana, LANGROVÁ, Jana, VÍT, František and SZANYI, Jana. Within-session reproducibility of motion-onset VEPs: Effect of adaptation/habituation or fatigue on N2 peak amplitude and latency. **Doc. Ophthalmol.**, 2007, vol 115, p. 95 - 103.

SZANYI, Jana, KUBOVÁ, Zuzana, KUBA, Miroslav, KREMLÁČEK, Jan, LANGROVÁ, Jana, TALÁB, Radomír, HONEGR, Karel and SZANYI, Juraj. Commparison of Visual Evoked Potentials in patients with Multiple Sclerosis and Neuroborreliosis (in Czech). **Čs. Neurol. Neurochir**., 2007, vol. 70, p. 658-664.

KUBA, Miroslav, LILÁKOVÁ, Dana, HEJCMANOVÁ, Dagmar, KREMLÁČEK, Jan, LANGROVÁ, Jana, KUBOVÁ, Zuzana. Ophthalmological examination and VEPs in preterm children with perinatal CNS involvement. **Doc. Ophthalmol**., 2008, vol. 117, p. 137 - 145. URBAN, Aleš, KREMLÁČEK, Jan, MASOPUST, Jiří, LIBIGER, Jan. Visual mismatch negativity among patients with schizophrenia. Schizophrenia Res., 2008, vol. 102, p. 320 - 328.

SZANYI, Jana, KUBA, Miroslav, KUBOVÁ, Zuzana, KREMLÁČEK, Jan, LANGROVÁ, Jana, JIRÁSKOVÁ, Naďa. Retrospective analysis of visual evoked potentials findings in acute retrobulbar neuritis (in Czech). **Čs. Neurol. Neurochir**., 2008, vol. 71, p. 317 - 323.

KUBOVÁ, Zuzana, KREMLÁČEK, Jan, VALIŠ, Martin, LANGROVÁ, Jana, SZANYI, Jana, VÍT, František, KUBA, Miroslav. Visual evoked potentials to pattern, motion and cognitive stimuli in Alzheimer's disease. **Doc. Ophthalmol.**, 2010, vol. 121, p. 37-49

JIRÁSKOVÁ, Naďa, KUBA, Miroslav, KREMLÁČEK, Jan, ROZSÍVAL, Pavel. Normal sensory and absent cognitive electrophysiological responses in functional visual loss following chemical eye burn. **Doc. Ophthalmol.**, 2011, vol. 123, p. 51 - 57.

SZANYI, Jana, KUBOVÁ, Zuzana, KREMLÁČEK, Jan, KUBA, Miroslav, LANGROVÁ, Jana, VÍT, František, SZANYI, Juraj, PLÍŠEK, Stanislav. Pattern and motion related visual evoked potentials in Neuroborreliosis: follow-up study. **J. Clin. Neurophysiol.**, 2012 – in press

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

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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 2011 results

Title of the presentation: Reduction of drusenoid retinal pigment epithelium detachment area in the dry form of age-related macular degeneration 2.5 years after rheohaemapheresis

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

Purpose: To evaluate changes in the area of drusenoid retinal pigment epithelium detachment (DPED) in the dry form of age-related macular degenereation (AMD) after rheohaemapheresis.

Methods: We investigated 22 eyes with DPED of 12 patients before and 2.5 years after rheohaemapheresis and randomised 18 eyes of 13 patients with DPED for a period of 2.5 years of natural course of dry form AMD. Each treated patient received a serie of 8 rheohaemaphereses of 1.5 plasma volume within 10 weeks. With Visupac method we measured the DPED area before and 2.5 years after the therapy and explained in mm^2. All patients were followed-up using ETDRS charts, optical coherence tomography, fluorescein angiography, electroretinography (ERG), multifocal ERG and by measured ocular blood flow.

Results: Initially, there was no statistically significant difference in the DPED area in both treated group and controls ($p = 0.605$), whereas after 2.5 years was very significant ($p < 0.0005$). In treated patients, best corrected visual acuity (BCVA) increased significantly from 0.61 (0.06-1.00) to 0.68 (0.35-1.00) after 2.5 years ($p = 0.035$). We found no significant changes and differencies in scotopic activity, whereas cone response and paramacular activity in the more peripheral region between 14° and 22° of eccentricity were significantly higher in treated patiens after 2.5 years.

Conclusion: Rheohaemapheresis reduced the area of DPED which increased during natural course of dry form of AMD, prevented the decline of BCVA and mainly, delayed progression to the wet form of AMD.

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Title of the project: The activity of selected platelet and coagulation markers after LDL-apheresis and rheopheresis

Grant Agency: Charles University ***Project Number:*** 373611

Principal Investigator: M. Lánská

Co-investigators: M. Bláha, I. Fátorová, M. Košťál, V. Mašín

Starting date: 22.3.2011 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 761

Summary of 2011 results

Title of the presentation: The activity of selected platelet and coagulation markers after LDL-apheresis and rheopheresis

Authors: M. Lánská (1), M. Bláha (1), I. Fátorová (1), M. Košťál (1), V. Mašín (2)
Fac. Med., Charles Univ., Hr. Králové: 2nd Dpt. of Internal Medicine, Haematology (1), Dpt. of Medical Biophysics (2)

Introduction: The aim of this research is to generate and verify an algorithm for optimal long-term follow-up of patients treated with extracorporeal elimination (LDL-apheresis and rheohemapheresis - RH). We utilized some special thrombocyte and coagulation parameters and want to correlate new results with our previous research.

Investigation schema: According to the grant research plan pair samples were collected before and after EE: a/ in cases of familial hypercholesterolemia (FH) after six months. b/ in other cases at the beginning and at the end of rheopheresis therapy.

EE therapy: a/ LDL-apheresis: adsorption Metod. b/ RH: Evaflux filter 4A.

Examination methods that were planned and used for the research: Immature platelet fraction – IPF, Activation of primary hemostasis using Impact-R device (DiaMed AG Morat, Switzerland), Platelet factor 4, β-thromboglobulin, Cytosolic kalcium, Tissue factor, Fragments F1, F2, Complexes thrombin-antithrombin (TAT) and Thrombomodulin.

Current results. First results show changes in activity of tests that up to date have not been described but may be of considerable clinical importance. After EE drop in thrombomodulin level was observed as well as in tissue factor and β-thromboglobulin; activation of primary coagulation decreased (but not yet significantly), only marginal drop of fragments F1, F2 and TAT was observed, and also a slight (non significant) increase in IPF percentage.

Conclusion. The first results support our hypothesis that a shift to normal values occurs in examined activated components of hemocoagulation system. Selected markers could be useful to follow-up patients treated with EE.

Project was supported by the Charles University, Grant Agency, No 373611.

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Title of the project: Pathophysiology of neuropsychiatric disorders and clinical applications: severe neuropsychiatric disorders their pathogenesis, prevention and treatment.

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

Principal Investigator: R. Rokyta

Co-investigators: J. Libiger, M. Kuba, J. Kremláček, I. Tůma, J. Hons, A. Urban, J. Masopust, J. Bažant

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

Total funds allocated for project - Kč (thousands): 414500

Summary of 2011 results

Title of the presentation: Summary of main research results in the period 2005-2011

Authors: J. Libiger, A. Urban. J. Hons, J. Masopust, J.Kremláček.R. Malý

The focus of research activities aimed at three principal targets: the significance of electrophysiological markers in patients with schizophrenia for understanding the pathophysiology of the disorder, the relationship between serum levels of excitatory amino acids and symptoms of schizophrenia and the pathophysiology and practical significance of vascular adverse effects related to antipsychotic treatment in schizophrenia.

We identified and published the deficit (missing or lower amplitude) of " mismatch negativity"(MMN) phenomenon in the motion induced ERPs of patients with schizophrenia. So far , this marker was investigated only in the auditory domain. Our finding supports the assumption that the poor MMN in the event related potentials of patients with schizophrenia may be a multimodal deficit related to early cognitive processing of sensory information. MMN can be linked to the NMDA receptor function in the brain and it was hypothesized to correspond with a working memory dysfunction. The decrease of MMN amplitude is detectable mainly among patients with the schizophrenia deficit syndrome.

The second main topic was the inquiry into the relationship of excitatory amino acids (EAA) , serine, glycine and D- serine, to the negative symptoms of schizophrenia. It was part of the search for markers in those patients who would benefit from adding EAA to their antipsychotic treatment, and thus help to overcome the poor effcacy of most antipsychotics in treating negative symptoms. We did not confirm the low levels of D- serine in serum of patients with schizophrenia. We found a significant negative relationship between the total serine levels and negative symptoms, and also low levels of glycine in comparison to healthy volunteers.

The evaluation of the venous tromoembolism risk among patients treated with antipsychotics was the third main topic of our research effort. Low plasma levels of P selectin and d-dimers were found in patients with schizophrenia relative to healthy volunteers. The activation of blood coagulation was detected also in first episode patients with schizophrenia, who were treatment naïve . We constructed and published the guidelines for evaluation of venous tromboembolism risk in restrained pychiatric patients as well as rules for monitoring and prevention of cardiovascular risks in antipsychotic treated patients .

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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): 521

Summary of 2011 results

Title of the presentation: Secretory and phagocytic activity of peritoneal macrophages 72 hours after laparoscopic and laparotomic surgery in rats

Authors: H. Lotková (1), L. Sákra (2), Červinková (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1), Regional Hospital Pardubice: Dept. of Surg. (2)

Literature data support that laparoscopic surgery can lead to the supression of local immune response. The aim of our study was to determine both the secretory and phagocytic activities of peritoneal macrophages isolated after laparoscopic surgery 72 hours after surgery.
Male Wistar rats underwent laparotomic or laparoscopic caecectomy, control animals anaesthesia. 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 TNFalpha, IL-1 and IL-6 were measured using ELISA kits. Phagocytic activity was evaluated using commercial kit (Vybrant Phagocytosis Kit, Molecular Probes). Statistical analysis was done using GraphPad Instant 3.06 for Windows (USA).
72 hours after surgery the basic production of TNF alpha and IL-1 intensified after laparoscopy and phagocytic activity was raised too. In the stimulated macrophages, laparoscopy attenuated increase in the production of TNF alpha, IL- 1 and IL-6 while phagocytosis did not change. After laparotomy the extensive production of cytokines was accompanied with reduced phagocytosis. Laparoscopic surgery could better prevent an intensive local inflammatory response, while preserving the phagocytic activity.

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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 (MM) and with monoclonal gammopathy of unknown significance (MGUS).

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

Principal Investigator: V. Maisnar

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

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

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

Summary of 2011 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, M. Tichý, J. Radocha, J. Vávrová, L. Zahradová, M. Holečková, R. Hájek

Our common project was focused on verifying the usefulness of Free Light Chains (FLC) analysis, which is the new marker of evaluation of the activity of monoclonal gammopathies. As a part of research and standardization activities of CMG we have a long term focus on methodology of parametres in myeloma patients. FLC evaluation is not a cheap method and thus we need to reconfirm the reliability of information provided by manufacturer for its use. Formulated goals of this project represented the most likely beneficial indications for FLC use in clinical practice. Therefore we standardized FLC analysis at first, then we examined benefit of this analysis in patients with MGUS and also for monitoring of disease activity in MM patients. The last part of the project was focused on analysis of patients in complete remission of the MM. For serum analysis we used standard FLC assay (The Binding Site, UK) together with the new ELISA method (BioVendor Laboratory Medicine, Inc., CR),.immunoturbidimetric assays were performed using analyser Modular P. We collaborated with Registry of Monoclonal Gammopathies databasis of Czech Republic (today, almost 4.000 pts). We were able to confirm validity of the prognostic model of MGUS stratification originally developed by Mayo Clinic. Moreover several other independent prognostic factors not included in the mentioned model have been identified, these we plan to include in the further analysis and development of more detailed prognostic model. For most patiens with monoclonal gammopathies, except of those with non-secretory/oligosecretory MM, measuring FLC is unlikely to have additional benefit for monitoring of disease activity. Normalization of FLC ratio was not connected to statistically significant prologation of complete remission duration, however trend towards it was observed. Longer follow-up is needed for these patients.

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Title of the project: Creating of prognostic panel in patients with monoclonal gammo-pathy 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: R. Hájek (3)

Co-investigators: V. Maisnar (1), M. Tichý (2), J. Radocha (1), M. Klincová (3), L. Kovářová (3), J. Vávrová (2)

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

Total funds allocated for project - Kč (thousands): 10038

Summary of 2011 results

Title of the presentation: Monoclonal Gammopathy of Undeterminated Significance: Current Clinical Issues

Authors: V. Maisnar (1), M. Klincová (2), J. Radocha (1), V. Sandecká (2), R. Hájek (3)

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

Monoclonal gammopathy of undetermined significance (MGUS) is a precancerosis comprising two different kinds of cancer: lymphoid/lymphoplasmocytoid MGUS and plasma cell MGUS that represents about 85% of all MGUS cases. This type of MGUS has low but persistent tendency to transform to malignant disease, mainly multiple myeloma (MM), with frequency of about 1% per year. Using known risk stratification models based on clinical parameters, it is possible to identify patients' groups with average rates of progression as low as 0.26% and as high as 12% per year. However, due to the lack of clear genetic and/or phenotypic markers distinguishing MGUS from MM, we are not able to predict if and when MGUS will progress to MM in individual patients. There are partially overlapping molecular pathogenic events shared by MGUS and MM.

Flow cytometry and genomic approaches have been useful in identification of subtypes of MGUS and MM with important clinical implications for prognosis and subsequent treatment. As the number of clonal PCs is an independent predictive marker of progression, focusing on its appropriate determination is very important. GEP (gene expression profiling) is a novel genomic method that would greatly improve current knowledge about changes in gene expression in MGUS in comparison to MM.

Better understanding of pathogenesis of MGUS and MM using molecular-genetic approaches will help disclose the mechanisms of myeloma genesis; it can be also useful for identification of novel molecular targets. The ultimate goal for the near future is to develop better markers for definition of high-risk MGUS patients who will be candidates for early treatment intervention.

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Title of the project: Racionalisation of therapy of multiple myeloma

Grant Agency: Ministry of Health ***Project Number:*** NT/12215-4

Principal Investigator: J. Bačovský

Co-investigators: V. Maisnar, I. Špička, E. Gregora, M. Krejčí

Starting date: 1.9.2011 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6479

Summary of 2011 results

Title of the presentation: RMG – Registry of Monoclonal Gammopathies

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

2nd Dept. of Medicine - Div. of Clin. Haematology, Faculty Hospital Hradec Králové (1), 3rd Dept. of Medicine, Faculty Hospital Olomouc (2), 1st Dept. of Medicine, General Faculty Hospital Prague (3), Dept. of Hematology, Faculty Hospital Královské Vinohrady Prague (4), Hemato-oncological Department., University Hospital Brno - Bohunice (5)

Registry of monoclonal gammopathies is one of the main Czech Myeloma Group projects. The purpose of this project is the prospective data analysis of monoclonal gammopathies patients in the region of the middle and also the east Europe including incidence of diseases, therapeutical modalities used, the treatment results and the most frequent adverse events of therapy.

Co-investigators and his datamanagers are responsible for data collection in their Faculty Hospitals, validation and digitalisation of data, which is necessary for planned analysis within different topics of the project. They will participate on statistical analysis, evaluation of different statistical analysis and their presentation at domestic and international scientific meetings according to the agreement with principal investigator.

Registry of monoclonal gammopathies together with the programme CRAB represent currently two main projects of the Czech Myeloma Group. Currently it is registered almost 4.000 patients with monoclonal gammopathies, 2401 patients with multiple myeloma and 1580 patients with monoclonal gammopathies of undetermined significance. It is ambitious project which could help us to improve the care about patients with monoclonal gammopathies in Czech republic.

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Title of the project: Direct oxymetric peripheral tissue perfusion monitoring during open heart surgery procedures with the use of miniinvasive cardiopulmonary bypass

Grant Agency: Ministry of Health ***Project Number:*** NS/10376-3

Principal Investigator: J. Manďák

Co-investigators: V. Lonský, V. Brzek, J. Kovalský , J. Kubíček, M. Volt

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

Total funds allocated for project - Kč (thousands): 4151

Summary of 2011 results

Title of the presentation: Peripheral tissue oxygenation during cardiac surgery using standard versus miniaturized cardiopulmonary bypass (CPB) - direct oxymetric tissue perfusion monitoring study

Authors: J. Manďák, V. Lonský (1), V. Brzek (1), J. Kovalský (2), J. Kubíček (1), M. Volt (1), M. Horák

(1) Department of Cardiac Surgery, (2) Department of Anesthesiology, Resuscitation and Intensive Care, Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Kralove, Czech Republic

Aim: The aim of this study was to compare the impact of standard cardiopulmonary bypass (CPB) and miniaturized CPB during cardiac surgery on peripheral tissue perfusion.

Methods: 40 patients were randomised into two groups – Group A (20 patients, operated using standard CPB) and Group B (20 patients, mini CPB). The measurement of oxygen tension was performed with an optical multiparametric sensor inserted into the patient´s deltoid muscle. Continuous measurement of interstitial tissue oxygen tension (ptO2) was made during the surgical procedure and postoperative period by special analyzer. Arterial blood pressure, blood flow during CPB, laboratory markers of tissue hypoperfusion, blood gases and body temperature were recorded.

Results: The both groups did not differ in the basic pre and peroperative characteristics. Lower priming volume in Group B (837 ± 221 mL) vs. Group A (1501 ± 48 mL) and significantly higher hematocrit (Group B 0.31 ± 1.1 % vs. Group A 0.25 ± 2.3 %) were recorded. Higher (than calculated) and continuous blood flow during CPB was analysed in Group A (4.9 ± 0.34 vs. 4.7 ± 0.45 L.min-1) and lower blood flow during CPB was found in Group B (3.8 ± 0.51 L.min-1 vs. 4.6 ± 0.38 L.min-1). Direct correlation between mean arterial pressure (MAP) and ptO2 was observed in Group A during CPB. Direct correlation between pump blood flow and MAP was found during CPB in Group B. Higher levels of ptO2 during CPB and surgery after CPB in comparison with initial levels were observed in Group B. Decreasing of ptO2 levels after surgery were found in both groups.

Conclusion:Miniaturized CPB enables perfusion with relatively low flow. The results of this study suggest that a flow decrease in mini CPB is well tolerated by the organism.

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Title of the project: Pathophysiological mechanisms of organ dysfunctions – new approaches in diagnostics and therapy.

Grant Agency: Charles University ***Project Number:*** 262901

Principal Investigator: S. Mičuda

Co-investigators: M. Červinka, V. Geršl, Z. Červinková, J. Hanuš, M. Řezáčová, J. Mokrý, Z. Fiala

Starting date: 1.1.2011 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1500

Summary of 2011 results

Title of the presentation: Pathophysiological mechanisms of organ dysfunctions – new approaches in diagnostics and therapy.

Authors: S. Mičuda (1), M. Červinka (2), V. Geršl (1), Z. Červinková (3), J. Hanuš (4), M. Řezáčová (5), J. Mokrý (6), Z. Fiala (7). Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology (1), Dept. of Medical Biology and Genetics (2), Dept. of Physiology (3), Dept. of Medical Biophysics (4), Dept. of Medical Biochemistry (5), Dept. of Histology and Embryology (6), Dept. of Hygiene and Preventive Medicine (7)

The aim of the present project was to support research activities of postgraduate students at the theoretical departments of Faculty of Medicine in Hradec Králové. Scientific target of the project was to contribute to current understanding of molecular mechanisms involved in the initiation and development of selected diseases together with the evaluation of new diagnostic and therapeutic possibilities. With respect to continuous activities of involved research groups, particular studies were focused on heart, liver, kidney, lung and other organ impairments which either were induced by administration of drugs (e.g. anthracycline chemotherapy), toxins (e.g. endotoxin of gram-negative bacteria) and food components (e.g. high-fat diet) or resulted from accumulation of endogenous compounds (e.g. bile acids during extrahepatic cholestasis). Furthermore, the project was also focused on the epidemiological studies of harmful influence of some physical and social factors on the health status of the population. Most salient data from the project contributed to understanding of molecular mechanisms of anthracycline cardiotoxicity, hepatoprotective effect of statins during cholestatic liver injury, capillary leak syndrome and its effect on pharmacokinetics of gentamicin, regeneration of liver parenchyma after partial hepatectomy and its impairment by fatty degeneration, and identification and detailed characterization of new promising cytostatic agents isolated from plants. Studies in humans suggested suitability of salivary cortisol as a indicator of different kind of stress.

Project was supported by the Charles University project, No SVV-2011-262901.

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Title of the project: Innovation of the histology and embryology practical classroom.

Grant Agency: Ministry of Education ***Project Number:*** 653 A

Principal Investigator: J. Mokrý

Co-investigators:

Starting date: 1.1.2011 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1440

Summary of 2011 results

Title of the presentation: Innovation of the practical classroom for Histology and Embryology

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

The aim of the project was to innovate the practical classroom of the Department of Histology and Embryology and to stimulate students to active work in practical classes and seminars. The practical classroom was equipped with 17 new microscopes CX21 that replaced old ones. 13 new microscopes were equipped with digital cameras projecting an image to students' LCD screen. This enabled the students to work in groups (e.g. by discussions when identifying structures they observed in monitors). The connection of cameras with students' computers enabled the student to download digitalized images of microscopical structures and use them in their self-study. Another innovation involved a purchase of the stereomicroscope SZX7 and a high resolution digital presenter UF-80 DX which enabled the teaching assistants to demonstrate macroscopic transparent as well as non-transparent structures (histological slides, animal embryos, educational models etc.). The practical classroom was also equipped with a wireless
Turning Point system consisting of 46 response card devices and a receiver which permits a rapid control of students‘ knowledge. Moreover, the Turning Point software allows to process and evaluate all the students‘ responses immediately and calculate the statistics of correct and false responses and present them in a graphic form. All these innovations improve conditions for the practical study of histology and increase motivation and activity of students during practical classes in histology.
Project was supported by the grant project FRVŠ 653/2011.

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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): 663

Summary of 2011 results

Title of the presentation: The use of fiducial markers for patient setup allows safety margin reduction and better sparing of organs at risk

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. Image-guided radiotherapy (IGRT) gives 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.

We used cone-beam CT (CBCT) to compare two different styles of IGRT: 29 patients (134 CBCTs) with bony landmark (BL) setup vs. 30 patients (177 CBCTs) with fiducial markers (FM) setup were assessed. We delineated clinical target volumes (prostate - CTV2, seminal vesicles - CTV1-2) 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 original treatment plan assuming 10mm margin. Fluences from hypothetical alternative plans considered tighter 7mm margins were also used for the dose reconstruction. Possibility of margin reduction was evaluated by means of calculated target coverage by the 95% isodose. CTV2 was underdosed in 9 % of reconstructed plans in case of BL and 10mm margin, 20 % in BL-7mm and 3 % in FM-7mm. CTV1-2 was underdosed in 2 % in BL-10mm, 10 % in BL-7mm and 7 % in FM-7mm. While the margin reduction in case of BL setup makes the prostate coverage significantly worse ($p=0.015$, Fisher's exact test), in case of FM setup with the reduced 7 mm margin the prostate coverage is even better compared to BL setup with 10mm margin ($p=0.049$, Fisher's exact test). Moreover, partial volumes of organs at risk irradiated with a specific doses can be significantly lowered ($p<0.0001$, unpaired t-test). This creates a potential for dose escalation in the future treatments.

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

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

Principal Investigator: P. Widimský

Co-investigators: R. Pudil, J. Ceral, J. Šťásek

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

Total funds allocated for project - Kč (thousands): 1900

Summary of 2011 results

Title of the presentation: Scientific results of the MSM 0020162817

Authors: all group of the MSM project and co-authors

Project activities were focused on the completion of research and publication.
In 2011, research activities were focused on the following areas:
1. cardio-markers and their use in diagnosis and treatment of cardiovascular diseases.
We evaluated high-sensitivity troponin T as a marker of myocardial injury after radiofrequency catheter ablation, the role of ischemia-modified albumin in patients with ST evaluation myocardial infarction. We studied the possibility to use combination of immune and myocardial structural markers in detection of hypertrophic cardiomyopathy.
2. Invasive procedures in cardiology
The group evaluated percutaneous closure of the left atrium appendage as a prevention of systemic embolisation. As a part of the reasearch team, we participated in two research projects: (1) Primary angioplasty in acute myocardial infarction with right bundle branch block: should new onset right bundle branch block be added to future guidelines as an indication for reperfusion therapy?, and (2) Routine upfront abciximab versus standard periprocedural therapy in patients undergoing primary percutaneous coronary intervention for cardiogenic shock: The PRAGUE-7 Study. An open randomized multicentre study.
3. Arterial hypertension research
The project was focused on difficult-to-control arterial hypertension or uncooperative patients. The main method was to assess the serum antihypertensive drug levels to differentiate non-responsiveness from non-adherence to recommended therapy.
In summary, the research project resulted in 4 original publications in impact factor journals, other three original publications in peer-reviewed journals and into two monographs in 2011.
The authors also participated in the preparation of one guideline.

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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 2011 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,

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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 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. We studied 114 patients (65 males, 49 females) of our database; the age was 6-18 years. Quantification of the peptides is performed by enzyme-linked immunosorbent assay (ELISA). Results. We found anemia (Hb < 120 g/l) in 54.4% of patients and serious anemia (Hb < 100 g/l) in 9,6%. We described microcytic anemia in 43.4% and hypochromic anemia in 46.3% of the patients. The iron level was decreased in 55.2%, ferritin was decreased in 53.3% and increased sTfR level was found in 45.1%. In 2 patients it was necessary to administrate transfusions of erythrocytes due to severity of anemia. Physical activity was affected in 5.3%. We performed ELISA test for hepcidin on 37 patients: 19 boys and 18 girls. The range of hepcidin level in blood was 31,9 – 133,7 ng/ml. Conclusions. Anemia was present in about a half of patients with IBD. The oral iron therapy is often ineffective and not well tolerated. One of the possible reasons could be the increased level of hepcidin. Increased hepcidin levels would be helpful in patients selection for parenteral iron therapy.

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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: R. Praus, P. Pařízek, V. Bláha, J. Popelka

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

Total funds allocated for project - Kč (thousands): 589

Summary of 2011 results

Title of the presentation: Echocardiographic changes after cardiac resynchronization therapy

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

Aim: The aim of the study was to evaluate echocardiographic changes in clinical responders and nonresponders after 3 and 15 months of cardiac resynchronization therapy (CRT).

Methods: 147 patients in whom a biventricular system was implanted from 7/2005 to 5/2010 were followed up at 3 and 15 months. Clinical and echocardiography parameters including systolic function of the right ventricle (RV) and ventricular dyssynchrony were assessed at baseline and after 3 and 15 months of CRT.

Results: In the responders group we found that except for the right ventricular systolic function, in which we had significant improvement only after 15 months (Sa 12.1 ± 3.2 cm/s to 13.1 ± 3.5 cm/s, $p < 0.05$, TAPSE 19.0 ± 4.6 mm to 20.5 ± 4.3 mm, $p < 0.001$), significant improvement of other important monitored parameters occurred 3 months after CRT implantation (left ventricle [LV] end-diastolic diameter 67.0 ± 8.1 mm to 62.8 ± 8.4 mm, $p < 0.001$, LV ejection fraction 23.8 ± 6.7 % to 31.9 ± 12.9 %, $p < 0.001$, pulmonary artery pressure [peak gradient of tricuspid regurgitation] 36.1 ± 13.7 mmHg to 27.9 ± 8.9 mmHg, $p < 0.001$, mitral regurgitation 2.6 ± 0.9 to 2.2 ± 0.9, $p < 0.001$). These changes did not occur in the group of nonresponders; on the contrary, after 15 months we saw significant progression of tricuspid regurgitation. These groups differed significantly in the degree of ventricular dyssynchrony and in the right ventricular systolic dysfunction initially.

Conclusion: In the group of responders significant changes of most monitored echocardiographic parameters were observed 3 months after CRT implantation. The only parameter which changed significantly only after 15 months was RV systolic function.
In the group of nonresponders these changes were not observed.

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Title of the project: Chatacteristic 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, J. Cmielová, R. Havelek, M. Seifrtová, A. Jiroutová, R. Kohlerová

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

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

Summary of 2011 results

Title of the presentation: Cisplatin-induced changes in dental pulp stem cells.

Authors: M. Seifrtová1, R. Havelek1, J. Ćmielová1, A. Jiroutová1, T. Soukup2, J. Mokrý2 , and M. Řezáčová1

Fac. Med., Charles Univ., Hr. Králové: Dept. of Med. Biochem..(1), Dept. of Histol. Embryol. (2)

Human dental pulp contains in adulthood stem cells (DPSCs) that are capable of differentiation into osteoblasts, odontoblasts, adipocytes and neuronal-like cells. These cells have potential use in periodontal tissue regeneration. We determined the response of dental pulp stem cells to DNA-damaging cytostatic cisplatin.

The cells were exposed to doses 5, 10, 20 and 40 umol/l cisplatin. Proliferation of affected cells was analyzed by Z2 Counter and viability by Vi-Cell XR using Trypan blue exclusion staining. Cell cycle distribution and induction of apoptosis were detected by flow cytometry. Induction of apoptosis was also determined by monitoring the activities of caspases. The expression of proteins was detected by electrophoresis and Western blotting.

Higher concentrations of cisplatin reduced the viability of DPSCs and induced the activation of caspases 3/7, and 9. The exposure of DPSCs to cisplatin provoked an increase in p53 and p21 expression and p53 phosphorylation of serine 15. All three main MAPK families – extracellular signal-regulated kinases (ERK), c-Jun-N-terminal kinase (JNK) and p38 were activated after treatment of DPSCs with cisplatin. This activation of MAPK pathways was not observed in equally treated human dermal fibroblasts.

Conclusion: Cisplatin in higher concentrations triggers activation of MAPK and apoptosis in DPSCs.

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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 2011 results

Title of the presentation: Detection of proinflamatory markers of oxidative stress in irradiated as an indicator of received dose of radiation

Authors: J. Chládek (2,3), A. Babicová (3), J. Vávrová, J. (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)

Text: The aim of the project was to investigate the changes in the level of oxidative and nitrosative stress induced in the circulation as well as in the airways of female SPF Wistar rats during an early stage (within 24 h) after whole body ionizing radiation. Furthermore, a delayed inflammatory phase of response to local irradiation of the chest (radiation pneumonitis) was investigated. The effets of potentially radioprotective agents acetyl-L-carnitine (ALC) and L-NG-Nitroarginine methyl ester (L-NAME) were studied. There were no differences between irradiated groups (IG) and controls in the concentration of exhaled nitric oxide (eNO) either during the early or late phase. Within 24 hours after irradiation, the plasma concentration of nitrite+nitrate (NOx) increased dose-dependently to a maximum level 4-fold higher than that of controls ($p < 0.001$). The non-specific inhibitor of NO synthases L-NAME increased MDA of controls but exerted no influence on its elevated level observed in irradiated rats unlike ALC which caused a reduction. On the contrary, only L-NAME decreased NOx in the plasma of irradiated rats. A trend towards a dose-dependent increase ($p = 0.06$) of MDA concentration in the bronchoalveolar lavage fluid was detected at seven weeks after local irradiation with 15Gy (+40%) and 20Gy (+70%), respectively. The development of radiation pneumonitis was demonstrated histologically by a decreased airness of the lungs and increased counts of phagocytic cells. The concentration of eNO did not change excluding the value of this noninvasive test either for biodosimetry or as a predicitve marker of radiation pneumonitis in rats. In the airways and lung, the changes in the metabolic pathway of L-arginine-NO caused by ionizing radiation are small.

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Title of the project: New methods and algorithms in diagnostics and search for predictive and prognostic markers in malignant tumors

Grant Agency: Charles University ***Project Number:*** 262902

Principal Investigator: A. Ryška

Co-investigators: J. Krejsek, V. Buchta, A. Krajina, P. Živný

Starting date: 1.1.2011 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1340

Summary of 2011 results

Title of the presentation: New methods and algorithms in diagnostics and search for predictive and prognostic markers in malignant tumors

Authors: A. Ryška, J. Krejsek, V. Buchta, A. Krajina, P. Živný

Current knowledge of tumor biology is still limited. As the diagnostic and therapeutic options are more and more oriented towards individualization, there is a strong need for specific biomarkers, which might predict the effect of particular therapy in each patient.
During the project, several research groups were looking for different biological markers which could fulfill the above mentioned needs. Among other results, there was studied role of T-lymphocytes in the pathogenesis of psoriasis, markers of inflammatory response in pregnant patients with premature rupture of amniotic membrane. Microbiology team has focused on study of in vitro activity of ftalocyanins on certain selected microbial and fungal pathogens. Radiology team has developed and introduced a new method into routine practice - chemoembolisation of hepatocellular carcinoma by corpuscules with active surface. Clinical biochemistry studied mutations C282Y, H63D and S65C in patients with suspicion on haematochromatosis. Pathology team studied several topics - breast cancer in young women, where it was found that these cases frequently display aggressive morphology, high proliferative activity, increased expression of p53 and a high incidence of lymph node metastasis. Triple-negative (TN) and HER2/neu positive phenotypes were over-represented in our sample of young women compared with the group of all women; in gastric carcinoma was studied the role of HER-2/neu protein in pathogenesis, in colorectal carcinoma, role of intercellular matrix - namely laminin expression and its correlation with the immune response was main topic.

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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 2011 results

Title of the presentation: Body Composition Monitoring in Chronic Hemodialysis and Kidney Transplant Patients

Authors: R.Šafránek (1), K.Petráňová (1), L.Habáňová (1), M.Kubišová (1), P.Moučka (1) S.Dusilová Sulková (2); 1 Dept. of Metabolic Care and Gerontology, University Hospital Hradec Králové, Czech Republic; 2 Institute of Clinical and Experimental Medicine, Prague, Czech Republic

Introduction: Chronic hemodialysis (HD) patients are at increased risk of malnutrition. Simple routine measurements, e.g. BMI are not suitable for HD patients. The aim of our work was to assess one-year monitoring of body composition of HD patients and compare it with kidney transplant patients. Methods: Body composition was assessed in 63 HD patients (22 females, 64 years) in two-month interval for one year. Bioimpedance spectroscopy (Body Composition Monitor) was used to estimate LTI (lean tissue index, kg/m^2),FTI (fat tissue index, kg/m^2). BMI (body mass index, kg/m^2) was calculated. Body composition of HD patients was compared with 100 kidney transplant patients (40 females, 53 (20; 99) months after kidney transplant, serum creatinine 123 (94; 156)µmol/l). Data are given as median (lower; upper quartile). Results: BMI, LTI, and FTI at the beginning of the study were 28.8 (26.2; 33.8) kg/m^2, 13 (11.4-14.8)kg/m^2, and 15.5 (10.8; 18.3)kg/m^2, in HD patients and 28.3 (25; 31.2)kg/m^2, 14.9 (12; 17.2)kg/m^2, 12.8 (8.7; 16.8)kg/m^2 in kidney transplant patients. Compared to reference range for normal population, we observed low lean tissue index in 44% and 33% of HD and kidney transplant patients and high fat tissue index in 65% and 60% of HD and kidney transplant patients, respectively. We observed no change in BMI during the course of the study in the group of HD patients, but changes in lean and fat tissue index in individual patients. Conclusions: Bioimpedance spectroscopy shows low lean tissue mass in half of HD patients and high fat amount in two thirds of HD patients. Similar results we obtained in a group of kidney transplant patients, which indicates profound defect in tissue composition regulation both in HD as well as in transplant status. We observed no influence of kidney graft function and time from kidney transplant on body composition. Body composition monitor is useful in both HD and transplant patients and reveals changes of body composition in individual patients that may help in management of the patients.

Supported by the Internal Grant Agency of the Ministry of Health, No. NT/11355-4.

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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 (1)

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

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

Total funds allocated for project - Kč (thousands): 423000

Summary of 2011 results

Title of the presentation: PurStem Project

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

Stem cells offer a promising avenue to therapy for a wide range of complaints. However, for this potential to be realized, a consistent and plentiful supply of well-characterised stem cells is essential. There has been relatively little progress in the development of new culture technologies for the large-scale manufacture of mesenchymal stem cells (MSCs). There is a strong possibility that this limited ability to produce stem cells will result in delays to the translation of new therapies to the clinic. This will have a direct negative effect on the health of European citizens suffering from diseases untreatable by conventional medical technology and delay European efforts to promote "NanoMedicine - Nanotechnology for Health". PurStem project is progressing the state of the art in the production of mesenchymal stem cells (MSCs) in large quantities. PurStem described the MSC "receptome" and used this repertoire of growth factor receptors to develop novel serum-free media for MSC production. PurStem also produce novel antibody reagents for specific MSC characterization and contribute to GMP manufacturing standards to enable rapid progression to production of serum-free MSC for clinical applications.

Team of the Charles University in Prague, Medical Faculty in Hradec Kralove (CUNI) was responsible for serum free media development. The combinatorial growth factor/ligand array developed from the output of PurStem team based in Leeds, UK and Galway, Ireland was initially tested on MSC isolated from bone marrow and dental pulp. The key results of CUNI team were summarized in a standardized procedure (SOP). Subsequent protocols serve as uniform methods of MSC serum-free isolation, culture and cryostorage.

The research leading to these results has received funding from the European Community's Seventh Framework Programme FP7/2007-2011 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 2011 results

Title of the presentation: The effect of succinate, coenzyme Q9, L-carnitine and darbepoetin alfa on toxic liver injury and on liver regeneration

Authors: P. Staňková (1) , O. Kučera (1) , T. Roušar (1) , H. Lotková (1), R. Endlicher (2), Z. Červinková (1)

Fac. Med., Charles Univ., Hr. Králové: Dept. of Physiology (1), Dept. of Anatomy (2)

The possibility to affect the process of liver regeneration could markedly contribute to treatment of toxic liver injury, which now becomes a significant healthy problem. The aim of this study was to describe the effect of exogenous succinate (a metabolite of Krebs cycle and a substrate of respiratory complex II) L-carnitine (a carrier of free fatty acid and a possible regulator of liver regeneration) coenzyme Q9 (an electrone carrier and a redox regulator) and darbepoetin alfa (a long acting analogue of erythorpoietin with diverse biological functions) on acute toxic liver injury and on liver regeneration.

Hepatocytes were isolated from male Wistar rats by two-step collagenase perfusion and incubated on collagen-coated Petri dishes. Toxic injury was induced by thioacetamide (TAA, 60 mM, 24h). Succinete (10 mM), L-carnitine (3 mM) and coenzyme Q9 (5 uM) were added together with TAA or 30 min before. We estimated LDH leakage, urea and albumin synthesis, GSH/GSSG content, mitochondrial respiration, mitochondrial membrane potential (MMP) and ROS production. Liver regeneration was induced *in vivo* by 2/3 partial hepatectomy (PH). Darbepoetin alfa (DA, 10μg/kg) was applied three days before PH and L-carnitine (100 mg/kg) was applied immediately after PH and six hours after PH. Animals were sacrificed 24 hours after surgery. In addition to routine serum analyses we measured mitochondrial respiration, MMP, ROS production, ATP content, mitochondrial swelling and GSH/GSSG content. Histopathological samples and samples for gene expression microarray analyses were also prepared.

We have not seen any protective effect of L-carnitine and succinate against hepatocytes injury induced by TAA *in vitro*. Succinate even increases LDH leakage, ROS production and decreases glutathione content. It seems that succinate behaves as a paracrine signal for liver damage and succinate dehydrogenase has specific function in superoxide handling. Coenzyme Q9 decreases LDH leakage and MDA production but did not affect mitochondrial functions. L-carnitine and DA demonstrate stimulating effect on liver regeneration induced by PH. Data from gene expression analyses are processed.

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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, M. Beneš, P. Drastich

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

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

Summary of 2011 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, T.Douda
Second Dpt. of Internal Medicine, University Hospital, Hradec Králové

The Czech Republic belongs to the countries with highest colorectal cancer (CRC) incidence and mortality. Screening is an important part of secondary prevention and the main aim is diagnosis and polypectomy of adenomas and early stages cancers. Our multicenter prospective study is focused on comparing efficiency of colonic capsule endoscopy and colonoscopy in detection of colorectal polyps and cancers. In years 2010 – 2014, 232 healthy people (asymptomatic individuals aged $\geq$ 50) will be examined in all 4 centres included into our study (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.

Our project started in October 2010. We developed new methodology and protocol of the capsule colonoscopy and organized first 16 investigations without any clinical complications. The capsule investigation failed due to the extremely fast peristalsis and capsule excretion in one patient. We identified 6 polyps (up to the 6 mm) by means of capsule endoscopy and 11 small polyps (from 2 to 6 mm) during the colonoscopy at all. The polyps missed by capsule endoscopy were localised mostly in right hemicolon and were tiny (from 2 to 5 mm). The colon preparation was sufficient in majority of patients. We did not identified any cancer during all investigations yet.

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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. Votroubková, H. Ulrychová

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

Total funds allocated for project - Kč (thousands): 416

Summary of 2011 results

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

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

The work of the research team was devoted to three activities in 2011 – writing further theoretical and review studies, translation and pilot verification of a new questionnaire for patients, and clinical verification of an abridged version of the world-wide most used questionnaire that explores patients´ perception of illness. Three theoretical and review studies were worked out: 1. Meaning of the disease from the perspective of patients: the study summarizes the current home and international psychological knowledge of the patients´ perception of the meaning of their illness and gives an overview of qualitative and quantitative diagnostic methods (incl. the six most common questionnaires); it reminds us that the knowledge of the meaning attached to the illness by patients may help individualize the provided health care, particularly in chronic or life-threatening diseases. 2. Patients´ perception of illness in the context of transcultural nursing – the study summarizes knowledge of the impact of the cultural background on perception of illness by various patients; it analyses to which extent the patients´ perception of illness is a part of transcultural nursing models. 3. Mental illness – subjective perspectives and factors that influence them: the study points to limits of the biomedical view on mental illness and summarizes international efforts to explore subjective experience of patients with their illness and results of research oriented in this direction. In the empirical part, the questionnaire MIQ – SR (Meaning of Illness Questionnaire - Self Report) was translated (with the content of G. Browne) and pilot verification was carried out in 100 patients suffering from a cardiovascular disease. We analyzed the psychometric characteristics of the Czech version of the questionnaire. The second empirical study summarizes clinical experience with the routine application of the Czech shortened version of the questionnaire IPQ-R-CZ Brief (*Revised-Illness Perception Questionnaire- Brief)* in the set of 103 patients with diagnoses such as myocardial infarction, angina pectoris, atherosclerotic disease, valvular disorders, and thrombosis of lower extremities. The experience of nurses with the abridged version is encouraging – the questionnaire is a useful tool for the completion of patients´ nursing care history and a guide for the individualization of the educational process for each of the patients.

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

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): 4507

Summary of 2011 results

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

Authors: Z. Vavera (1), J. Vojáček (1), J. Malý (2), R. Pudil (1), P. Eliáš (3)
University Hospital Hradec Kralove: 1st. Dept. of Cardiovascular Medicine (1), 2nd Dept. of Medicine (2), Dept. Diagnostic Radiology (3)

Objective: Description of a relationships among simply and routinely aviable data (morphologic, laboratory, clinic, anamnestic) and risk of chronic thromboembolic pulmonary hypertension (CTEPH) as a chronic complication of an acute pulmonary embolism (PE). CTEPH incidence.

Methods: 120 consecutive patients (60 women) of age 19-85 (mean 57,9 ± 16,2) with proved acute PE as a first thromboembolic event underwent 2-year follow-up with echocardiography at the time of diagnosis, discharge, and at 6-, 12- and 24-months visit. NT-proBNP and troponin-T were assessed on admission and, if abnormal, at the time of discharge. 6 months since acute state a computer tomography pulmonary arteriography (CTAG) was performed, concerned to residua of thromboembolic masses and signs of pulmonary hypertension (PH).

Results: Echocardiographic signs of PH were present at the time of discharge in more than one half (50,4 %) of patients. Predictors of persisting PH at this time were initial pulmonary hypertension, high initial NT-proBNP levels (OR 6,5) and age. At 6-, 12-, and 24-months visit there were echocardiographic signs of PH present in 27 of 103 (26,2 %), 19 of 101 (18,8 %) and 6 of 97 (6,19 %) patients respectively. Five of them were considered as CTEPH. Risky appeared especially persistence of NT-proBNP and right ventricle (RV) dilatation at the discharge time and thromboembolic residua on CTAG.

Conclusion: NT-proBNP levels on admission can be used not only for an initial risk stratification, but also as an indicator for effective follow-up of patients in risk of PH persistence. Persisting high NT-proBNP levels and right ventricle dilatation at the discharge are risk factors for CTEPH as well as thromboembolic residua in the CTAG. Incidence of CTEPH in our cohort was 5,15 %.

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Title of the project: Development of production technology and application forms of glutathione with high bioavailability for the suppression of oxidative stress (radiation, chemotherapy)

Grant Agency: Ministry of Commerce ***Project Number:*** FR-TI3/496

Principal Investigator: Z. Zadák

Co-investigators: R. Hyšpler, A. Tichá, I. Svobodová, J. Krejcarová, M. Vacková, S. Janáčková

Starting date: 1.3.2011 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 19700

Summary of 2011 results

Title of the presentation: Glutathione in different preventive and clinical applications

Authors: Z. Zadák, R. Hyšpler, A. Tichá, I. Svobodová, J. Krejcarová, M. Vacková, S. Janáčková

Dept. of Research and Development, University Hospital Hradec Králové

Glutathione (GSH) is a tripeptide (g-Glu-Cys-Gly) and can be found in animal and plant cells. GSH is synthetized in the liver, delivered to peripheral tissues, functions as a principal intracellular antioxidant and also has an effect on reparation of the nucleic acids and toxin conjugation. Supplements containing glutathione are important in antioxidant protection of human organism and there are rich sources of GSH in industrial scale. Yeast biomass is a source of GSH in this project.

The doses 50 – 600 mg (average 250 mg) daily are recommended for humans. The major problem is biological availability of orally administered glutathione, which is very low. The aim of this starting project is a development of strategies to increase glutathione absorption and intracellular transport by encapsulation, hydrolysis protection, suitable carrier binding (transport enhancers) or by minor changes in molecular structure. The bioavailability of different forms of GSH will be tested using in vivo experiments.

Literature:

1. ANDERSON, M. E. Glutathione and glutathione delivery compounds. *Adv Pharmacol,* 1997, 38, p. 65-78.

2. LOMAESTRO, B. M., MALONE, M. Glutathione in health and disease: pharmacotherapeutic issues. *Ann Pharmacother,* 1995, 29, p. 1263-1273.

3. FRATERNALE, A., PAOLETTI, M. F., CASABIANCA, A. et al. Antiviral and immunomodulatory properties of new pro-glutathione (GSH) molecules. *Curr Med Chem,* 2006, 13(15), p. 1749-1755.

4. LIANG, G., WANG, B., XIE, J., MO, Y. Novel pH control strategy for glutathione overproduction in batch cultivation of *Candida utilis. Afric J Biotechnol*, 2009, 8(22), p. 6337-6345.

Project was supported by Ministry of Commerce, No FR-TI3/496.

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Title of the project: Leptin and it's relation to grow and bone density of eutrophic and hypotrophic pre-term newborns

Grant Agency: Charles University ***Project Number:*** 432411

Principal Investigator: P. Kanioková Veselá

Co-investigators: M. Bayer

Starting date: 1.1.2011 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 553

Summary of 2011 results

Title of the presentation: Prematurity and bone metabolism

Authors: P. Kanioková Veselá
Fac.Med., Charles Univ., Hr. Králové: Dept. of Paediatrics

Osteopenia of prematurity is a metabolic bone disease, refers to the hypomineralized skeleton of the premature infant. Just as the extrauterine growth rate lags behind the intrauterine rate, the extrauterine rate of the skeletal mineralization is delayed in comparison with that of the corresponding fetal skeleton. Data on the processes of osteopenia of prematurity are very limited. The cause is multifactorial.
Growth and development of bone structure during intrauterine life have predictive quality for development during childhood until the adulthood. From the studies with term born newborns we know that the leptin influence the maturation of chondrocytes and osteoblasts. There are little information about pre-term newborns, who are in higher risk of low bone density.
This study is prospective. We investigate 61 pre-term newborns – slight and mild immaturity.
We analyse Ca, P, ALP, AST, 25-OH D, leptin, osteocalcin in cord blood and each 6 months until 2 years of corrected age. We measure weight, length and placenta average too.
We measure bone mineral density by DXA in two years of life. Until now we investigate 9 children by DXA. Eight investigated children have bone mineral density in normal lag, one bone density was at the lower range of normal.
Literature: A.R. Spitzer et al.: Intensive care of the fetus and neonate, 1179–1197, 2005
Project was supported by the Charles University grant Agency, No 432411.

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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

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

Total funds allocated for project - Kč (thousands): 272730

Summary of 2011 results

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

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Prof. Zadák, Dr. Hyšpler – The framework of the project included the examination of depletion of cholesterol and the related new methods introduced into clinical practice, primarily the analyses of non-cholesterol sterols and squalene using the GC-MSD method, and the determination of cholesterol synthesis by the liver after administration of D_2O. Analytical methods were developed and validated for the determination of a "^{13}C isotope excess" in the glucose and lactate molecule for clinical research in various metabolic situations. Another topic under examination was the determination of plasma derivatives of hydrogen sulphide. Hydrogen sulphide ranks among the most recently discovered gasotransmitters and the determination of its plasma derivatives seems to be very topical also for other areas of research. New technologies were introduced for HPLC analyses for the determination of cis- and trans-retinoic acid in the serum and vitamin E in erythrocyte membranes. The team working on the theme of energy requirements in gravidity elaborated a revised Harrison-Benedict equation, whose formulation was published in the journal *Nutrition International*. **Prof. Černý** – The goals of the partial research task primarily included: (1) introduction of the methodology of examination of microcirculation in selected organs and tissues using the method of direct visualization in real time, (2) evaluation of the degree of the disorder in microcirculation in individual tissues and organs on experimental models of selected pathological conditions, evaluation of the effect of selected interventions into microcirculation in an experiment and clinical practice, 3) evaluation of the influence of anaesthesiological techniques and procedures on microcirculation in an experiment and clinical practice. New technologies enabling direct examination and analysis of microcirculation and regional tissue perfusions were introduced in an experiment and subsequently into clinical research – the methods Sidestream Dark Field (SDF) imaging and Laser Doppler Flowmetry (LDF), an inseparable part of these techniques being a software analysis of obtained data by means of newly available analytical programmes (AVA 1 and AVA 3, CapImage). The results from the experiment and human studies exert a direct

influence on the evaluation of the effect of a number of medical procedures (therapeutic interventions into microcirculation). The second principal contribution is the obtaining of priority data concerning the behaviour of microcirculation in various organs and tissues. The third contribution is the introduction of priority methods and models, quoted in world literature. **Assoc. Prof. Tošnerová** – The investigation within the framework of the project resulted in an introduction of new methods into clinical practice: 1) methods of complex functional and nutritional examination in hospitalized geriatric patients, 2) methods of early rehabilitation and supplementary nutrition in hospitalized geriatric patients, 3) methods of supplementary exercise to increase the muscular strength of arms and thighs. An aid for sensomotoric training was developed and tested; in hospitalized seniors it was developed as a training device. **Prof. Malý** – The principal research lines in 2011 included: examination of endothelial dysfunction in patients endangered by arterial thrombosis, examination of the response to pharmacoprevention of arterial thrombosis, monitoring of antiaggregation treatment, monitoring of new signs in the pathogenesis of venous thrombosis in psychiatric patients, validation of biomarkers of myocardial damage in cytostatic treatment and in thyroid gland diseases in pregnant women, and the formulation of the guidelines for the treatment of malignant lymphomas. **Prof. Bureš** – Our gastroentorologic team performed a multicentric epidemiological study of the prevalence of infection with *Helicobacter pylori* and dyspepsia in the Czech Republic. The functional changes in the small intestine after short-term administration of large doses of indomethacin with or without the probiotic bacteria *Escherichia coli* Nissle 1917 were also tested. Capsule endoscopy of the small intestine in patients with rheumatoid arthritis and osteoartrosis with microcytic anaemia and without anaemia, ex-vivo confocal laser endomicroscopy of gastro- and enteropathy due to nonsteroidal antiphlogistics, and computer-assisted morphometry of the small intestine after nonsteroidal antiphlogistics were performed. **Prof. Petera** – In the year 2011, oncological research dealt with the analyses of new biomarkers of damage in oncological treatment and the determination of advances in brachytherapy of oncological diseases. Also the quality of life in oncological patients was evaluated. **Prof. Palička** – The subgroup concerned with tissue metabolism and examination of the changes in the tissues and organs with the use of microdialysis techniques published priority results concerning an impairment of the tissue mechanism of metabolism of the submucosa of the gastric wall in an ischemia-reperfusion model. The previous years of research were linked up with the papers on the effect of Goeckerman therapy of psoriasis on immunity markers (demonstration of a change in sCH30, but not sCD30L); the whole area of research in this field resulted in a monograph dedicated to the Research Task. An extensive research and subsequently publishing activity accompanies the examination of the effects of antihypertensive agents and statins on the metabolism of the bone tissue. A considerable part of the activities of the team was focused on the examination of different gene variations and their clinical impact. Very prestigious findings are the results of the bond between gene variants of catechol-O-methyltransferase (Val158Meth) and the development and heaviness of dependence on methamphetamine, and also the findings concerning the genetic substrate and hyperplasia of the gingiva after therapy with immunosuppressive agents (cyclosporine A) are of a priority nature; the processing of the results is still in progress.

In the year 2011, 76 articles with the impact factor (total IF: 151.9) and 5 monographs were publicated and accepted.

Supported by the Research project MZO 00179906.

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