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

XXI. VĚDECKÁ KONFERENCE

P R O G R A M

25. ledna 2017

Velká posluchárna budovy Teoretických ústavů Lékařské fakulty
v Hradci Králové

Vydavatel: Univerzita Karlova, Lékařská fakulta v Hradci Králové
ISBN: 978-80-906595-0-6

T e c h n i c k é p o k y n y

V programu jsou uvedeny názvy řešených projektů a jména odpovědných řešitelů.

Věcná část publikovaných abstrakt dodaných řešiteli nebyla editována.

Ústní sdělení

1. Doba sdělení 10 minut, diskuse 5 minut.
2. K dispozici je dataprojekce.

Plakátová sdělení

Postery budou vyvěšeny po celou dobu konání konference. Prohlídka plakátových sdělení je možná v průběhu přestávek.

XXI. vědecká konference Lékařské fakulty v Hradci Králové a Fakultní nemocnice Hradec Králové 25. ledna 2017

09.00 - 09.15 Z a h á j e n í k o n f e r e n c e
prof. MUDr. RNDr. Miroslav Červinka, CSc. děkan lékařské fakulty
prof. MUDr. Vladimír Palička, CSc., dr. h. c. ředitel fakultní nemocnice

Sekce I Předsedající: **prof. Ing. Zdeněk Fiala, CSc.**

09.15 - 09.30 Testování a porovnání prokrvení anastomózy tračníku konstruované tkáňovým lepidlem ve vztahu k tradičně používaným technikám vytváření anastomóz
MUDr. Slavomír Blažej (přednášející: doc. MUDr. RNDr. Milan Kaška, Ph.D.)
GA UK 187515 (LF)

09.30 - 09.45 Vulnerabilita a možnosti reparace peroperačního iatrogenního poškození chámovodu v experimentu
MUDr. Radek Štichhauer
GA UK 160315 (LF)

09.45 - 10.00 Optimization of Treatment and Management of Schizophrenia in Europe
prof. MUDr. Jan Libiger, CSc.
(odp. řešitel: prof. dr. René S. Kahn - University Medical Center Utrecht)
FP7 OPTiMiSE (LF)

10.00 - 10.15 Finalizace technických a uživatelských vlastností pelet s řízeným uvolňováním glukózy
MUDr. David Neumann, Ph.D.
TA ČR GAMA TG02010020-1(FN)

10.15 - 10.30 Sledování změn v metylaci DNA u sinonazálního karcinomu
Mgr. Marcela Chmelařová, Ph.D.
MZ ČR RVO-FNHK/2016-1 (FN)

10.30 - 10.45 Proprotein kináza PCSK9 – diagnostický, terapeutický a prognostický potenciál při léčbě věkem podmíněné makulární degenerace reoferézou
prof. MUDr. Milan Bláha, CSc.
MZ ČR RVO-FNHK/2016-2 (FN)

10.45 - 11.00 *Přestávka – občerstvení*

Sekce II Předsedající: **prof. MUDr. Jiří Manďák, Ph.D.**

11.00 - 11.15 Detekce rezistence k acykloviru u izolátů herpes simplex viru ve Fakultní nemocnici Hradec Králové pomocí metod sekvenční analýzy
MUDr. Miroslav Fajfr, Ph.D.
MZ ČR RVO-FNHK/2016-3 (FN)

11.15 - 11.30	Sledování změn koncentrace donepezilu v mozkomíšním moku po orální aplikaci **doc. MUDr. Martin Vališ, Ph.D.** MZ ČR RVO-FNHK/2016-15 (FN)
11.30 - 11.45	Hodnocení myokardiálního poškození při dobutaminové a dynamické zátěžové echokardiografii **MUDr. Karel Mědílek, FRCP** MZ ČR RVO-FNHK/2016-5 (FN)
11.45 - 12.00	Letalita traumatické ruptury hrudní aorty v regionu traumacentra FN Hradec Králové **MUDr. Jan Trlica, Ph.D.** MZ ČR RVO-FNHK/2016-6 (FN)
12.00 - 12.15	Vliv selektivní ventilace na koncentraci antibiotika v plicní tkáni - mikrodialyzační studie **prof. MUDr. Jiří Manďák, Ph.D.** MZ ČR RVO-FNHK/2016-7 (FN)
12.15 - 12.30	Význam epigenetických změn u urologických malignit **MUDr. Petr Hušek** MZ ČR RVO-FNHK/2016-8 (FN)
12.30 - 12.45	Sledování metylace DNA pomocí NGS u ovariálního karcinomu **Mgr. Ing. Ivana Bubancová** MZ ČR RVO-FNHK/2016-9 (FN)
12.45 - 13.30	*Přestávka na oběd*
Sekce III	Předsedající: **prof. MUDr. Sylvie Dusilová Sulková, DrSc.**
13.30 - 13.45	Korelace dysregulovaných proteinů a receptorů s imunohistochemickým vyšetřením u adenomů hypofýzy **MUDr. Filip Gabalec, Ph.D.** MZ ČR RVO-FNHK/2016-10 (FN)
13.45 - 14.00	Léčba náhlé ztráty rovnováhy pomocí reohemaferézy **MUDr. Jakub Dršata, Ph.D.** MZ ČR RVO-FNHK/2016-11 (FN)
14.00 - 14.15	Mikroskopická analýza buněk, extracelulární matrix a novotvorby kosti po implantaci MSCs do kostního defektu experimentálního zvířete **MUDr. Lukáš Školoudík, Ph.D.** MZ ČR RVO-FNHK/2016-12 (FN)
14.15 - 14.30	Změna mikroRNA profilu v periferní krvi spojená se standardní intravenózní léčbou glukokortikoidy u pacientů s revmatickým onemocněním **MUDr. Tomáš Soukup, Ph.D.** MZ ČR RVO-FNHK/2016-13 (FN)
14.30 - 14.45	„Frailty patient" - vliv suplementace silných kationtů **RNDr. Alena Tichá, Ph.D.** MZ ČR RVO-FNHK/2016-14 (FN)

14.45 - 15.00	Preklinické testování pomůcky k aplikaci přesně definovaného přítlaku obvazu na chirurgickou ránu **MUDr. Jan Mejzlík, Ph.D.** MZ ČR RVO-FNHK/2016-4 (FN)
15.00 - 15.15	Využití Near-Infrared Spectroscopy k monitoraci mozkové perfuze během karotické endarterektomie **MUDr. Igor Guňka, Ph.D.** MZ ČR RVO-FNHK/2016-16 (FN)
15.15 - 15.30	Využití paralelního masivního sekvenování (MPS) pro sledování klonálních přestaveb receptorů na povrchu B lymfocytů **Ing. Kateřina Hrochová** MZ ČR RVO-FNHK/2016-17 (FN)
15.30 - 15.45	Exprese biotransformačních enzymů u hepatocelulárního karcinomu **RNDr. Jana Nekvindová, Ph.D.** MZ ČR RVO-FNHK/2016-18 (FN)
15.45 - 16.00	Změny v metylaci DNA u hyperplasie endometria **MUDr. Ondřej Dvořák** MZ ČR RVO-FNHK/2016-19 (FN)
16.00 - 16.15	Molekulární vodík - metabolismus a antioxidační působení **MUDr. Radomír Hyšpler, Ph.D.** MZ ČR RVO-FNHK/2016-20 (FN)
16.15 - 16.30	U k o n č e n í k o n f e r e n c e **prof. MUDr. RNDr. Miroslav Červinka, CSc.** děkan lékařské fakulty **prof. MUDr. Vladimír Palička, CSc., dr. h. c.** ředitel fakultní nemocnice

Projekty prezentované formou plakátových sdělení

Thromboembolic complications in relation to a selected antithrombotic therapy in patients with atrial fibrillation
V. Závalová, V. Knoblochová, A. Dvořáková, M. Kuneš
MZ ČR RVO-FNHK/2016-21 **(FN)**

Topografická anatomie horní končetiny – elektronické knihy
B. Doubková, T. Filipský, M. Pös, E. Šmahelová, K. Vaňková, P. Hájek
MŠMT IP 2016-2018 62 **(LF)**

NA LF V HRADCI KRÁLOVÉ A VE FN HRADEC KRÁLOVÉ SE V ROCE 2016 DÁLE ŘEŠILY NÁSLEDUJÍCÍ PROJEKTY

(abecedně podle jmen řešitelů)

V tomto přehledu jsou uvedeny ostatní smluvně podložené projekty a spolupráce na projektech.

Péče o nemocné staršího věku (>65 let) s Non-Hodgkinovými lymfomy – analýza faktorů ovlivňujících volbu léčby a osod nemocných
MUDr. David Belada, Ph.D.
(odp. řešitel: prof. MUDr. Marek Trněný, CSc. – VFN Praha)
AZV MZ ČR 16-31092A **(FN)**

Studium vaginální mikrobioty ve vztahu k rekurentnímu vulvovaginílnímu dyskomfortu
doc. RNDr. Vladimír Buchta, CSc.
AZV MZ ČR 15-29225A **(FN)**

Biomarkery a nové metodické přístupy v diagnostice a terapii v interních oborech
prof. MUDr. Jan Bureš, CSc.
SVV 260286 **(LF)**

Alterace glykokalyx v kritických stavech a během velkých operačních výkonů a možnosti její protekce
prof. MUDr. Vladimír Černý, Ph.D., FCCM
AZV MZ ČR 15-31881A **(FN)**

Nové postupy v diagnostice a terapii civilizačních chorob a onemocnění spojených se stárnutím populace
prof. MUDr. RNDr. Miroslav Červinka, CSc.
PRVOUK P37 **(LF)**

Interaktivní a zpětnovazebné prvky v podpoře výuky topografické anatomie
Barbora Doubková
MŠMT IP 2016-2018 62 **(LF)**

Remodelace svalu na podkladě extracelulární matrix osázené funkčně charakterizovanými buňkami
prof. MUDr. Stanislav Filip, Ph.D.
GA ČR 15-09161S **(LF)**

Lipozomy (drug delivery systems) v kineticky řízené léčbě platinarezistentního karcinomu ovarií doxorubicinem pomocí plazmafiltrace
prof. MUDr. Stanislav Filip, Ph.D.
AZV MZ ČR 16-30366A **(LF)**

Centrum pro výzkum toxických a protektivních účinků léčiv na kardiovaskulární systém
doc. PharmDr. Martin Štěrba, Ph.D. (prof. MUDr. Vladimír Geršl, CSc.)
(odp. řešitel: doc. PharmDr. Tomáš Šimůnek, Ph.D. – UK FaF UK)
UNCE 204019/304019 **(LF)**

Testování protinádorového účinku isochinolinových alkaloidů
Mgr. Klára Habartová
GA UK 932616 **(LF)**

Trajektorie kvality života seniorů v počáteční fázi demence
prof. MUDr. Roman Herzig, Ph.D.
(odp. řešitel: PhDr. Helena Kisvetrová Ph.D. – UP FZV Olomouc)
AZV MZ ČR 16-28628A **(FN)**

Mikrovaskulární abnormality jakožto endofenotyp schizofrenie
prof. MUDr. Ladislav Hosák, Ph.D.
AZV MZ ČR 16-27243A **(LF)**

Neinvazivní detekce intraamniální infekce stanovením dominantní bakterie v cervikální tekutině
doc. MUDr. Marian Kacerovský, Ph.D.
AZV MZ ČR 16-28587A **(FN)**

Rozvojem perioperační péče o nemocné k úspěšné chirugické terapii
doc. MUDr. RNDr. Milan Kaška, Ph.D.
SVV 260288 **(LF)**

Vytváření multiplatformových systémů pro podporu výuky včetně nástrojů pro uživatelsky přívětivou zpětnou vazbu
RNDr. David Kordek, Ph.D.
MŠMT IP 2016-2018 63 **(LF)**

Charakteristika aterosklerotického plátu a riziko mozkové ischemie při stentingu vnitřní karotidy
prof. MUDr. Antonín Krajina, CSc.
(odp. řešitel: prof. MUDr. David Školoudík Ph.D., FESO – FN Ostrava)
AZV MZ ČR 16-30965A **(FN)**

Koncept nekvarterních reaktivátorů AChE jakožto antidot otrav organofosfáty – nová naděje či slepá cesta?
prof. Ing. Kamil Kuča, Ph.D.
GA ČR 15-16701S **(FN)**

Vývoj multifunkčního léčiva na Alzheimerovu nemoc: kombinace inhibitoru AChE a derivátu melatoninu
prof. Ing. Kamil Kuča, Ph.D.
AZV MZ ČR 15-30954A **(FN)**

Plasmonické nanočástice pro teranostiku s laditelnými optotermálními parametry
prof. Ing. Kamil Kuča, Ph.D.
GA ČR 16-13967S **(FN)**

Biomedicínská fotonická zařízení pro pokročilou lékařskou diagnostiku a terapii
RNDr. Martin Kuneš, Ph.D.
AZV MZ ČR 15-33459A **(FN)**

Utilization of cellular reprogramming technology in current medicine research and drug screening
RNDr. David Kunke, CSc.
Norské fondy NF-CZ07-ICP-4-290-2015 **(LF)**

Péče zaměřená na zvláštnosti pacienta: individualizovaná péče (teorie, diagnostika, intervence)
prof. PhDr. Jiří Mareš, CSc.
AZV MZ ČR 16-28174A **(LF)**

Studium patofyziologických mechanizmů vnitřních onemocnění, rizikových faktorů a nových diagnostických a terapeutických intervencí – pokračování 2016
prof. MUDr. Stanislav Mičuda, Ph.D.
SVV 260287 **(LF)**

Modulátory mitochondriálních enzymů k léčbě neurodegenerativních onemocnění
doc. PharmDr. Kamil Musílek, Ph.D.
AZV MZ ČR 15-28967A **(FN)**

Vývoj nových insekticidů proti komárům přenášejícím malárií šetrných pro životní prostředí
doc. PharmDr. Kamil Musílek, Ph.D.
AZV MZ ČR 16-34390A (**FN)**

Manipulační a bezpečnostní pás k vysokému chodítku
Mgr. Tomáš Osladil
TA ČR GAMA TG02010020-3 **(FN)**

Conferences in medical sciences 2016/13th International medical Postgraduate conference
prof. MUDr. Vladimír Palička, CSc., dr.h.c.
SVV 260290 **(LF)**

Spectroscopy as a diagnostic tool for assaying disease specific molecules
Rishikaysh Pisal, M.Sc.
MŠMT IP 2016-2018 64 **(LF)**

Studium kardioprotektivních účinků ACE-inhibitoru, dexrazoxanu a jeho nových derivátů vůči chronické antracyklinové kardiotoxicitě u králíka
Mgr. Zuzana Pokorná
GA UK 680216 **(LF)**

Testování nových protinádorových terapeutik na bázi nízkomolekulárních inhibitorů DNA-PK
Ing. Monika Pospíšilová
GA UK 932516 **(LF)**

Racionální design nových immunomodulátorů – potenciální adjuvans pro vakcíny – na bázi ligandů TLR4
prof. MUDr. Roman Prymula, CSc., Ph.D.
GA ČR 15-11776S **(FN)**

Nové metody a postupy v diagnostice a hledání prediktivních a prognostických markerů nádorových onemocnění
prof. MUDr. Aleš Ryška, Ph.D.
SVV 260289 **(LF)**

Banka klinických vzorků
prof. MUDr. Aleš Ryška, Ph.D.
(odp. řešitel: prof. MUDr. Dalibor Valík, Ph.D. – MOÚ Brno)
MŠMT BBMRI_CZ **(LF)**

Detekce velmi časných projevů kardiotoxicity chemoterapie s využitím pokročilých echokardiografických technik, proteinových kardiomarkerů a cirkulujících mikroRNA
MUDr. Mikuláš Skála
GA UK 814316 **(LF)**

Analýza klonální heterogenity chronické lymfocytární leukemie pomocí sekvenování nové generace genu B-buněčný receptor. Národní studie.
doc. MUDr. Lukáš Smolej, Ph.D.
(odp. řešitel: prof. MUDr. Michael Doubek, Ph.D. – FN Brno)
AZV MZ ČR 15-30015A **(FN)** – abstrakt nebyl dodán

Vývoj nových dezinfekčních činidel proti patogenům vyskytujících se v nemocničním prostředí
PharmDr. Ondřej Soukup, Ph.D.
AZV MZ ČR 15-31847A **(FN)**

Nové hybridní molekuly v léčbě kognitivních poruch spojených s neurodegenerací
PharmDr. Ondřej Soukup, Ph.D.
(odp. řešitel: Mgr. Martin Horák Ph.D. – Fyziol. úst. AV ČR)
GA ČR 16-08554S **(FN)**

Využití Narrow Band Imaging v diagnostice slizničních nádorů hlavy a krku
MUDr. Jana Šatanková
MŠMT IP 2016-2018 65 **(LF)**

Vliv funkčních polymorfismů ovlivňujících zánět a oxidační stres na průběh chronické lymfocytární leukémie a volbu individuální léčebné strategie
MUDr. Martin Šimkovič
(odp. řešitel: prof. MUDr. Tomáš Papajík, CSc. – UPOL LF)
AZV MZ ČR 16-32339A **(FN)**

Inovace metody kryokonzervace pro klinické využití autologních multipotentních mesenchymálních kmenových buněk (MSCs) k léčení rozsáhlých defektů skeletu při reimplantaci totální endoprotézy kyčelního kloubu
doc. MUDr. Pavel Šponer, Ph.D.
TA ČR GAMA TG02010020-4 **(FN)**

Katetrizační uzávěr ouška levé síně versus terapie novými orálními antikoagulancii u rizikových pacientů s fibrilací síní (studie PRAGUE-17)
doc. MUDr. Josef Šťásek, Ph.D.
(odp. řešitel: doc. MUDr. Pavel Osmančík, Ph.D. – 3. LF UK Praha)
AZV MZ ČR 15-29565A **(FN)**

Porovnání účinnosti kolonické kapslové endoskopie a optické kolonoskopie u osob s pozitivním imunochemickým testem na okultní krvácení do stolice
MUDr. Ilja Tachecí, Ph.D.
(odp. řešitel: MUDr. Štěpán Suchánek, Ph.D. – ÚVN Praha)
AZV MZ ČR 16-29614A **(LF)**

Role oxidačního stresu ve vztahu mezi buněčnou senescence a apoptózou
Mgr. Vojtěch Tambor, Ph.D.
(odp. řešitel: MUDr. Zdeněk Hodný, CSc. – ÚMG AV Praha)
GA ČR 15-03379S **(FN)**

Nové analytické metody pro efektivní stanovování biologických markerů.
prof. MUDr. Zdeněk Zadák, CSc.
(odp. řešitel: Ing. Michal Bartoš – Výzkumný ústav organických syntéz, a.s.)
TA ČR TA04010954 **(FN)**

Inovace infuzních roztoků podle nejnovějších poznatků s protektivním účinkem na glykokalyx
prof. MUDr. Zdeněk Zadák, CSc.
(odp. řešitel: Ing. Milan Máchal – Ardeapharma a.s. Ševětín)
MPO ČR FV10454 **(FN)**

Národní program studia mutací a klonality leukemických buněk u pacientů s akutní myeloidní leukémií
doc. MUDr. Pavel Žák, Ph.D.
(odp. řešitel: doc. MUDr. Zdeněk Ráčil, Ph.D. - FN Brno)
AZV MZ ČR 15-25809A **(FN)** – abstrakt nebyl dodán

Specific methods for detection of antibiotic resistance mechanisms in the clinical microbiology laboratory
doc. MUDr. Helena Žemličková, Ph.D.
Norské fondy NF-CZ07-MOP-4-254-2015 **(LF)**

SOUHRNY VÝZKUMNÝCH ÚKOLŮ

ŘEŠENÝCH NA LF UK A VE FN V HRADCI KRÁLOVÉ

(ABECEDNĚ)

Title of the project: Population of Elderly Patients with Non-Hodgkin´s Lymphomas- the Analysis of Factors Affecting the Management and Outcome.

Grant Agency: Ministry of Health ***Project Number:*** 16-31092A

Principal Investigator: M. Trněný

Co-investigators: D. Belada, A. Janíková, V. Procházka, H. Móciková, K. Kubáčková, J. Ďuraš, S. Vokurka

Starting date: 1.4.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 13402

Summary of 2016 results

Title of the presentation: Population of Elderly Patients with Non-Hodgkin´s Lymphomas- the Analysis of Factors Affecting the Management and Outcome.

Authors: dr. David Belada, doc. Andrea Janíková, doc. Vít Procházka, Dr. Heidi Móciková, Dr. Kateřina Kubáčková, Dr. J. Ďuraš, doc. S. Vokurka and prof. Marek Trněný

Elderly project is focused on patients with Non-Hodgkin´s lymphoma (NHL). Median age at the diagnosis is between 60-65 years. The data on the management, therapy, effect, toxicity is limited, especially at the age ≥ 80 years. Project is based on prospective Lymphoma Program of Czech Lymphoma Study Group. There has been already registered 6171 patient at the age ≥ 60 years. The inclusion about 1000 patients is anticipated in the prospective part of the project. Geriatric assessment will be performed in these patients. The project objectives are:

1. Analysis of currently included patients according to diagnosis (NHLS subtype), biological characteristic, age, furthe prognostic factors, analysis of therapy, its effect and toxicity, farmacoeconomy of targeted therapy.
2. In prospective part the from 2015 the geriatric assessment, activity of daily living, will be included (currently not reported) and will part of the analysis
3. National guidelines for management of lymphoma in old age will be prepared.

During 2016 we started several analysis of elderly patients with NHL from the Czech Lymphoma Registry. We discussed results of treatment of elderly patients with lymphoma focusing on patients with DLBCL, primary CNS lymphoma, Hodgkins lymphoma, peripheral T-cell lymphoma and follicular lymphoma during annual meeting of Czech Lymphoma Study Group. Several publications on this topic are now prepared and will be published during next months. We also prepare prospective assessment of activity of daily living, this will be incorporated into clinical practice during 2017.

Address for correspondence: David Belada, M.D., Ph.D., Charles University and Medical School, 4-th Clinic of Internal Medicine, Haematology Dept., Sokolska street 581, Hradec Kralove 5, 500 05, Czech Republic; david.belada@fnhk.cz

Title of the project: Proprotein convertase subtilisin/kexin 9 (PCSK9) in the pathophysiology and treatment of age-related macular degeneration

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-2

Principal Investigator: M. Bláha

Co-investigators: H. Langrová, M. Lánská, V. Bláha, A. Stepanov, C. Andrýs

Starting date: 1.1.2016 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation: Proprotein convertase subtilisin/kexin 9 (PCSK9) in the pathophysiology and treatment of age-related macular degeneration

Authors: M. Bláha (1), H.Langrová (2), M. Lánská (1), V. Bláha (3), A. Stepanov (2), C. Andrýs (4)

Fac. Med. and Teach. Hosp., Charl. Univ., Hr. Králové: 4th Dpt. of Medic. (1), Dept. of Ophthalmology (2), 3th Dept. of Medicine (3), Inst. of Immun. Allergol. (4).

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a secreted regulator of cell surface LDL receptor (LDLR) levels, whose function results in elevation of plasma LDL levels. Decrease of PCSK9 is an active and promising area of therapeutic development for hypercholesterolemia. A major target of these drug development efforts is aimed at reducing levels of PCSK9 by using monoclonal antibodies. More than 30% of plasma PCSK9 is bound to LDL, and thus rheohemapheresis could also reduce plasma PCSK9 levels by removing LDL. However, the PCSK9 reducing effects of rheohemapheresis on the course of AMD have not yet been studied. It was the aim of our project as a research probe to the grant proposal.

Group of patients: 19 AMD patients were treated with rheopheresis in the last three years and the PCSK9 level was measured – at the beginning and after finishing the therapeutic serie of 8 rheopheretic procedures.

Results: PCSK9 level is increased in patients compared to the controls. It decreases after procedures significantly. PCSK9 level correlates with total cholesterol but does not correlate with HDL cholesterol, fibrinogen, plasma and blood viscosity, IgM and alfa2-macroglobuline. We have not found also any correlation between patients with therapeutic success and non-successfully treated patients.

Conclusion: Our results show that lipoprotein metabolism disturbances can be connected with the development or progress of AMD. Currently, it is also clinically important - novel pharmacological treatments which enable inhibition of PCSK9 activity have provided for unprecedented lowering of plasma LDL-C (up to 70%) along with evidence of a reduction in the rate of cardiovascular event in ongoing clinical trials**.**

Address for correspondence: Prof. MUDr Milan Bláha, CSc., 4th Department of Internal Medicine - Haematology, Faculty Hospital Hradec Králové, Sokolská 481, 50005 Hradec Králové

Title of the project: Testing and comparison of microcirculation in tissue by glue constructed colon anastomosis in relation to commonly used techniques for colon anastomosis

Grant Agency: Charles University ***Project Number:*** 187515

Principal Investigator: S. Blažej

Co-investigators: J. Páral, Z. Turek, A. Ryška, M. Pavlík, V. Radochová, B. Jegorov, M. Kaška

Starting date: 1.5.2015 ***Duration (years):*** 2

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

Summary of 2016 results

Title of the presentation: Segmental perianastomic microcirculation at various types of bowel anastomosis in experiment

Authors: S. Blažej et al. (above mentioned)

Background: Contemporary abdominal surgery is facing an increasing number of malignant diseases of the large bowel. Its surgical therapy is based on resection and reconstruction using common methods such as suture and stapling. A potentially usable method seems to be the application of a special glue. A microcirculation intensity is one of the limiting factors of bowel anastomotic healing.

Methods: The experimental study included 27 female pigs from the controlled breed of mean weight 40kg. Two microcirculation detection probes (Laser Doppler Flowmetry - LDF) were placed to the anastomotic site and a basic microcirculation status was measured. The bowel was transected and anastomosis was performed using an absorbable knitted thread for standard sewing technique or a stapler or the glue (Glubran2). Microcirculation intensity was measured 60 and 120 min after the construction of anastomosis. After 7 days relaparotomy in general anaesthesia was performed. The biopsy was examined by H-E staining microscopically focusing on the status of healing anastomosis. Measured values of blood microcirculation intensity were statistically processed using the software SigmaPlot 13.0.

Results: 27 animals were enrolled in 3 subgroups counting 9 animals each. It was found out that the gentlest method of all from the actual microcirculation level impact point of view is suture. The drop in microcirculation intensity at the anastomotic site was of 38.01% from maximum to the original values during 120 min after anastomosis construction. A more significant drop in microcirculation from the original values was in stapling and gluing of 52.42% and 52.53%, respectively. Macroscopically there was no disorder in healing in any animal recorded and microscopic examination showed satisfactory healing in all cases.

Conclusions: Partial microcirculation drop of the large bowel tissues in the first 120 min after anastomosis construction is significantly higher in stapling and gluing. Function and biopsy results do not show significant differences in healing of the bowel whether the reconnection was performed by stapling, sewing or gluing.

Address for correspondence: MUDr. Slavomír Blažej, Korunní 104, 101 00 Praha 10, sblazej@azet.sk

Title of the project: Monitoring of DNA methylation using NGS in ovarian cancer

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-9

Principal Investigator: I. Bubancová

Co-investigators: M. Chmelařová, H. Kovaříková, J. Laco, E. Ruszová, O. Dvořák

Starting date: 1.2.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 405

Summary of 2016 results

Title of the presentation: Monitoring of DNA methylation using NGS in ovarian cancer

Authors: I. Bubancová (1), H. Kovaříková (1), J. Laco (2), E. Ruszová (3), O. Dvořák (4), V. Palička (1), M. Chmelařová (1)

University Hospital Hr. Králové: Inst. for Clinical Biochemistry and Diagnostics (1), The Fingerland Dpt. of Pathology (2), Dpt. of Clinical Genetics (3), Dpt. of Obstetrics and Gynecology (4)

DNA methylation is well-known to be associated with ovarian cancer (OC) and has great potential to serve as biomarker in monitoring response to therapy and for disease screening. The purpose of this study was to investigate methylation of the HNF1B and GATA4 genes and correlate detected methylation with clinicopathological characteristic of OC patients.

Study group consisted of 64 patients with OC and 35 patients with normal ovarium. To determine the most important sites of HNF1B and GATA4 we used next generation sequencing. For further confirmation of detected methylation of selected regions we used high-resolution melting analysis and methylation-specific real-time PCR.

Selected regions of HNF1B and GATA4 were completely methylation free in all control samples, whereas methylation-positive pattern was observed in 32.8 % (HNF1B) and 45.3 % (GATA4) of OC samples. Evaluating both genes together we were able to detect methylation in 65.6 % of OC patients. We observed statistically significant difference in HNF1B methylation between samples with different stages of OC. We also detected subtype specific methylation in GATA4 and decrease of methylation in late stages of OC. Combination of unmethylated HNF1B and methylated GATA4 was associated with longer overall survival.

In our study we employed innovative approach of methylation analysis of HNF1B and GATA4 in searching of possible epigenetic biomarkers. We confirmed significance of the HNF1B and GATA4 hypermethylation with emphasis on the need of selecting the most relevant sites for analysis. We suggest selected CpGs to be further examined as potential positive prognostic factor.

Address for correspondence: I. Bubancová, Institute for Clinical Biochemistry and Diagnostics, University Hospital Hradec Králové, Sokolská 581, Hradec Králové 500 05, Czech Republic

Title of the project: Study of vaginal microbiota and its relationship to recurrent vulvovaginal discomfort

Grant Agency: Ministry of Health ***Project Number:*** 15-29225A

Principal Investigator: V. Buchta

Co-investigators: M. Kacerovský, J. Nekvindová, M. Drahošová, J. Vávrová

Starting date: 1.5.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 12754

Summary of 2016 results

Title of the presentation: Study of vaginal microbiota of women with chronic vulvovaginal discomfort

Authors: V. Buchta (1), C. Andrýs (2), L. Plíšková J (3) , J. Vávrová (3), D. Leško (4), J. Špaček (4), L. Nováková (5), et al.
University Hospital, Hradec Králové, Dept. of Clinical Microbiology (1)
University Hospital, Hradec Králové, Dept. of Clinical Immunology and Allergy (2)
University Hospital, Hradec Králové, Dept. of Clinical Biochemistry and Diagnostics (3)
University Hospital, Hradec Králové, Dept. of Obstetrics and Gynecology (4)
Charles University in Prague, Fac. Pharm., Dept. of Analytical Chemistry (5)

Vaginal microbiota represents a finely balanced microbial ecosystem with specific microbe-microbe relationships, relevant effects of sexual hormones and tunning immune system all of which have a substantial impact on the vaginal health and disease (1,2). The study of women with chronic vulvovaginal discomfort (CVD) revealed different hormonal profile in the patients and control group in the blood. CVD patients had a relatively higher levels of estradiol and progesterone - 298.5 ± 286.7 pmol/l and 10.7 ± 16.12 nmol/L, respectively, compared to the controls - 161.4 ± 379.33 pmol/l of estradiol and 0.3 ± 0.14 nmol/l of progesterone. Immunological investigation showed variabilty of individual parameter in CVD patients, including L and D isomer of lactic acid, EMMPRIN, MMP8, TLR2, TLR4, and beta-defensin 2. Comparison of women with and without positive findings of a yeast in the vagina suggested higher level of EMMPRIN, TLR4, and beta-defensinu 2 in positive-yeast samples and lower concentration of L-lactate when no yeast were detected in vaginal fluid. Nevertheless, the number of CVD patients and controls was relatively low to draw definitive conclusions.

Literature:

(1) Špaček J, Kestřánek J, Jílek P, Leško D, Plucnarová S, Buchta V. Comparison of two long-term gestagen regimens in the management of recurrent vulvovaginal candidiasis: a pilot study. Mycoses, accepted to press

(2) Buchta V, Jílek P, Špaček J. Aktuální pohled na problematiku kvasinek ve vulvovaginální oblasti. Acta Medicinae, 2016, 4:70-73.

Project was supported by Ministry of Health of the Czech Republic, grant nr. 15-29225A. All rights reserved.

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

Title of the project: Biomarkers and new methodical approach in diagnostics and therapy in internal medicine

Grant Agency: Ministry of Education ***Project Number:*** 260286

Principal Investigator: J. Bureš

Co-investigators: J. Horáček, J. Šťásek, M. Kopáčová, L. Sobotka, P. Žák, J. Čáp, F. Salajka, S. Filip, M. Vališ, L. Hosák, S. Skálová and 17 students of doctoral studies

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 1289

Summary of 2016 results

Title of the presentation: Biomarkers and new methodical approach in diagnostics and therapy in internal medicine

Authors: J. Bureš (1), J. Horáček (2), J. Šťásek (3), M. Kopáčová (4), L. Sobotka (5), P. Žák (2), J. Čáp (2), F. Salajka (6), S. Filip (7), M. Vališ (8), L. Hosák (9), S. Skálová (10) and 17 students of doctoral studies

Charles Univ Fac Med, Acad Dept Int Med (1), 4th (2), 1st (3), 2nd (4), 3rd (5) Dept Med, Dept Pneumol (6), Dept Oncol Radioter (7), Dept Neurol (8), Dept Psych (9), Dept Pediat (10)

Specific university research programme supports experimental and clinical research of students of doctoral studies. In total, 17 students and their doctoral projects were supported in 2016 coming from internal medicine, paediatrics, oncology and radiotherapy, neurology and psychiatry.

Doctoral projects focused on the changes of CD8+ tumour infiltrating lymphocytes after neoadjuvant radiochemotherapy in rectal adenocarcinoma, S100 proteins in colorectal neoplasia and inflammatory bowel disease, microsatellite instability in melanoma, palliative cancer care, hepatic encephalopathy after transjugular intrahepatic portosystemic shunt, sarcopenia as a prognostic survival factor in transjugular intrahepatic portosystemic shunt, proteomic analysis of cerebrospinal fluid for relapsing-remitting multiple sclerosis, microvascular abnormalities in depression, urinary iodine concentrations in mothers and their term newborns in country with sufficient iodine supply, markers of bone metabolism, serum leptin levels and bone mineral density in preterm babies, chronic obstructive pulmonary disease prognostic score and radioiodine administration in papillary thyroid multifocal microcarcinoma.

Specific university research programme helps doctoral students to carry out their scientific projects, to get required data and to obtain original results for their theses. Doctoral research achieved a significant impact on diagnostics and therapy in internal medicine.

Address for correspondence: J. Bureš, Acad Dept Internal Medicine, Charles University Faculty of Medicine in Hradec Králové, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic. E-mail: bures@lfhk.cuni.cz

Title of the project: Alterations of glycocalyx in critical illness and during major surgery and approaches for glycocalyx protection

Grant Agency: Ministry of Health ***Project Number:*** 15-31881A

Principal Investigator: V. Černý

Co-investigators: D. Astapenko, J. Beneš, Z. Turek, R. Pařízková, P. Dostál, I. Abdo, J. Martínková, R. Škulec, R. Hyšpler, Z. Zadák

Starting date: 1.1.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 8340

Summary of 2016 results

Title of the presentation: Effect of general and regional anesthesia on glycocalyx thickness in patients undergoing hip/knee replacement - a prospective observational study.

Authors: V. Cerny (1,2), D. Astapenko (1), J. Pouska (3), J. Benes (2), Ch. Lehmann (4)

Dept. of Research and Development Univ. Hosp. H. Kralove (1), Dept. of Anesth. and Intensive Care, Charles Univ., Fac. of Medicine, H. Kralove (2), Dept. of Anesth. and Intensive Care, Charles Univ., Fac. of Medicine, Pilsen (3), Dept. of Anesthesia, Pain Management and Perioperative Medicine, Dalhousie University, Halifax, Canada (4).

Background: Alteration of EG has been described during surgery, but the effect of anesthesia remains unknown. Perfused boundary region (PBR) is supposed to enable intra-vital assessment of EG dimension. The aim of the study was to evaluate the changes of PBR in patients undergoing elective hip/knee surgery under general (GA) and regional anesthesia (RA). Our primary hypothesis was that RA affects PBR less than GA.

Materials and Methods: Adult patients ASA 2-3 undergoing elective total knee/hip replacement under GA or RA were included in this multicentric prospective observational study. PBR in the sublingual microcirculation was recorded in each patient at two time points - before surgery and 24 hours after surgery. Specialized software (GlycoCheck, the Netherlands) was used for automatic PBR analysis.

Statistical plan and analysis: Sample size calculation indicated 52 patients; we aimed to enroll 60 consecutive patients (30 per each group). Data were tested for normality; t-test (paired/unpaired) was used for group comparison. A value of $p \leq 0.05$ was considered as statistically significant.

Results and Discussion: 60 patients were examined. Before surgery, there was no significant difference in baseline PBR (mean ± SEM) between groups (1.95 ± 0.03 in RA vs. 2.02 ± 0.03 in GA). However, after surgery PBR significantly increased in both groups with respect to baseline values (RA: 2.09 ± 0.02, $p < 0.001$; GA: 2.20 ± 0.03, $p < 0.001$). In addition, PBR significantly differed between RA and GA group 24 hours after surgery (2.09 ± 0.02 vs. 2.20 ± 0.03, $p = 0.006$).

Conclusion(s): Hip/knee surgery led to significant changes of PBR dimensions. Patients in GA group had significantly higher PBR 24 hours after surgery compared to RA group. According to our data, RA may exert a possibly protective effect on the PBR and supposedly on the EG.

Address for correspondence: V. Cerny, Dept. of Research and Development Univ. Hosp. H. Kralove and Dept. of Anesth. and Intensive Care, Charles Univ., Fac. of Medicine, H. Kralove, Czech Republic

Title of the project: New procedures in diagnostics and therapy of lifestyle diseases and diseases connected with population ageing

Grant Agency: Charles University ***Project Number:*** P 37

Principal Investigator: M. Červinka

Co-investigators: Z. Červinková, Z. Fiala, M. Kaška, J. Krejsek, M. Kuba, J. Malý, S. Mičuda, J. Mokrý, A. Ryška, E. Rudolf, R. Pudil, L. Sobotka, R. Slezák

Starting date: 1.7.2012 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 37876

Summary of 2016 results

Title of the presentation: New procedures in diagnostics and therapy of lifestyle diseases and diseases connected with ageing of population

Authors: M. Červinka, Z. Červinková, Z. Fiala, M. Kaška, J. Krejsek, M. Kuba, J. Malý, S. Mičuda, J. Mokrý, A. Ryška, E. Rudolf, R. Pudil, L. Sobotka, R. Slezák

This complex research project is focused on central problems of European medicine. The leading idea of the project is the fact that population in the Czech Republic is aging. The main health issue associated with this demographic facts is an increasing number of people suffering from lifestyle diseases. Therefore we need intensive and complex research in these areas.

1. Research in the sphere of lifestyle diseases affecting cardiovascular system is focused on problems of ischemic heart disease, sudden heart death and specification of risks, but also on a non-pharmacological and pharmacological prevention of such events. Other spheres of the research are the conditions for regeneration of myocardium in the experiment and clinical practice and diseases affecting gastrointestinal system.
2. The area of oncology and haemato-oncology: The study of prediction of the impact and toxicity of the treatment, importance of individual dosage regulation of medicaments and prediction of the response to these medical procedures. Significant attention is paid to oncological problems of a digestive tract.
3. The issue of aging and related health concerns including the study of regeneration on all levels. The research will be aimed at major metabolic and molecular manifestation of aging and on reparation and restoration processes. An accompanying sphere of interest is the study of damage and repair on the level of DNA, cell and organ level, including possibilities to influence such processes.

The research work in all three mentioned interconnected areas has been fulfilled by 13 working groups. The results of the whole project are 660 original research papers and publications, dedicated to this project. From these 328 was published in journals with impact factor. Summary IF is equal 701, the mean IF = 2.18, and maximum IF = 29.35.

Address for correspondence: M. Červinka, Charles University, Faculty of Medicine in Hradec Králové, Šimkova 870, 50038 Hradec Králové, Czech Republic

Title of the project: Interactive and feedback elements to support the topographic anatomy education

Grant Agency: Ministry of Education ***Project Number:*** IP 2016-2018 62

Principal Investigator: B. Doubková

Co-investigators: T. Filipský, M. Pös, E. Šmahelová, K. Vaňková, P. Hájek

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 836

Summary of 2016 results

Title of the presentation: E-books Topographic Anatomy of the Upper Limb

Authors: B. Doubková, T. Filipský, M. Pös, E. Šmahelová, K. Vaňková, P. Hájek

With respect to the modern form of education, we work to publish four Czech and English e-books of topographic anatomy. The first one, finished in both languages, is Topographic Anatomy of the Upper Limb followed by the Head and neck, ready to be published.

The books are based on our E-learning courses of topographic anatomy running in LMS Moodle for almost one decade. Back then, we found out that time stress made students to focus on the systematic anatomy and to sidetrack the topographical anatomy. For this reason, the courses were supposed to complete current contents of the practical classes. The feedback, as a part of our Moodle courses, made us to modify the source.

New e-books are available on the web site https://elfhk.publi.cz. The product is a result of teamwork of students, the tutor and a software development company “Code Creator”. All the chapters in each book contain study material (text, interactive pictures, schemes, photos and videos) and they are concluded by a quiz as a feedback. All the texts are original work based on literature knowledge and also our own dissection experience. The texts are clearly structured by bullets, anatomic terms are highlighted and interactive pictures made from students´ illustrations which display structures in a picture by coloring or an arrow after clicking on the legend. This material is meant to serve both as a study material and to self-examination. Last but not least, the book is equipped with an interactive glossary containing a list of anatomic terms with a description of about 3 sentences.

The product is in a form of an application, optimized for tablets with bookmarks and many other tricks, however it can work on PCs as well. For students who hate using electronic devices, a PDF document is placed at the top of each chapter. It is an abstract in the extent of about 3 A4 pages, fits to be printed and put into a pocket of a laboratory coat.

We hope, that the result of our work will be valuable and helpful academic material for the future generations of students.

The project is supported by Charles University, IP 2016-2018.

Address for correspondence: B. Doubková, Dept. of Anatomy, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Treatment of Acute Unilateral Vestibular Failure with Rheohaemapheresis

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-11

Principal Investigator: J. Dršata

Co-investigators: M. Bláha, M. Janouch, J. Mejzlík, V. Chrobok, M. Košťál

Starting date: 24.5.2016 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation: Treatment of Acute Unilateral Vestibular Failure with Rheohaemapheresis - results of pilot study

Authors: J. Dršata (1), M. Bláha (2), J. Mejzlík (1), M. Janouch (1), V. Chrobok (1), M. Košťál (2). Dpt. of ORL HNS (1), 4th Dpt. of Internal Medicine (2); University Hospital Hradec Kralove, Charles University in Prague, Faculty of Medicine in Hradec Kralove.

Acute Unilateral Vestibular Failure (AUVF) is an acute clinically-defined disease of vestibular labyrinth with unknown etiology. Among supposed etiological factors, vascular theory seems to play a significant role.

Aim of the study was a pilot probe into detailed function of the vestibular organ (individual ampullae of semicircular canals, maculae of sacculus and utriculus) in patients with AUVF, treated with rheopheresis (RF).

Patients and methods: in the course of a 6-month project in 2016, we included 5 patients to the rheopheretic arm and 3 patients to the corticosteroid arm as controls. While no fixed standards for audio-vestibular examinations exist, we examined a group of healthy volunteers for establishing the cut-off values of the normal range. Methods used were patient's history and clinical investigation, and special audio-vestibular instrumental examinations (videooculography, video-head-impulse-test, vestibular evoked myogenic potentials) before and 1 month after therapy. Besides, haematological parameters were studied.

Results in the rheopheretic group showed improvement in the most of the ampullae, sacculi and utriculi function, while in the corticosteroid group this effect was not observed. Disputable is however the interpretation of the results due to a limited number of patients studied. The project is to be therefore extended for 2017, to get a larger set of data for statistical analysis.

Address for correspondence: Jakub Dršata, MD, Ph.D., Dpt. of ORL-HNS, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Králové, Czech Republic; jakub.drsata@fnhk.cz.

Title of the project: Changes in DNA methylation of tumor suppressor genes of endometrial hyperplasia

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-19

Principal Investigator: O. Dvořák

Co-investigators: M. Chmelařová, E. Dvořáková, J. Laco, J. Špaček,

Starting date: 1.1.2015 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 580

Summary of 2016 results

Title of the presentation: Changes in DNA methylation of tumor suppressor genes of endometrial hyperplasia

Authors: O. Dvořák (1), M. Chmelařová (2), E. Dvořáková (1), J. Laco (3), J. Špaček (1),
University Hospital and Charles University Medical School, Hradec Kralove, Dept. of Obstetrics and Gynecology (1), Inst. of Clin. Bioch. and Diagnostic(2), The Fingerland Dept. of Pathology (3)

Endometrial hyperplasia (EH) is a condition of excessive proliferation of the cells of the endometrium. EH is usually based on high levels of free estrogens, combined with insufficient levels of the progesteron-like hormones with opposite effect to estrogen's proliferative effects on this tissue. The gland-forming cells of a EH may undergo progressive changes leading to transformation to endometrial cancer (EC). A cross-sectional study was performed. Formalin-fixed and paraffin-embedded samples of endometrial hyperplasia and the samples of normal endometrial tissue we analysed.EC and EH are complex diseases in which formation is involving by many factors, including genetic and epigenetic alterations. DNA methylation, one of the first alterations in the development of carcinogenesis, is also one of the most common epigenetic changes and probably has a key role in carcinogenesis of endometrial tissue. For monitoring of DNA methylation changes we used the MS-MLPA (Methylation-specific Multiplex ligation-dependent probe amplification), probe set ME002 and ME004 (MRC-Holland, Amsterdam, The Netherlands) to analyze the DNA methylation changes in the promoter regions of selected tumor suppressor genes. The aim of research was to identify eventual changes in selected tumor suppressor genes. We observed significantly higher methylation in CDH13 gene in the premalignant endometrial tissue group compared to samples of control nonneoplastic endometrium. According to level seriousness of changes in endometrial premalignant samples, we also observed significantly higher methylation in PTEN, and MSH6 in group of hyperplasia with atypia, compared to next two examinated groups. These findings suggest the importance of hypermethylation of these genes in endometrial carcinogenesis. Detailed examination and understanding of these changes could be in future used for the detection of patients with high risk of endometrial carcinoma and could be helpful for finding new diagnostic and therapeutic options, for these patient

Address for correspondence: O. Dvořák, Dept. of Obstetrics and gynecology, University Hospital and Charles University Medical School Sokolska tr. 581, 50005 Hradec Kralove Hradec Kralove, Czech Republic

Title of the project: Detection of herpes simplex virus resistance to acyclovir in the University hospital Hradec Kralove by the using of sequence analysis methods

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-3

Principal Investigator: M. Fajfr

Co-investigators: L. Plíšková, R. Kutová, J. Radocha

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 301

Summary of 2016 results

Title of the presentation: Detection of herpes simplex virus resistance to acyclovir in the University hospital Hradec Kralove by the using of sequence analysis methods

Authors: M. Fajfr (1), L. Plíšková (2), R. Kutová (2), J. Radocha (3)

Institute of Clinical Microbiology, Charles University, University Hospital in Hradec Kralove (1), Dep. of molecular biology, Institute of Clinical biochemistry and diagnostic, University Hospital in Hradec Kralove (2), 4th Department of Internal Medicine - Haematology, Charles University, University Hospital Hradec Kralove (3)

Project aim: The aim of project is the development of a methodology for detection of Herpes simplex virus (HSV) resistance to the nucleoside analogues by using of gene sequencing and examination of 20 samples from patients hospitalized in the Faculty hospital in Hradec Králové.

Material and Methods: During the evaluation period was an extensive search of work in order to find all the currently known polymorphisms associated with resistance to HSV from UL23 gene (gene for viral thimidine kinase) and UL30 gene (gene for viral polymerase) and the databases of all known polymorphism was created. After this phase, there were designed the primer sets which allow amplification of HSV gene parts with all known mutation associated with resistance to antivirals. There were prepared primer sets both for classical gene sequencing according to Sanger and for next generation sequencing (NGS). A totally 19 HSV1 strains from patients were collected for next examination.

Results: We developed a novel method for detection of HSV resistance to antivirals and our method was certificated by QCMD control. From 19 HSV1 strains was evaluated in 7 cases D672N mutation in UL30 gene, which is associated with resistance to aciclovir and foscarnet. Except this mutation there were found also many other polymorphisms both in UL23 and UL30 genes, but with no association with resistance to antivirals. Both methods, the sequencing accordint to Sanger and NGS gave comparable resuls.

Conclusion: The reason for this high resistance level is unclear and under investigation

Address for correspondence: M. Fajfr; Institute of Clinical Microbiology, University Hospital in Hradec Kralove, Sokolska 581, 500 05, Czech Republic; email address: miroslav.fajfr@fnhk.cz,

Title of the project: Muscle remodelling on the basis of extracellular matrix seeded with functionally characterized cells.

Grant Agency: Czech Republic ***Project Number:*** 15-09161S

Principal Investigator: S. Filip

Co-investigators: J. Mokrý, G. Dayanithi, O. Forostyak, H. Hrebíková, R. Pisal, J. Chvátalová, D. Čížková

Starting date: 1.1.2015 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 5794

Summary of 2016 results

Title of the presentation: Muscle remodelling on the basis of extracellular matrix seeded with functionally characterized cells.

Authors: S. Filip (1), J. Mokrý (2), G. Dayanithi (2), O. Forostyak (2), H. Hrebíková (2), R. Pisal (2), J. Chvátalová (2), D. Čížková (2).
Department of Oncology and Radiotherapy (1); Department of Histology and Embryology (2).

The extracellular matrix (ECM) consists mainly of proteins, glycosaminoglycans and glycoproteins. A large number of ECM producing cells, fibroblasts and cells of connective tissue ECM enables attachment of cells, intercellular communication, and plays a role in cell differentiation, growth, migration, and function on the basis of the dynamic interaction of chemical and physical factors microenvironment. Forming cells microenvironment and circulating cells are very sensitive to these interactions and ECM represents a complementary system responsible for the process of regeneration and carcinogenesis. In our work we discussed about methodological approach to dissect the Ca2+ clearance mechanisms myogenic and non-myogenic cells. The aim of this perspective is to confront diversity by considering how the activation mechanisms for generating Ca2+ signals have been adapted to control different muscle cells (MCs) functions. To our knowledge, the functional physiological properties in terms of Ca2+ signalling mechanisms and their activation are not well studied. Experiments based on colonization of the decellularised ECM isolated from the skeletal muscle with muscle-derived cells could reconstruct the muscle organ. For this purpose new methodological procedures based on increasingly monitor Ca2+ signaling mechanisms in skeletal muscle cells and their effect on homeostasis of ECM have to be introduced. To this end new methodological approaches that will allow this monitoring need to be developed. The results could be exploited for skeletal muscle reconstruction as well as repair of other organs.

Keywords: Extracellular matrix, Ca2+ signaling, homeostasis, skeletal muscles, muscle remodeling

Address for correspondence: S. Filip, Dept. of Oncology and Radiotherapy, Charles University in Prague. Faculty of Medicine and Faculty Hospital in Hradec Králové, Šimkova 870, 50038 Hradec Králové, Czech Republic, fillip@fnhk.cz

Title of the project: Liposomes (drug delivery systems) in kinetically guided therapy of ovarian platinum-resistant cancers with doxorubicin using plasmafiltration

Grant Agency: Ministry of Health ***Project Number:*** 16-30366A

Principal Investigator: S. Filip

Co-investigators: M. Bláha, J. Martínková, O. Kubeček, M. Hodek, J. Maláková, M. Lánská, J. Špaček

Starting date: 1.5.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 10446

Summary of 2016 results

Title of the presentation: Liposoms (drug delivery systems) in kinetically guided therapy of ovarian platinum-resistent carcinoma with doxorubicin using plasmafiltration.

Authors: S.Filip (1), M. Bláha (2), Jiřina Martínková (1), Ondřej Kubeček (1), Miroslav Hodek (1), Jana Maláková (3), Miriam Lánská (2), Jiří Špaček (4)

1. Dept. of Oncology and Radiotherapy; 2. IV. Internal Clinic; 3. Dept. of Clinical Biochemistry; 4. Dept. of Gynecology.

Our primary aim is to demonstrate that the safety and efficiency of extracorporeal elimination of PLD is dependent on its kinetically guided regulation, i.e. extracorporeal removal should be individualized according to the patient´s elimination capacity. An anti-cancer drug, doxorubicin, encapsulated in small-sized pegylated liposomes (PLD) is indicated for second-line treatment of ovarian cancer and AIDS-related Kaposi´s sarcome. PLD shows a lower uptake by RES, and consequently, increased tumor uptake. Long-circulating lipozomes (as drug delivery systems) appear to offer a double advantage: toxicity buffering (mainly serious cardiotoxicity, less myelotoxicity) and selective tumor accumulation leading to an enhanced antitumour activity. Liposome longevity might be risky for mucocutaneous toxicities (mucositis and hand-foot syndrom) due to the unique pharmacokinetics of liposomes. A sufficient time gap between saturation of tumor tissue with PLD and accumulation in skin has been found which allows to eliminate a remaining dose circulating in the plasma by a system of extracorporeal plasma filtration. Our project solves the fundamental question of detoxification focused on drugs encapsulated to nanoparticles, their safe and effective clinical use in the treatment of cancer patients.

Key words: liposoms; drug delivery system; liposomal doxorubicine; toxicity; chemotherapy; tumors; plasmafiltration; nanotechnologies

Address for correspondence: S. Filip, Dept. of Oncology and Radiotherapy, Charles University in Prague. Faculty of Medicine and Faculty Hospital in Hradec Králové, Šimkova 870, 50038 Hradec Králové, Czech Republic, fillip@fnhk.cz

Title of the project: Correlation of different methods evaluating dysregulated proteins and somatostatine receptors in pituitary adenomas

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-10

Principal Investigator: F. Gabalec

Co-investigators: J. Soukup, P. Kašparová, J. Čáp

Starting date: 1.4.2016 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation: Correlation of different methods evaluating dysregulated proteins and somatostatine receptors in pituitary adenomas

Authors: Filip Gabalec

Transsphenoidal surgery often leads to uncomplete removal of adenoma. Residual tissue grows in 11-46% of cases. Biomarker predicting tumor growth has not been found yet. In the last years we found 3 dysregulated protein in aggressive/non-agressive pituitary adenomas: Ubiquitin carboxyl-terminal hydrolase isozyme L1, Alpha-internexin and Tubulin polymerization-promoting protein family member 3. The goal of this pilot project was to confirm this dysregulation by immunohistochemistry (ICC). The second part was to confirm correlation between somatostatine receptor mRNA expression and ICC in acromegaly.

Results: 1) TPPP3 expression was negative in all cases. Internexin expression was negative in non-aggressive tumours and positive in aggressive tumours, but not significantly. Ubiquitin was expressed in both groups. We did not found correlation between proteomic and ICC results in aggressive and non-aggressive adenomas. 2) Somatostatine receptor 2 was expressed in all cases. 1+ in 4, 2+ in 6 and 3+ in 4 cases. SSTR5 was 0+ in 1, 1+ in 8, 2+ in 3 and 3+ in 4 cases. We did not found a correlation between somatostatin receptor mRNA expression and expression evaluated by ICC.

Conlusion: We did not confirmed dysregulated proteins in aggressive/non aggressive clinically non funtioing pituitary adenomas by ICC. Somatostatine receptors expression evaluted by ICC and real-time PCR did not correlate.

Address for correspondence: filip.gabalec@fnhk.cz

Title of the project: Research Center for the Study of Toxic and Protective Effects of Drugs on Cardiovascular System

Grant Agency: Ministry of Education ***Project Number:*** UNCE 204019/304019

Principal Investigator: T. Šimůnek

Co-investigators: M. Štěrba, E. Jirkovský, O. Lenčová, M. Hroch, K. Vávrová, P. Nachtigal, P. Zimčík, P. Kovaříková, A. Jirkovská, I. Němečková, H. Jansová, G. Karabanovich, J. Roh

Starting date: 1.1.2012 ***Duration (years):*** 6

Total funds allocated for project - Kč (thousands): 18152

Summary of 2016 results

Title of the presentation: Examination of cardioprotective effects of new N,N-dimethyl derivative of dexrazoxane against chronic anthracycline cardiotoxicity in vivo.

Authors: P. Brázdová, E. Jirkovský, Z. Pokorná, O. Lenčová (1), G. Karabanovich, J. Roh (2), A. Jirkovská (3), M. Hroch (4), K. Vávrová (2), T. Šimůnek (3), P. Kovaříková (5), M. Štěrba (1). Charles Univ.; Fac. Med., Hr. Králové: Dept. of Pharmacol.(1), Dept. of Biochem.(4); Fac. Pharm., Hr. Králové: Dept. of Inorg. and Org. Chemist.(2), Dept. of Biochem. Sci.(3), Dept. of Pharm. Chem. and Pharm. Anal.(5).

Dexrazoxane (DEX) is the only drug with well-established cardioprotective efficacy against anthracycline (ANT) cardiotoxicity in both clinical and experimental settings. Unfortunately, it is only seldom used in current clinical practice due to the concerns related to its adverse effects. However, only limited information is available about possibilities of optimization of pharmacological profile of this drug via targeted modification of its chemical structure. Hence, the aim of the present study was to prepare close derivatives of DEX and study their safety and cardioprotective efficacy on a well-established in vivo model. This effort yielded in optimized synthesis of N,N-dimethyl derivative of DEX which was available in the amount suitable for chronic in vivo experiment. This new metabolite was designed to be hydrolysable in vivo to open-rings metabolite which should have similar metal chelating properties as the corresponding metabolite of DEX. Cardioprotective effects of the N,N-dimethyl derivative of DEX were then studied on a model of chronic ANT cardiotoxicity in rabbits (daunorubicin, DAU, 3 mg/kg, weekly for 10 weeks). The new derivative was administered in the same dose and schedule as DEX (60 mg/kg, i.p. 30 min before each DAU dose) which was previously shown highly effective on this model. Interestingly, unlike DEX, its N,N-dimethyl derivative showed no protective effect against DAU-induced mortality, cardiac dysfunction and cardiac troponin T rise in plasma. Following pharmacokinetic investigation confirmed that the difference in cardioprotective effects is not due to the significantly different PK profile of the parent drug or its metabolite in plasma or myocardium as compared to DEX. Instead, pilot data suggest that unlike DEX, N,N-dimethyl derivative of DEX lacks the interaction with topoisomerase IIβ, which is proposed to be a new major target for ANT in the heart. This study also shows that free (unsubstituted) imide nitrogens in DEX molecule are required for this interaction and are indispensable for cardioprotective effects.

Address for correspondence: M. Štěrba, Department of Pharmacology, Charles University, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 03 Hradec Králové, Czech Republic.

Title of the project: The role of near-infrared spectroscopy (NIRS) for monitoring cerebral perfusion during carotid endarterectomy

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-16

Principal Investigator: I. Guňka

Co-investigators: M. Leško, D. Krajíčková, M. Lojík, J. Raupach

Starting date: 1.6.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 122

Summary of 2016 results

Title of the presentation: The role of near-infrared spectroscopy (NIRS) for monitoring cerebral perfusion during carotid endarterectomy

Authors: I. Guňka (1), M. Leško (1), D. Krajíčková (2), M. Lojík (3), J. Raupach (3)
Department of Surgery, University Hospital Hradec Králové (1), Department of Neurology, University Hospital Hradec Králové (2), Department of Radiology, University Hospital Hradec Králové (3)

Carotid endarterectomy (CEA) is the treatment of choice to prevent ischemic cerebrovascular events in patients with symptomatic internal carotid artery (ICA) stenosis ≥ 50% and selected patients with asymptomatic ICA stenosis ≥ 70%. However, this precedure itself carries a risk of stroke. Strokes with an onset during CEA are mainly caused by thrombosis, peripheral embolism or intraoperative ischemia related to hypoperfusion during carotid artery cross-clamping. Cerebral ischemia during CEA may be prevented by placement of an intraluminal shunt. The brain monitoring is of utmost importance for detection of cerebral ischemia during carotid cross-clamping and thus for avoidance of unnecessary shunt placement. Use of locoregional anesthesia allows monitoring function in awake patients. Patients operated under general anesthesia require alternative methods of cerebral perfusion monitoring such as trans-cranial doppler (TCD), electroencephalography (EEG), Somato-sensory evoked potentials (SSEP) or ICA stump pressure. However, TCD cannot be performed in all patients, since a temporal bone window in missing in up to 15% of patients. EEG measurements can be influenced by anesthetic agents or electrocautery. Moreover, pre-existing EEG abnormalities in patients with severe stroke make interpretation very difficult or even impossible. Cerebral oxymetry is a simple non-invasive method to detect cerebral ischemia by monitoring changes in regional cerebral oxygen saturation (rSO2) in the frontal lobes by NIRS. However, at present, the evidence defining clear cut-off values for presence of intraoperative cerebral ischemia is limited.

The aim of present study was to analyse the role of NIRS in combination with awake testing in detecting cerebral ischemia in patients undergoing CEA under local anesthesia. During the study period, 25 patients undergoing CEA were peroperatively monitored with cerebral oxymeter. Cerebral ischemia was assessed by awake testing in conjunction with rSO2. Shunting was based solely on deterioration in the neurological status. The correlation between awake testing and percentage fall in rSO2 was analysed. Drop in rSO2 more than 20% seems to have high sensitivity and specificity.

Address for correspondence: I. Guňka, Department of Surgery, University Hospital Hradec Králové, Sokolská 581, Hradec Králové, 500 05, Czech Republic.

Title of the project: Antitumor activity of isoquinoline alkaloids

Grant Agency: Charles University ***Project Number:*** 932616

Principal Investigator: K. Habartová

Co-investigators: M. Pospíšilová, R. Havelek, M. Řezáčová

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 247

Summary of 2016 results

Title of the presentation: Biological activity of isoquinoline alkaloid scoulerine

Authors: K. Habartová (1), R. Havelek (1), L. Cahlíková (2), M. Řezáčová (1)
(1) Department of Medical Biochemistry, Faculty of Medicine in Hradec Kralove, Charles University; (2) ADINACO Research group, Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Charles University

Natural products and their derivatives are important sources of new drug leads. In the area of cancer therapy, up to 80% of approved drugs is derived from natural products (e.g. Vinca alkaloids). Among various species investigated in search of small molecule constituents with potential in the therapy, plants of the Fumariaceae family have been particularly fruitful. One of these potential drug leads is scoulerine, which can be found in small amounts in Croton flavens or Corydalis cava. Scoulerine possesses a vast amount of biological properties, such as antitussive and antiemetic, has affinity to the GABA receptor and protects α-adrenoreceptors against irreversible blockade by phenoxybenzamine. Results of our experiments show that scoulerine reduces viability and proliferation of Jurkat and MOLT-4 cells in dose dependent manner within 24 hours of treatment. Moreover, the reduction of cell viability was more pronounced 48 hours after scoulerine aplication than after 24 hours. The decrease in cell viability following 24 hours of treatment was caused by activation of apoptosis, determined by Annexin V binding and activation of caspases-3/7, -8 and -9. To further understand the mechanism by which scoulerine affects cell proliferation, we performed cell cycle and phospho-histone H3 (Ser10) analysis, which revealed accumulation of cells in G2 phase with mitotic arrest. Western blot analysis showed activation of cell cycle regulatory proteins e.g. p53 in MOLT-4 cells and pChk1 and pChk2 in Jurkat cells. All acquired results suggest that scoulerine has very promising activity and it would be worthwhile to subject it to further evaluation.

This project was financially supported by the Grant Agency of Charles University (Project No. 932616)

Address for correspondence: K. Habartová, Department of Medical Biochemistry, Charles University, Faculty of Medicine in Hradec Králové, Zborovská 2089, 500 03 Hradec Králové, Czech Republic

Title of the project: Trajectories of quality of life by the elderly in early phase of dementia

Grant Agency: Ministry of Health ***Project Number:*** 16-28628A

Principal Investigator: H. Kisvetrová

Co-investigators: D. Školoudík, R. Herzig, P. Ressner, M. Vališ, K. Žiaková, Y. Yamada, B. Jurašková, P. Krulová, K. Langová

Starting date: 1.4.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 7610

Summary of 2016 results

Title of the presentation: Trajectories of quality of life by the elderly in early phase of dementia

Authors: R. Herzig (1), H. Kisvetrová (2), D. Školoudík (2), P. Ressner (3), M. Vališ (1), K. Žiaková (2), Y. Yamada (2), B. Jurašková (1), P. Krulová (3), K. Langová (4)

(1) University Hospital, Hradec Králové; (2) Faculty of Health Sciences, Palacký University, Olomouc; (3) University Hospital, Ostrava; (4) Faculty of Medicine and Dentistry, Palacký University, Olomouc, Czech Republic

Maintaining or improving the quality of life amongst the elderly with dementia is currently one of the key aims of health care. The main aim of the project is to ascertain how quality of life changes amongst Czech elderly patients with early-phase dementia (quality of life trajectory). To achieve the main aim, five sub-aims have been set, which should support the investigation of quality of life in patients with a nervous system disease, namely amongst the elderly patients at an early phase of dementia. (1) Translation and validation of Czech versions of standardised Quality of Life – Alzheimer's Disease (QOL–AD) and Patient Dignity Inventory (PDI) questionnaires. (2) Identification of the most important factors contributing to the changes in quality of life among Czech elderly patients with early-phase dementia. (3) Creation of a profile of quality of life trajectory amongst Czech elderly patients at an early phase of dementia. (4) Comparison of the trajectory of Czech elderly patients with early-phase dementia to the trajectory of the elderly not diagnosed with dementia. (5) Creation of a database for subsequent longitudinal trajectory tracking the quality of life of older people with dementia. In the year 2016, 228 out of the 290 selected elderly patients with a diagnosed dementia at an early phase and, 290 out of the 290 selected control group subjects (elderly subjects without dementia) were enrolled. PDI questionnaire was translated to the Czech language and the process of its validation has been commenced – 239 out of the 421 selected subjects have completed the questionnaire. For the validation of the Czech version of the QOL–AD questionnaire, a set of 200 pairs (each consisting of elderly patient with dementia and his/her family caregiver) was created.

Address for correspondence: Prof. Roman Herzig, MD, PhD, FESO, FEAN, Department of Neurology, Charles University Faculty of Medicine and University Hospital, Sokolská 581, CZ-500 05 Hradec Králové, Czech Republic

Title of the project: Microvascular abnormality as an endophenotype of schizophrenia

Grant Agency: Ministry of Health ***Project Number:*** 16-27243A

Principal Investigator: L. Hosák

Co-investigators: O. Šerý, J. Studnička, D. Bayer

Starting date: 1.5.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 5910

Summary of 2016 results

Title of the presentation: Microvascular abnormality as an endophenotype of schizophrenia

Authors: L. Hosak (1), O. Sery (2), J. Studnicka (3), D. Bayer (1)

Dpt. of Psychiatry, Charles University School of Medicine and University Hospital Hradec Kralove (1), Institute of Animal Physiology and Genetics AV CR (2), Dpt. of Ophthalmology, Charles University School of Medicine and University Hospital Hradec Kralove (3)

The research project is aimed to assess whether microvascular abnormality evaluated by retinal imaging is an endophenotype of schizophrenia. If yes, it should be found significantly more frequently in the patients´ healthy relatives than in the general population. The project should contribute to our knowledge of schizophrenia etiopathogenesis in an innovative way. The following hypotheses are tested:

Microvascular abnormality detected by retinal imaging is significantly more frequent in the schizophrenia patients´ healthy relatives than in the healthy controls.

Micovascular abnormality has a genetic background related to angiogenesis and inflammation.

Assessment of microvascular abnormality and genetic examination should be performed in 80 patients of schizophrenia, their 80 healthy first-degree relatives, and 80 unrelated healthy controls.

Till the end of 2016, 20 patient-relative duos are expected to be involved in the research. At the beginning of December 2016, 14 these duos have already been examined.

The results of the research will only be available in 2018, because all genetic examinations will be performed at the same time at the end of the study due to economic reasons, and ophthalmological examinations must be compared to healthy controls, which will only be involved in 2018.

Address for correspondence: Prof. L. Hosák, M.D., Ph.D., Dpt. of Psychiatry, University Hospital, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Use of next generation sequencing (NGS) for monitoring the clonal rearrangements receptors on the surface of T lymphocytes

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-17

Principal Investigator: K. Hrochová

Co-investigators: F. Vrbacký, L. Petrová, D. Belada, M. Šimkovič

Starting date: 1.2.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 226

Summary of 2016 results

Title of the presentation: NGS technology as a way for monitoring of IgVH clones in ALL patients - pilot study

Authors: K. Hrochová1, F. Vrbacký2,L. Petrová1, D.Belada2, J. Horáček2, M. Šimkovič2, P. Žák2 1 Institute of clinical biochemistry and diagnostics, University Hospital, Hradec Králové, 2 4th Department of Internal Medicine - Hematology , University Hospital, Hradec Králove

Acute lymphoblastic leukaemia (ALL) is the most common form of haematological cancer in children but it also affects adults. The clinical course of ALL is highly variable. Nowadays, determination of the mutational status of rearranged immunoglobulin heavy chain variable (IGHV) genes in large series of patients ALL has shown powerful and independent prognostic value. Newly introduced massively parallel sequencing technology enables through deep sequencing of rearranged IgVH CDR3 regions analysis of a previously inaccessible level of BCR repertoire. The CDR3 diversity reflects clonal composition, the potential antigenic recognition spectrum, and quantity of available B cell responses. The aim of our study is using NGS profiling to follow up minimal residual disease (MRD) in samples from our ALL patients. Materials and methods: Peripheral blood mononuclear cells were isolated by Ficoll-Paque density gradient centrifugation. For IGHV analysis we used both complementary DNA (cDNA) and genomic DNA (gDNA). First step was fragment analysis with Biomed2 primers for clonality testing. Samples for NGS we amplified with Biomed2 primers (FR1 and FR2) with adapters for Multiplicom MIDs. Prepared libraries were analyzed using paired-end Illumina MiSeq sequencing. Raw data were processed by our own pipeline and on-line Vidjil software (http://www.vidjil.org/). If sensitivities beyond 10-4 are desired, it is important to note that there are approximately 6,5 pg of DNA in each cell. We analyzed samples gained from 13 patients with ALL in time. We were able to set up the method's sensitivity up to 1x10-5. In conclusion NGS showed the potential to be a very sensitive method for MRD monitoring. Furthermore, within this monitoring we can detect a new potential pathologic clone. The interpretation of results seems to be very complicated. There is need to discussed how to set up the sensitivity threshold. The other question for discussion is how to handle with observed discrepant results within DNA and cDNA.

references: JJM van Dongen. Report of the BIOMED-2 Concerted Action BMH4-CT98-3936, Leukemia (2003) 17, 2257–2317

Address for correspondence: K. Hrochová, Dept. of molecular biology, Institute of clinical biochemistry and diagnostics, Sokolská 581, University hospital, 500 05 Hradec Králové, Czech Republic

Title of the project: The role of epigenetic changes in the pathogenesis of the urological malignancies

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-8

Principal Investigator: P. Hušek

Co-investigators: J. Pacovský, M. Chmelařová, M. Podhola, M. Broďák

Starting date: 1.1.2015 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 536

Summary of 2016 results

Title of the presentation: The role of epigenetic changes in the pathogenesis of the urological malignancies

Authors: Petr Husek(1), Jaroslav Pacovsky(1), Marcela Chmelarova(2), Miroslav Podhola(3) and Milos Brodak(1)

University Hospital and Charles University Medical School, Hradec Kralove, Dept. of Urology (1), Inst. of Clin. Bioch. and Diagnostic (2), The Fingerland Dept. of Pathology (3)

The aim of the research was the evaluation of methylation status for prediction of BCG (Bacillus Calmette-Guérin) response in patients with high grade non-muscle-invasive bladder tumor (NMIBC). We evaluated 82 patients with high grade non-muscle-invasive bladder tumor (stage Ta, T1, CIS) who had undergone BCG instillation therapy and 13 specimens of normal urotel (bladder tissue) as control samples. We compared epigenetic methylation status differences between BCG-responsive and BCG-failure groups. The present study used the MS-MLPA (Methylation-Specific Multiplex Ligation-Dependent Probe Amplification) probe sets ME001 and ME004 and bisulfite conversion based techniques such as NGS (Next generation sequencing) and MSP (Methylation specific PCR). Newly identified methylations in high grade NMIBC were found in MUS81a, NTRK1 and PCCA. The methylation status of CDKN2B ($p=0.00312$) and MUS81a ($p=0.0191$) was associated with clinical outcomes of BCG instillation therapy response. CDKN2B and MUS81a unmethylation was found in BCG failure patients. Genetic and epigenetic alterations play an important role in urothelial cancer pathogenesis. Deeper understanding of these processes could help us achieve better diagnosis and management of this life-threatening disease. According to our research results we can conclude that the methylation status of selected tumor suppressor genes (TSGs) has the potential for predicting BCG response in patients with NMIBC high grade tumors. Tumor suppressor genes such as CDKN2b, MUS81a, are very promising for future research.

Address for correspondence: P. Husek, Dept. of Urology University Hospital and Charles University Medical School Sokolska tr. 581, 50005 Hradec Kralove Hradec Kralove, Czech Republic

Title of the project: The influence of hydrogen on intraluminal production of reactive oxygen species in large bowel

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-20

Principal Investigator: R. Hyšpler

Co-investigators: A. Tichá, Z. Zadák

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 193

Summary of 2016 results

Title of the presentation: Molecular hydrogen - metabolism and antioxidative action

Authors: MUDr. Radomír Hyšpler, Ph.D., RNDr. Alena Tichá, PhD, prof. MUDr. Zdeněk Zadák, CSc.

Introduction:

Despite a significant interest in molecular hydrogen as an antioxidant during the last nine years, its quantitative metabolic parameters and reactivity in vivo are still lacking. It is formed naturally in the human large bowel during soluble fiber fermentation.

Methods:

Intraperitoneally-applied deuterium gas was used as a metabolic tracer and deuterium enrichment was determined in the body water pool in rats. In another experiment the effect of diet enriched with 10 % of inulin (fermentable fiber used as a source of hydrogen) on oxidative damage of colonic mucosal cells was evaluated. The oxidative damage of cellular DNA was determined using Comet assay (expressed as tail DNA percentage).

Results:

A significant oxidation of about 10% of the applied dose was found under physiological conditions in rats, proving its antioxidant properties. Hypoxia or endotoxin application did not exert any effect, whilst pure oxygen inhalation reduced deuterium oxidation. Increased soluble fiber content (presumably via molecular hydrogen production) reduced oxidative DNA damage in colonocytes (35,8 +- 7,7 % vs. 48,7 +- 11,6 %).

Conclusions:

According to our findings, hydrogen may be an efficient, non-toxic, highly bio-available and low-cost antioxidant supplement for patients with pathological conditions involving ROS-induced oxidative stress.

Address for correspondence: Radomír Hyšpler, M.D., Ph.D., Dept. of Research and Development, University Hospital, Sokolska 581, 500 05 Hradec Králové, Czech Republic, e-mail: rhyspler@lfhk.cuni.cz

Title of the project: Monitoring of DNA methylation changes in sinonasal cancer

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-1

Principal Investigator: M. Chmelařová

Co-investigators: H. Kovaříková, J. Laco, I. Bubancová, K. Sieglová, M. Vošmik

Starting date: 1.2.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 387

Summary of 2016 results

Title of the presentation: Monitoring of DNA methylation changes in sinonasal cancer.

Authors: M. Chmelařová (1), H. Kovaříková (1), J. Laco (2), I. Bubancová (1), K. Sieglová (2), M. Vošmik (3)

(1) Institute for Clinical Biochemistry and Diagnostics, (2) The Fingerland Department of Pathology, (3) Department of Oncology and Radiotherapy, Charles University in Prague, Faculty of Medicine in Hradec Kralove and University Hospital Hradec Kralove.

Malignant tumors of the sinonasal tract are generally uncommon tumors of the head and neck area that account for approximately 3% to 5% of all upper respiratory tract malignancies. Diagnosis and treatment of these tumors pose several problems due to their very low incidence, histological diversity, largely unknown molecular profiles and production of non-specific symptoms in the early stages that can simulate an inflammatory process. Because sinonasal carcinomas are a group of aggressive tumors it is very important to know molecular parameters to establish diagnostic strategies and individualized therapies. The aim of this study was to investigate promoter methylation of specific genes in sinonasal carcinoma by comparison with normal sinonasal tissue. To search for epigenetic events we used MS–MLPA (Methylation-specific Multiplex ligation-dependent probe amplification) to compare methylation status of 57 formalin fixed, paraffin embedded tissue samples of sinonasal carcinomas with 18 control samples. The most important changes in DNA methylation were confirmed using MSP (Methylation specific PCR). Using MSP for SMARCB1 gene we also search for methylation changes in SMARCB1/INI1-deficient sinonasal carcinomas. No methylation was found in SMARCB1 gene. Using MS-MLPA, we observed significantly higher methylation in GATA5 ($p=0.0005$), THBS1 ($p=0.0002$) and PAX5 ($p=0.03$) genes in the sinonasal cancer group compared to the control group. Methylation in THBS1 gene was significantly higher in samples of high grade carcinoma compared to low grade carcinoma ($p=0.028$). Using MSP, we confirmed significantly higher methylation of GATA5 gene in samples of sinonasal carcinoma in comparison with control samples of non-cancerous sinonasal tissue ($p= 0.03$). These observations provide evidence that changes in methylation of these genes may be one of the major mechanisms in sinonasal carcinogenesis. In addition, changes in methylation could potentially be used in screening of sinonasal cancer and may have implications for future individualized therapy based on epigenetic changes.

Address for correspondence: M. Chmelařová, Institute for Clinical Biochemistry and Diagnostics, Sokolská 581, Hradec Králové 500 05, marcela.chmelarova@fnhk.cz

Title of the project: Non-invasive detection of intraamniotic infection based on identification of dominant bacteria in cervical fluid

Grant Agency: Ministry of Health ***Project Number:*** 16-28587A

Principal Investigator: M. Kacerovský

Co-investigators: O. Šimetka, P. Janků

Starting date: 1.5.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6430

Summary of 2016 results

Title of the presentation: Cervical fluid calreticulin and cathepsin-G in pregnancies complicated by preterm prelabor rupture of membranes

Authors: Marian Kacerovsky, Ivana Musilova, Ondrej Soucek, Marcela Drahosova, Lenka Pliskova, and Ctirad Andrys

Objective: The study aimed to determine the cervical calreticulin and cathepsin-G concentrations in pregnancies complicated by preterm prelabor rupture of membranes (PPROM) with respect to the presence of microbial invasion of the amniotic cavity (MIAC) and intra-amniotic inflammation (IAI).

Methods: Eighty women with singleton pregnancies complicated by PPROM were included in this study. Cervical and amniotic fluids were obtained at the time of admission, and concentrations of calreticulin and cathepsin-G in cervical fluid were determined using ELISA. The MIAC was defined as a positive PCR analysis for Ureaplasma species, Mycoplasma hominis, and/or Chlamydia trachomatis and/or by positivity for the 16S rRNA gene. IAI was defined as amniotic fluid bedside IL-6 concentrations ≥ 745 pg/mL

Result: Neither women with MIAC nor with IAI had different cervical fluid concentrations of calreticulin (with MIAC: median 18.9 pg/mL vs. without MIAC: median 14.7 pg/mL, p=0.28; with IAI: median 14.3 pg/mL vs. without IAI: median 15.6 pg/mL, p=0.57;) or of cathepsin-G (with MIAC: median 30.7 pg/mL vs. without MIAC: median 24.7 pg/mL, p=0.28; with IAI: median 27.3 pg/mL vs. without IAI: median 25.1 pg/mL, p=0.80) than women without those complications. No associations between amniotic fluid IL-6 concentrations, gestational age at sampling, and cervical fluid calreticulin and cathepsin-G concentrations were found.

Conclusions: Cervical fluid calreticulin and cathepsin-G concentrations did not reflect the presence of MIAC or IAI in women with PPROM.

Address for correspondence: Marian Kacerovsky, Department of Obstetrics and Gynecology, University Hospital, Sokolska 581, Hradec Kralove, 500 05

Title of the project: Development of a perioperative care for successful therapy of surgical patients

Grant Agency: Ministry of Education ***Project Number:*** 260288

Principal Investigator: M. Kaška

Co-investigators: A. Ferko, J. Harrer, M. Broďák, P. Šponer, S. Řehák, M. Košťál, P. Rozsíval, V. Chrobok, R. Slezák, V. Černý, J. Manďák, J. Vojáček, P. Žáček, M. Kanta, P. Dostál, J. Špaček, H. Langrová, N. Jirásková, A. Šimůnek, R. Koberová-Ivančaková, F. Raiskup, M. Kacerovský, J. Studnička, P. Čelakovský, I. Sedláková, Z. Turek, J. Pacovský, and the Doctor Study Program students in 1st - 4th year of their study

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 892

Summary of 2016 results

Title of the presentation: Application of modern therapeutic methods in daily surgial practice

Authors: M. Kaška et al. (above-mentioned co-investigators)

The students of DSP groups in individual surgical disciplines were focused on very recent applied methods of specific medical management during perioperative phase of this processes during 2016 year. Surgeons were performing experiments with laboratory animals – pigs and rats with preparations of manuscripts for publication of results performing experiments and clinical complications of large bowel resection as the possible influence of lipid-lowering drugs on liver functions. Gynecologists focused on neonatal outcomes in subgroup of women with preterm prelabor rupture of membranes and infection complications of obstetric and gynecologic surgery. Cardio-surgeons and thoracic surgeons published their useful experiences for clinical daily practice in quality of life after aortic valve repair. They had found this is similar to Ross patients and superior to mechanical valve replacement in a cross-sectional trial. Neurosurgeons published some findings in the treatment of giant aneurysm after failure of endovascular and surgical reconstruction and in surgical therapy of hydrocephalus in adults. Ophthalmologists described anterior ischemic chamber migration of dexamethasone intravitreal implant through basal iridectomy in pseudophakic patients, clinical cases in possibilities in management of injuries in ophthalmology as is branch retinal artery occlusion after transcatheter closure of foramen ovale and some clinical experience in long-term follow up of posterior capsule opacification after AquaLase and NeoSoniX phacoemulsification. Dentists published new clinical experiences with use Cyclin D1 expression in ectomesenchymal chondromyxoid tumor of the anterior tongue, proliferative capacity and phenotypical alteration of multipotent ecto-mesenchymal stem cells, and protocols for dental-relates stem cells isolation, amplification and differentiation. Urologists announced experience and assessment in using tissue cyanoacrylate glue in partial nephrectomy. The anesthetists published clinical findings about rescuer fatigue does not correlate to energy expenditure during simulated basic life support. ORL performed study of narrow-band imaging endoscopy in optical biopsy of vocal cord biopsy etc.

Address for correspondence: Address for correspondence: Milan Kaška, Academic Department of Surgery, Medical Faculty and Surgical Department of Teaching Hospital, Hradec Králové, e-mail: kaskam@lfhk.cuni.cz

Title of the project: Creating of multiplatform systems for education support

Grant Agency: Ministry of Education ***Project Number:*** IP 2016-2018 63

Principal Investigator: D. Kordek

Co-investigators: M. Kopeček, K. Čáňová, K. Habartová, M. Pospíšilová

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 881

Summary of 2016 results

Title of the presentation: Creating of multiplatform systems for education support

Authors: D. Kordek (1), M. Kopeček (1), K. Čáňová (2), K. Habartová (3), M. Pospíšilová (3)
Fac. Med., Charles University, Hradec Králové: Department of Medical Biophysics (1), Department of Medical Biology and Genetics (2), Department of Medical Biochemistry (3)

Project goal is to create digital study materials for the subject of biology, biochemistry and biophysics. The materials will be optimalized for the mobile devices and students will be able to use them without online internet connection. The support courses will be realized in the environment of the program Moodle. The Project is focused for the students 1. 2. and the 3. year of General Medicine. The questionnaire was made in year 2016 and detects the most Important topics from the biology, biochemistry and biophysics, which students prefer as a supplement of electronic study materials. Students did not have to anyhow justify their choose. Of the three subjects it was chosen from each of four subjects with the highest incidence (ie topics that students have pointed out frequently). Topics from Biology has been selected: Epigenetics, Gene Therapy, invasiveness and metastasis, Fundamentals of biological therapy. Topics from biochemistry has been selected: Vitamins as cofactors for enzymes, biological oxidation + RONS, Acid-base balance, composition and basic urinalysis. Topics from biophysics has been selected: Biomechanics of muscle and bone, biomechanics of breathing, astigmatism as the aberration of an optical system Selected chapters of modern physics. It was also created interactive add-on module "book" in Moodle, which allows students to interact and answer questions in Moodle Mobile. Currently the above-mentioned selected topics are processed by research team (According to plan). The electronic courses will be created and optimalized for the mobile devices to the end of 2017.

Address for correspondence: D. Kordek, Dept. of Medical Biophysics, Charles University, Faculty of Medicine in Hradec Kralové, Šimkova 870, 50003 Hradec Králové, Czech Republic

Title of the project: Atherosclerotic plaque characteristics and the risk of brain ischemia during internal carotid artery stenting

Grant Agency: Ministry of Health ***Project Number:*** 16-30965A

Principal Investigator: F. Charvát

Co-investigators: A. Krajina, D. Školoudík, F. Cihlář

Starting date: 1.6.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 3485

Summary of 2016 results

Title of the presentation:

Authors: A. Krajina (1), V.Chovanec (1), J. Raupach (1), D. Krajickova (2), R. Herzig (2), Z. Belobradek (1), D. Školoudik (3) , F.Cihlář (4), F.Charvát (5)

1) Dept of Radiology, 2) Dept of Neurology, University Hospital Hradec Kralove, 3) Dept of Neurology, University Hospital Ostrava, 4) Dept of Radiology, University Hospital Ustí nad Labem, 5) Dept of Radiology, Military University Hospital Prague

Stroke is one of the leading causes of morbidity, mortality and long-term disability worldwide. The incidence rates varied between 200 and 500 per 100.000 inhabitants. Vulnerable plaque is a term that represent susceptibility of a plaque to rupture and release material which is source of cerebral ischemia. Large clinical trials demonstrated that carotid endarterectomy is a benefical therapy for selected patients with a symptomatic ICA stenosis ≥ 50% or asymptomatic ICA stenosis ≥ 60%. In the last decade, carotid angioplasty with stenting (CAS) has become an alternative treatment for carotid stenosis because general anesthesia and surgical incisions can be avoided. The aim of this study is to verify a hypothesis that ultrasonography, CT and MRI can identify risky plaques with an increased periprocedural risk of brain infarction detected by brain MRI in patiets indicated to CAS. Total of 240 patients with atherosclerotic plaques in the carotid bifurcation and internal carotid artery causing stenosis ≥ 70% detected by sonography will be included in the study - 120 patients indicated for CAS and 120 patients indicated to conservative treatment. Carotid atherosclerotic plaques will be investigated and assessed by duplex sonography, CT angiography and MRI. Patients selected for CAS group will undergo control MRI of brain after procedure to determine a new ischemic lesions. During the first year of this project from 1.4.2016 to 31.12.2016 were created evaluation protocols of ultrasonography, CT and MR imaging. Sixty patients with stenosis of the internal carotid artery ≥ 70%, 30 patients were indicated for CAS and 30 as a control group. All 30 patient in control group had planned diagnostic imaging. All imaging evaluations were done in the CAS group and the CAS has beed performed in 18 of them.

Fist interim results will be analysed during 2017 year.

Address for correspondence: prof. Antonin Krajina, M.D., CSc., EBIR; Dept. of Radiology, University Hospital Hradec Kralove, Charles University in Prague, Sokolska street 581, Hradec Kralove, email: antonin.krajina@fnhk.cz

Title of the project: Concept of non-quaternary reactivators AChE as the antidotes of organophsophorus poisoning - a new hope or a blind way?

Grant Agency: Czech Republic ***Project Number:*** 15-16701S

Principal Investigator: K. Kuča

Co-investigators: K. Musílek, O. Soukup, J. Korábečný, E. Nepovimová, D. Jun, P. Jošt, V. Šepsová, M. Pavlík, M. Hrabinová

Starting date: 1.1.2015 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 7049

Summary of 2016 results

Title of the presentation: Progress in concept of uncharged acetylcholiensterase reactivators

Authors: K. Kuča (1,2), K. Musílek (1,2), O. Soukup (1,2), J. Korábečný (1,2), E. Nepovimová (1,2), D. Jun (1,2), P. Jošt (1,2), V. Šepsová (1,2), M. Pavlík (1,2), M. Hrabinová (1,2)

Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic (1), Department of Toxicology and Military Pharmacy, Faculty of Military Health Sciences, Trebesska 1575, 500 01 Hradec Kralove, Czech Republic (2)

Acute intoxication with organophosphorus (OP) inhibitor can be managed either via the pre-treatment using e.g. acetylcholinesterase inhibitors or ex-post therapy using parasympatholytic agent, oxime reactivator and anticonvulsant drug. During the past decades, several reactivators based on pyridinium scaffold have been synthesized and evaluated for their reactivation potency against different types of OP inhibitors. However, these structures possess two major drawbacks - low BBB permeation and narrow therapeutic window in case of intoxication after different OP.

In 2016, we reviewed new efforts leading to the development of novel reactivators and to proposition of promising strategies to design novel and effective antidotes. Structure–activity relationship of recently proposed acetylcholinesterase reactivators were also discussed and summarized. The main attention was paid to dual binding site ligands of AChE as the current mainstream strategy. We also discussed new chemical entities as potential replacement of oxime functional group. According to the method described earlier, we have prepared the last uncharged reactivator denoted as K1358. We further continue with in vitro assessment establishing inhibition potency of standard reactivators and uncharged reactivators. We have also performed reactivation potency after intoxication with tabun, sarin and paraoxon. Moreover, cytotoxicity was also established comparing pyridinium oximes with uncharged reactivators on different cell lines (ACHN, HepG2, SH-SY5Y). We have compared permeation through BBB using PAMPA assay of all the derivatives under the study. Finally, maximum tolerated dose (MTD) of selected cholinesterase reactivators (standard vs. novel compounds) was evaluated in BALB/c mice.

Address for correspondence: K. Kuča, Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Title of the project: Development of multi-target drugs for Alzheimer´s disease: combination of AChE inhibitor and melatonin derivative

Grant Agency: Ministry of Health ***Project Number:*** 15-30954A

Principal Investigator: K. Kuča

Co-investigators: O. Soukup, M. Hrabinová, E. Nepovímová, J. Říčný, D. Řípová, Z. Krištofíková, J. Korábečný, K. Špilovská

Starting date: 1.5.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 9105

Summary of 2016 results

Title of the presentation: Synthesis and in vitro evaluation of novel cholinesterase inhibitors combined with tryptophan as potential multi-target drugs for Alzheimer's disease

Authors: Kamil Kuca (1), Ondrej Soukup (1,2), Martina Hrabinova (1), Eugenie Nepovimova (1), Jan Ricny (2), Daniela Ripova (2), Zdena Kristofikova (2), Jan Korabecny (1,2), Katarina Spilovska (1,2)

Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic (1); National Institute of Mental Health, Topolova 748, 250 67 Klecany, Czech Republic (2)

Alzheimer's disease (AD) is an age-related multifactorial progressive neurodegenerative disorder accompined with several pathological changes including synaptic and neuronal loss, oxidative damage, activated inflammatory cells, extracellular amyloid plaques mainly composed by Aβ peptide, and intracellular neurofibrillary tangles comprised of hyperphosphorylated aggregates of the microtubule-associated protein Tau. There is demand on the researchers around the world for more effective and safe drugs or novel therapeutic approaches available for AD treatment preferably aiming to more than one target in the pathological cascade of the AD. Firstly, we have synthesized 21 novel hybrids comprising of tacrine derivatives and tryptophan tethered by different length of alkyl chains. Furthermore, we countinued with our synthesis by changing tacrine scaffold for huprine to obtain six novel huprine-related hybrids. During the second year of the presented project, we have also evaluated effects of novel tacrine-tryptophan hybrids on the activity of neuronal nitric oxide synthase (nNOS) in vitro. Observed changes showed that the activity of nNOS can be enhanced, dropped or steady-stated depending on the type of the linker and/or tacrine moiety used within the scaffold. Furthermore, we predicted the potential bioavailability of the compounds in the central compartment since the penetration across the BBB is an essential property for compounds targeting the CNS. In order to predict passive blood-brain penetration of novel compounds, modification of the parallel artificial membrane permeation assay (PAMPA) has been used. Moreover, Hurpine X (K1354) and huprine-tryptophan hybrids (K1355-K1357) were evaluated according to the spectrophotometric Ellman's assay to determine their AChE/BChE inhibition profile.

Address for correspondence: Kamil Kuca, Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Title of the project: Plasmonic nanoparticles for theranostics with tunable optothermal properties

Grant Agency: Czech Republic ***Project Number:*** 16-13967S

Principal Investigator: K. Kuča

Co-investigators: K. Musílek, D. Maliňák, Š. Salajková, R. Doležal, J. Bartek, J. Proska, M. Žárská, F. Novotný, F. Havel

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 8537

Summary of 2016 results

Title of the presentation: Plasmonic nanoparticles for theranostics with tunable optothermal properties

Authors: K. Kuča (1), K. Musílek (1), D. Malinak (1), Š. Salajková (1), R. Doležal (1), Bartek J., Proska J., Zarska M., Novotný F., Havel F.

Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic (1), Ústav molekulární genetiky, Akademie věd ČR (2)

Appropriate surface coating and functionalization together with excellent plasmonic tenability predispose gold nanorods to be used in biomedicine, where the specific targeting is a major challenge for applications in many different areas, from diagnostics, imaging and sensing to therapy. Main objective is to develop gold nanoparticles optothermally tuned “on demand” for specific areas of their application.

Nanoparticles retaining spectral properties under femtosecond laser-induced heating for applications in theranostics are of special interest. Novel homologous surfactants for gold nanorods passivation are synthesized with both thiol and quaternary moieties involved in one molecule. Particular aim is to estimate the effects of bulky aromatic quaternary moieties in molecule of surfactant on thermal dissipation in ligated gold nanorods and to understand the in vitro processes of nanoparticle uptake by cancer and normal cells.

Principal objective is to develop non-toxic gold nanoparticles optothermally tuned “on demand” for specific areas of their application in theranostics and biomedical imaging. Surfaces of the gold nanorods and gold nanodumb-bells are modified with de novo synthesized ligands. During the first year all methods and technical approaches necessary for the project were established (1). As a pilot experiment, gold nanoparticles (GNRs) modified by (16-mercaptohexadecyl)trimethylammonium bromide (MTAB) were chosen as a model for their excellent absorption by cells.

(1) Zarska, M., Novotny, F., Havel, F., Sramek, M., Babelova, A., Benada, O., Novotny, M., Saran, H., Kuca, K., Musilek, K. and Hvezdova, Z., 2016. Two-Step Mechanism of Cellular Uptake of Cationic Gold Nanoparticles Modified by (16-Mercaptohexadecyl) trimethylammonium Bromide. Bioconjugate Chemistry, 27(10), pp.2558-2574.

Address for correspondence: K. Kuča, Biomedical Research Centre, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Title of the project: Biomedical photonic devices for advanced medicinal diagnostics and therapy

Grant Agency: Ministry of Health ***Project Number:*** 15-33459A

Principal Investigator: M. Kuneš

Co-investigators: O. Podrazký, O. Lyutakov

Starting date: 1.6.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 17137

Summary of 2016 results

Title of the presentation: Testing of surface functionalized biodegradable polymers

Authors: M. Kuneš (1), V. Závalová (1), K. Bastekova (2), O. Lyutakov (2), O. Guselnikova (2), V. Svorcik (2)

University Hospital Hradec Králové: Biomedical Research Centre (1), Institute of Chemical Technology (2)

Biodegradable synthetic polymers are omnipresence in tissue apparatus designs. Prevailingly, commercially available acid proformas are incorporated into the polyester group, e.g. polylactic (PLLA) and polyglycolic (PLGA) acids, and their copolymers - poly-L-lactid acid (PLGA). Most of the known polymers show suitable properties to be applicable within the patient's body, from the point of materials toxicity and withdrawal of degradation products from organisms. A surface of PLLA film was modified by grafting with different functional organic groups (OFGs), changing the PLLA surface properties (contact angle, morphology, zeta potential, chemistry, mechanical). (i) exposing one-side PLLA film to the plasma discharge with the aim to activate polymer surface, (ii) soaking the PLLA in the arenediazonium tosylates salts (ADT) and NaBH4 solution resulting in radicals bonding to PLLA surface, and (iii) PLLA plasma treatment followed by soaking in the chemically activated ADT salts. The tested samples (50um thick; 0.5 x 0.5 cm) were inserted into the microtubes with physiological saline or heparinized pig blood and shaken at 37°C for 7 consecutive days. After finishing the experiment, the weight loss of the samples was measured. The surface of tested samples was evaluated using confocal microscope to detect the potential biofouling (cell repelent property) or sites of cell adhesion. Biodegradation and bioadhesive/repellent properties in the physiological solution and blood were evaluated using the gravimetry and optical microscopy. The plasma treatment enhances the biodegradation of PLLA in saline solution, because of higher hydrophilicity in this case. Grafting with hydrophilic OFGs also increase the degradation rate. Oppositely, grafting with -C18H37 and -C8F17 prevents the sample contact with physiological solution and as a result it decreases the degradation rate. A somewhat different situation was observed in the case of PLLA degradation in blood. It was shown, that proposed technique allows preparation of biorepellent or biabsorptive surfaces, tuning of PLLA biodegradation rate and nanomechanical properties which are important for PLLA use in medical applications.

Address for correspondence: M. Kuneš, Biomedical Research Centre, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové, Czech Republic

Title of the project: Utilization of cellular reprogramming technology in current medicine research and drug screening.

Grant Agency: Ministry of Education ***Project Number:*** NF-CZ07-ICP-4-290-2015

Principal Investigator: D. Kunke

Co-investigators: E. Macourková

Starting date: 1.3.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 987

Summary of 2016 results

Title of the presentation: Utilization of cellular reprogramming technology

Authors: J.Mokrý (1), D.Kunke (1) and G.J.Sullivan (2)
Fac. Med., Charles Univ., Hr. Králové: Department of Histology and Embryology (1) and Fac. Med., University of Oslo, Institute of Basic Medical Sciences (2)

The project had two main targets; the first to establish induced pluripotent stem cells (iPSCs) technology in our laboratory and test iPSCs array cards. The second goal was presented in collaboration with our Norwegian partner state of the art of cellular reprogramming technology in Hradec Kralove area to find other collaborators in the field of biomedical research and education, particularly with interest in drug discovery and compound screening, personalised medicine, regenerative medicine and related subjects. The final workshop entitled "The most promising and effective approach for reprogramming somatic cells are non-integrating cell reprogramming methods" was held in Hradec Králové in September 8-9, 2016 (see SCAN, 4/2016, 14).

iPSCs are genetically reprogrammed (in our set-up human) somatic cells exhibiting a pluripotent stem cell-like state similar to embryonic stem cells. Thus, they are an invaluable new source of pluripotent cells for drug discovery, cell therapy and basic research. Cellular reprogramming technology and personalized medicine with regard to modelling diseases poses promising ad hoc utilization of iPSCs in a clinical approach with focus on studies that have demonstrated a disease phenotype in the tissue of interest.

The iPSCs PCR array card should profile the expression of the key genes involved in iPSC research. To control the procedure of cell reprogramming and conversion to pluripotency, the expression of multiple gene classes included on the array has to be monitored simultaneously: representative parental cell line genes, the ectopically expressed transcription factors, markers of iPSCs, and markers of the redifferentiation into cells of ectodermal, endodermal, and mesodermal origin. Namely, we can “functionally” divide tested genes into 5 groups: expression in undifferentiated cell, maintenance of pluripotency, correlation to stemness, differentiation markers, endogenous controls - housekeeping genes.Using real-time PCR, research studies can easily and reliably analyse the expression of a focused panel of genes involved in the induced pluripotent stem cell dedifferentiation and redifferentiation processes with this array.

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

Title of the project: Optimisation of Treatment and Management of Schizophrenia in Europe: Optimise Trial

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

Principal Investigator: R. Kahn

Co-investigators: J. Libiger, R. Köhler

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

Total funds allocated for project - Kč (thousands): 4871

Summary of 2016 results

Title of the presentation: What is the optimal sequence of steps in treating treatment non-responding patients with schizophrenia?: the elusive question.

Authors: Libiger J.

Optimise is a large scale multinational clinical investigation designed by European Group for Research in Schizophrenia and financed in the 7th FP EU, that has completed the data collection in 2016. In accordance with the agreement between the the EU and the Trial Consortium the anlysis of data and publicaton of results is scheduled in 2017.The main objective was to test the optimal sequence of tretament with the most effective second generation antipsychotics and to answer the question whether the dose adaptation and continuation on the initial monotherapy or a switch to another antipsychotic drug are the preferrable options in treatment of a partially responsive or non-responsive patients with schizophrenia. The trial was reported in abstracts at the Faculty Research Conferences in 2014 and 2015 already, however it has reached the final stage this year, and some preliminary results will be discussed.The clinical trial ran at 19 European sites, including Israel, and it has been supplemented by a parallel double blind trial that tested the efficacy of cannabidiol in schizophrenia and by collection of MRI scans and also biochemical and genetic biological material for assessments of predictive markers for specific types of treatment response. The Czech centre was in Hradec Králové. Thirteen Czech patients were recruited and completed the trial. However, all of them met the criteria for symptomatic response already after 4 weeks of treatment with the initial antipsychotic- amisulpride. Because the trial was powered for at least 100 patients who would complete the double-blind Phase II, which included the comparison of the starting medication with a new antipsychotic (amisulpride vs. olanzapin), the centres that were successful in reaching the full remission in phase I were closed.This applied to the Czech site at Hradec Králové as well as to sites in 5 other countries.The final number of recruits was reached in May 2016. There are data from 500 patients in phase I ,more than 100 patients in phase II,and as of the end of 2015, 28 patients in phase III (clozapine treated patients). The preliminary results will be presented and current state of knowledge discussed in the presentation.

Address for correspondence: prof. MUDr. Jan Libiger, CSc, Dept. of Psychiatry, Charles University Faculty of Medicine and Faculty Hospital at Hradec Králové, 500 05 Hradec Králové, Czechia

Title of the project: Impact of selective ventilation on antibiotic levels in lung tissue - microdialysis study

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-7

Principal Investigator: J. Manďák

Co-investigators: M. Děrgel, M. Voborník, M. Pojar

Starting date: 1.6.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 80

Summary of 2016 results

Title of the presentation: Impact of selective ventilation on antibiotic levels in lung tissue

Authors: J. Manďák (1), M. Děrgel (1), M. Voborník (1), M. Pojar (1), Z. Turek (2), J. Maláková (3), V. Závalová (4)

Univ Hosp, Hradec Kralove, Dept. of Cardiac Surgery (1), Dept. of Anesthesiology, Resuscitation and Intensive Medicine (2), Dept. of Clinical Biochemistry (3), Dept. of Surgery (4)

Introduction: Mini-thoracotomy in cardiac and thoracic surgery is more and more freguent, safe and effective procedure being equivalent to the traditional medial sternotomy. These operations are performed using the technique called one lung ventilation by two-lumen endotracheal intubation in order to fully expose to the surgery field.

Aim: The aim of this pilot study was to monitor the impact of selective ventilation and atelectasis of lung on tissue concentrations of prophylactic antibiotic (cefuroxim) in peripheral lung tissue during surgery by interstitial microdialysis. These antibiotic concentrations were compared with plasma concentrations of cefuroxim.

Materials: The sample of this pilot animal study included 5 healthy pigs of 35 kg weight.

Methods: After introduction of anestethesia a double-lumen endotracheal cannula with blocker was inserted and the left main bronchus was obturated. Than bilateral mini-thoracotomy with explosure of lung were performed and special microdialysis probes (CMA) were inserted into the lung tissue and continuously perfused. Cefuroxim in dose 40mg/1 kg of weight was administered intravenously and microdialysis was started. Samples of dialysates and samples of blood were collected in the same intervals, each 30 minutes to the end of surgery. Concentrations of cefuroxim were determined by the fluid chromatography method and corrected by in vivo recoveries of the microdialysis probes.

Results: Final analysis of all samples of five studies are not comleted yet. Reached results showed higher concentrations of cefuroxim in plasma than the MIC-minimum inhibitory concentration in 210 minutes only and lower in later phases. Average concentrations in dialysates were higher in tissue of ventilated lung than in colapsed one but higher than MIC on both side.

Conclusion: This method can bring new information about administering drugs in lung tissue during the surgery with atelectasis of the lung. The finalization of our results and further research in this field is necessary.

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

Title of the project: Health Care Focused on Particularities of Patients: Individualized Care (Theory, Assessment, Interventions)

Grant Agency: Ministry of Health ***Project Number:*** 16-28174A

Principal Investigator: J. Mareš

Co-investigators: V. Gigalová, L. Hodačová, D. Skorunka, M. Štefančíková, E. Vachková, D. Vaňková

Starting date: 1.4.2016 ***Duration (years):*** 4

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

Summary of 2016 results

Title of the presentation: Health Care Focused on Particularities of Patients: Individualized Care (Theory, Assessment, Interventions)

Authors: J. Mareš
Faculty of Medicine, Charles University, Hradec Králové: Department of Social Medicine

Translation and validation of assessment methods: Contacts with foreign colleagues have been established and their consent was acquired with: a) translation of the original questionnaires; b) standardisation of the questionnaires for the Czech conditions; c) application of the questionnaires in clinical practice. Following questionnaires were used: SDM (Kriston, 2010), DES (Stalmeier, 2005), ICS-P, ICS-N (Suhonen, 2013), CCCQ (De Witte, 2006), PPES (Erickson, 2009). Questionnaires were translated to Czech language by two independent translators. After discussions, final version of each questionnaire was made. The questionnaires were administered at the Faculty Hospital in Hradec Králové. Heads of 7 selected clinical departments were informed about the project by a formal letter. A pilot validation of 3 questionnaires (ICS-N; ICS-P; SDM-Q) was carried out at those clinics.

Detailed review of quantitative methods for assessment of individualized care from the perspectives of patients, nurses, and physicians. A review study was written and publisher. It reviews detailed findings about 16 questionnaires for patients, 2 questionnaires for family members, 10 questionnaires for care assistants and nurses, and 4 questionnaires for physicians.

Detailed review of those variables, which are used in studies abroad for assessment of the size of individualized care in hospitals. The study focuses on data about 22 generic variables used in questionnaires for patients; data about 34 specific variables used in questionnaires for patients; data about 12 generic variables used in questionnaires for care assistants and nurses; data about 13 generic variables used in questionnaires for physicians.

Detailed review of findings about an extent of shared decision making with patients about planned treatment. A review study about models of shared decision making between physicians and patients were published. It describes history of the term, its definition, various models and factors, which influence the process of shared decision making.

Address for correspondence: J. Mareš, Faculty of Medicine, Charles University, Hradec Králové: Department of Social Medicine

Title of the project: New device for improvement of surgical wound healing based on the principles of mechanical support of capillary microperfusion

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-4

Principal Investigator: J. Mejzlík

Co-investigators: V. Chrobok

Starting date: 1.1.2016 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation: New device for improvement of surgical wound healing based on the principles of mechanical support of capillary microperfusion

Authors: Mejzlik, J., Chrobok, V.

Objective: This preclinical animal study examines the use of a device for improvement of surgical wound healing. The device is based on the principles of mechanical support of capillary microperfusion.

Method: The study involved experimentation on 10 pigs. Each animal had two standardized surgical wounds. A cuff that delivered pneumatic 3-second micro-pulses was applied to the wound designated as “experimental wound”. The pressure inside the cuff was continuously kept at a level below 10mmHg so that during the pulse, the pressure would decrease by one third of the established value. The second wound, designated as “control wound”, was covered with a standard dressing. Over the course of 5 days, the pressure inside the cuff was monitored. After 5 days, the experimental animal was killed, and two specimens were collected for histological analysis (one sample from the experimental wound and the other one from the control wound). Wound healing parameters for the experimental and control arms were established by the pathologist. Statistical analysis was performed using IBM PSPP statistical software. The level of statistical significance alfa was always set at 0.05.

Results: The average pressure in the cuffs was 8.46mmHg (SD 3.86). No disparities in wound healing were observed in cases of different average pressures in the experimental arm. With respect to the wound healing parameters, a statistically significant difference was found in favour of the experimental arm. The occurrence of histological signs of deranged healing was identical in both study arms.

Conclusion: The study shows that a device for the support of capillary microperfusion of the surgical wound had a positive effect. It was confirmed that the mechanical support system of capillary microperfusion was safe and reliable.

Declaration of interest: The authors have no conflicts of interest to declare.

Key words: microcirculation; wound healing; animal study; dressing; compression

Address for correspondence: Jan Mejzlik, ENT, University Hospital Hradec Kralove, Charles University in Prague, Faculty of Medicine in Hradec Kralove ,Sokolska 581, 500 05 Hradec Kralove, Czech Republic. jan.mejzlik@hotmail.com

Title of the project: Assessment of Myocardial Damage in Dynamic and Dobutamine Stress Echocardiography

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-5

Principal Investigator: K. Mědílek

Co-investigators: L. Žaloudková, R. Pudil

Starting date: 1.3.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 261

Summary of 2016 results

Title of the presentation: Assessment of Myocardial Damage in Dynamic and Dobutamine Stress Echocardiography

Authors: K. Medilek (1), L. Zaloudkova (2), R. Pudil (1), University Hospital Hradec Kralove: Dept. of Cardio-angiology (1), Dept. of Clinical Biochemistry (2)

In stress echocardiography dynamic stress (ExSE) or dobutamine stress (DSE) are considered as equal. High dose i.v. dobutamine (30-40 ug/kg/min.) is used in DSE. The rate of adverse events (arrhytmias, hypotension, myocardial infarction) is considerably higher in DSE on comparison to ExSE, likely due to the pharmacological effect of dobutamine. ExSE is therefore preferred stress method according to the present guidelines. We studied myocardial damage during ExSE and DSE measured by high sensitive troponin T (hsTnT).

Methods: 16 DSE and 17 ExSE adult patients with normal stress test result performed according to the standard protocols (including i.v. atropine or/and handgrip shall need) were included. Patients with LV EF<50%, previous ACS/PCI, epicardial coronary artery stenosis >50%, left venctricle hypertrophy (>13 mm), pulmonary hypertension (systolic PA pressure >45 mmHg), uncontrolled hypertension (rest BP >160/100 mmHg) and severe valvular disease were excluded.

Results: All patients had diagnostic test and achieved target heart rate. Both groups were comparable in following baseline characteristics: use of i.v. contrast Sonovue (94% vs 72%), gender (male 44% vs 29%), eGFR (1,33 vs 1,31 mL/min/1.73m2) and IHD risk (37% vs 28%). Pacients in DSE group were older (65 vs 54 years) and they more frequently suffered from diabetes (38% vs 6%) and systemic hypertension (81% vs 35%). Serum hsTnT (ng/l) in DSE patients was significantly higher before, 60, 120 and 180min. after the stress test respectively (10,20 vs 6,58 p=0,05; 15,04 vs 6,58 p=0,02; 29,17 vs 7,23 p=0,01; 35,21 vs 9,01 p=0,01). The result did not differ if ExSE and DSE tests in which atropine was used were not considered.

Discussion: The results of study can be influenced by higher risk of DSE group (age, diabetes, hypertension), also demonstrated by higher basal level of hsTnT, which is known risk factor. Extended study will aim for ellimination of these effects and should confirm if higher hsTnT levels are linked to the stress method itself or patients´ risk profile.

Address for correspondence: K. Medilek, Dept. of Cardio-angiology, University Hospital Hradec Kralove, Sokolska 581, Hradec Kralove 500 05, Czech Republic, karel.medilek@fnhk.cz

Title of the project: Pathophysiological mechanisms of diseases – possibilities of prevention, diagnosis, and therapy - year 2016

Grant Agency: Ministry of Education ***Project Number:*** 260287

Principal Investigator: S. Mičuda

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

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 892

Summary of 2016 results

Title of the presentation: Pathophysiological mechanisms of diseases – possibilities of prevention, diagnosis, and therapy - year 2016

Authors: S. Mičuda (1), M. Červinka (2), 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 rationale of the present project was to support scientific activities of postgraduate students at the Theoretical Departments of Faculty of Medicine in Hradec Králové during the year 2016. Finally, 36 postgraduate students and 16 of their mentors have participated to the project. The central theme of the research was to assess the new diagnostic or therapeutic strategies in various diseases and the possibilities of their prevention. The project spanned over preclinical as well as clinical topics with the respect to continuous activities of involved research groups. The preclinical works focused mainly to research of malignancies of different origin and to the pathologies of the heart, and the liver induced by drug administration (e.g. anthracycline cardiotoxicity, and acetaminophen hepatotoxicity) or by special diets (e.g. high-fat diet). Cellular studies were mainly concentrated on the elucidation of cancer cell biology, their regulatory pathways and the effect of new promising chemotherapeutics, but tissue regeneration and role of stem cells was also investigated. The characteristics of different materials for prosthetic devices were studied using biophysical methods. Clinical part of the project has been performed by the epidemiological studies of harmful influence of occupational stress. Toxicological implications of medicinal tar in Goeckerman therapy have been evaluated in patients with psoriasis. Taking together, project supported completion of 17 articles in journals with impact factor and 3 postgraduate students from the team successfully finished their theses in the year 2016.

Project was supported by the Charles University project No. SVV-2016-260287.

Address for correspondence: S. Mičuda, 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: Modulators of mitochondrial enzymes for treatment of neurodegenerative disorders

Grant Agency: Ministry of Health ***Project Number:*** 15-28967A

Principal Investigator: K. Musílek

Co-investigators: Z. Fišar

Starting date: 1.5.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 8134

Summary of 2016 results

Title of the presentation: Modulators of mitochondrial enzymes for treatment of neurodegenerative disorders

Authors: K. Musilek, O. Benek, L. Hroch, R. Dolezal, M. Hrabinova, M. Kunes, Z. Fisar, J. Hroudova, J. Raboch

The mitochondrial enzymes seem to be next target for molecular design in term of Alzheimer Disease (AD) treatment. They are well known for their interaction with β-amyloid and they are subsequently responsible for disruption of cell homeostasis and cell death. The inhibition of β-amyloid interaction with mitochondrial enzymes by small modulators might prevent neuronal cell loss and thus improve progress of AD. Up-todate, only few mitochondrial enzyme modulators were published and their design, synthesis and evaluation will be highly progressive in near future. The main aim of the project is development of convenient candidates (small molecules) for further preclinical research.

Results:

Design, synthesis and in vitro evaluation of benzothiazole-based ureas as potential ABAD/17β-HSD10 modulators for Alzheimer's disease treatment. Hroch L, Benek O, Guest P, Aitken L, Soukup O, Janockova J, Musil K, Dohnal V, Dolezal R, Kuca K, Smith TK, Gunn-Moore F, Musilek K. Bioorg Med Chem Lett. 2016 Aug 1;26(15):3675-8. doi: 10.1016/j.bmcl.2016.05.087.

Progress in drug development for Alzheimer's disease: An overview in relation to mitochondrial energy metabolism. Hroudová J, Singh N, Fišar Z, Ghosh KK. Eur J Med Chem. 2016 Oct 4;121:774-84. doi: 10.1016/j.ejmech.2016.03.084.

Drugs related to monoamine oxidase activity. Fišar Z. Prog Neuropsychopharmacol Biol Psychiatry. 2016 Aug 1;69:112-24. doi: 10.1016/j.pnpbp.2016.02.012.

Pig Brain Mitochondria as a Biological Model for Study of Mitochondrial Respiration. Fišar Z, Hroudová J. Folia Biol (Praha). 2016;62(1):15-25.

Effect of Simvastatin, Coenzyme Q10, Resveratrol, Acetylcysteine and Acetylcarnitine on Mitochondrial Respiration. Fišar Z, Hroudová J, Singh N, Kopřivová A, Macečková D. Folia Biol (Praha). 2016;62(2):53-66.

Address for correspondence: University Hospital, Biomedical Research Center, Sokolska 581, 500 05 Hradec Kralove, Czech Republic; kamil.musilek@gmail.com

Title of the project: Development of new environment friendly insecticides against malaria mosquito

Grant Agency: Ministry of Health ***Project Number:*** 16-34390A

Principal Investigator: K. Musílek

Co-investigators:

Starting date: 1.4.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 6729

Summary of 2016 results

Title of the presentation: Development of new environment friendly insecticides against malaria mosquito

Authors: K. Musilek, D. Jun, J. Korabecny, R. Prymula, R. Dolezal, M. Hrabinova, M. Schmidt, V. Hrabcova, V. Tambor

Use of pesticides as a preventive measure is still the most important element in the integrated management approach to malaria and other vector-borne disease parasitoses. In this project, new insecticides with enhanced selectivity to insects and not toxic to mammals and other animal species will be developed. Twenty new acetylcholinesterase (AChE; EC 3.1.1.7) inhibitors, which will covalently bond to the insect specific cysteine, will be prepared. This cysteine is located at the rim of active side in insect and is mutated to phenylalanine in other species. In this project, the synthesis of novel compounds and the structure-biological activity relationship SAR) evaluation will be performed. Then, selection of the best insecticide candidates and their in vivo safety validation will be carried out and subsequent patent protection before commercialization will be applied.

Address for correspondence: University Hospital, Biomedical Research Center, Sokolska 581, 500 05 Hradec Kralove, Czech Republic; kamil.musilek@gmail.com

Title of the project: Regulation of gene expression in hepatocellular carcinoma - potential role of microRNAs in regulation of cytochrome P450 expression

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-18

Principal Investigator: J. Nekvindová

Co-investigators: A. Mrkvicová

Starting date: 1.2.2016 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation: Altered expression of biotransformation enzymes in hepatocellular carcinoma.

Authors: J. Nekvindová
Inst. for Clin. Biochem. and Diagnostics, University Hospital Hradec Kralove, Czech Republic

Malign transformation of the liver tissue may affect its function. Among others, ability to express biotransformation enzymes can be expected due to e.g. loss of tissue architecture and close cell communication which is required by hepatocytes to maintain their proper functions.
Hepatocellular carcinoma (HCC) is one of the high-rank tumours in terms of worldwide morbidity and mortality, affecting around 750.000 individuals yearly. Only 10-30% patients are estimated to be eligible for surgical treatment, 5-year survival is 15-20%. Most of the patients die because of progressive liver failure. As the liver is the major organ of biotransformation (which includes drug metabolism), we have concerns about the efficiency and safety of pharmacotherapy namely in patients with large liver tumours and impaired liver function caused by underlying liver condition such as cirrhosis, chronic hepatitis, hemochromatosis or NASH. To address these concerns, we have analyzed the expression of major biotransformation enzymes in paired tumour and surrounding non-cancerous tissue from patients with HCC to better understand the changes of their biotransformation capacity.
The study included assessment of a number of genes coding for most important enzymes of both phase I and II of biotransformation at the mRNA (qPCR, TaqMan Low Density Arrays) and protein level (immunoblotting) in 17 HCC patients; and correlation of their expression with clinicopathological characteristics of the tumors and patients. Quantitative PCR analysis revealed a significant down-regulation of expression of most of the genes/enzymes in approximately half of the patients which was confirmed at the protein level. Expression of the cytochromes P450 correlated with expression of their nuclear receptors and clinically with histological grade of the tumors (namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1, CYP3A4, CYP3A5, GSTA1, NAT1, NAT2, SULT1E1, SULT2A1 and UGT1A10 at p=0.05). The findings suggest that patients with large high-grade tumors could be at a higher risk of adverse drug reactions (toxicity) and/or ineffective pharmacotherapy compared to common population, especially for drugs metabolized by cytochromes P450.

Address for correspondence: J. Nekvindova, Institute for Clinical Biochemistry and Diagnostics, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic. Tel.: +420 495 832 767, E-mail: nekvindova@fnhk.cz

Title of the project: Finalization of technical and user properties of the pellets with controlled release of glucose

Grant Agency: Czech Republic ***Project Number:*** TG02010020-1

Principal Investigator: D. Neumann

Co-investigators: A. Franc, J. Muselík, D. Sabadková, I. Růžička

Starting date: 1.11.2015 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 790

Summary of 2016 results

Title of the presentation: Mixed meal influence on palatability and glucose release from pellets with control release of glucose

Authors: D. Neumann (1), A. Franc (2), J. Muselík (2), D. Sabadková (2), I. Růžička (3)
Fac. Med., Charles Univ., Univ. Hospit. Hr. Králové, Dp. of Pediatrics (1), Univ. Veterinar. Pharmaceut. Sci. Brno, Dpt. of Pharmaceutics (2), Univ. Hradec Králové, Hr. Králové, Dpt. of Physid Educ. Sport (3)

Real-life reactions of people with diabetes mellitus type 1 to maintain near normal glycemia need flexible insulin dosing and food with suitable properties. We made effort to create personalized form of glucose with known delay in its release to mimic "snack eaten in advance", which allows to diabetics eat in socially convenient situations. Thus, we developed (2012-14) and clinically tested (2015) pellets with control release of glucose in previous IGA MH CZ and Preseed projects. We introduced 13C-glucose breath test in in-vivo testing and confirmed stable glucose release under different modes of physical activity.

The palatibility and influence of mixed meal were tested in 2016 in group of 11 children. The palatability significantly improved when pellets were incorporated into chocolate bar. The tests pointed dominant impact of chewing, as highly individual activity, on glucose release.

We conclude, that appropriate food form is needed to preserve pharmaceutical properties of the pellets.

Address for correspondence: D. Neumann, Dpt. of Pediatrics, University Hospital Hradec Králové, Sokolská 581, 50005 Hradec Králové, Czech Republic. david.neumann@fnhk.cz

Title of the project: Manipulation and safety device for high wheel walker

Grant Agency: Czech Republic ***Project Number:*** TG02010020-3

Principal Investigator: T. Osladil

Co-investigators:

Starting date: 1.3.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 215

Summary of 2016 results

Title of the presentation: Manipulation and safety device for high wheel walker

Authors: T. Osladil

The device reduces manual effort of medical personnel and makes high walkers more efficient and more secure tool for gait and balance training. The device also can be used alone to facilitate handling of the patient (movements in the bed, in a sitting position to a standing position).

The device is composed from the seat belt and from the solid and elastic straps, which can be attached to any high wheel walker. Elastic suspension serves as a supporting force and as stimulation during training walk. Solid suspension is used to determine the maximum range of motion and reduce the risk of falling.

The device is currently completely ready and after intellectual property protection it will be offered for production. Areas of use includes hospitals, aftercare facilities, rehabilitation departments, nursing homes or homes for the elderly.

Address for correspondence: tomas.osladil@fnhk.cz

Title of the project: International Conferences in Medical Sciences 2016 - 13th International Medical Postgraduate Conference

Grant Agency: Ministry of Education ***Project Number:*** 260290

Principal Investigator: V. Palička

Co-investigators: M. Červinka, E. Rudolf, S. Mičuda, M. Řezáčová, J. Laco, J. Čáp

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 639

Summary of 2016 results

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

Authors: M. Červinka, V. Palička

The 13th International Medical Postgraduate Conference took place in Hradec Kralove on November 24-25, 2016 under the auspices of Rector of the Charles University, Prague. Medical schools across the Europe nominated 34 students of medical doctoral study programmes in biomedicine from 10 European countries (Austria, Georgia, Germany, United Kingdom, Hungary, Poland, Portugal, The Netherlands, Slovak and Czech Republic). International Evaluation Committee was represented by the experts from 8 countries including the President of the Association of Medical Schools in Europe.

Most of presentations were published in the conference proceedings.

We consider this particular meeting of postgraduate students in biomedicine very important from the point of international harmonisation of PhD studies in Europe and for its multidisciplinary spirit. The participants often find that other fields of medicine are interesting and beneficial for them and realize how knowledge of other areas of medicine may be valuable.

The other conference aims were also fulfilled, namely comparison of achieved results and demands on PhD programmes in different countries and on different medical schools. Quality of the presentations of scientific results, meeting of the students and experts from European countries and the opportunity to discuss common problems were deeply appreciated by all participants.

The best three students (according the evaluation of the scientific quality, style of presentation and ability to defend the results in the discussion) received a diploma with financial award, to support their activities in future.

Address for correspondence: V. Palička, Charles University, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 03 Hradec Králové, Czech Republic

Title of the project: Spectroscopy as a diagnostic tool for assaying disease specific molecules

Grant Agency: Ministry of Education ***Project Number:*** IP 2016-2018 64

Principal Investigator: R. Pisal

Co-investigators: H. Hrebíková, J. Chvátalová

Starting date: 7.4.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 115

Summary of 2016 results

Title of the presentation: Spectroscopy as a diagnostic tool for assaying disease specific molecules

Authors: R.V. Pisal (1) , H. Hrebíková (2) , J. Chvátalová (3)

In order to create conditions that allows students to get acquainted with the technique as well as to get hands on experience of performing the test using the instrument we have introduced one small topic in "Stem cell and regenerative medicine" on use of spectrophotometer in clinical diagnostics, this theory lecture will be followed by a practical session where students will get to perform the test and analyze the results using the spectrophotometer.

This approach will generate interest among students as they will get to know a new technique with practical know how.

We have prepared a presentation for students describing the principle, working and application of spectrophotometer. Major focus of the presentation is based on medical application of spectrophotometer.

For detail study a short chapter has been prepared which will help students to understand the instrument and its varied application in the field of medicine.

A demonstrative protocol for measuring hydroxyproline content of decellularized skeletal muscle tissue has been prepared. Protocol has been conceptualized in such a way that each student will be able to perform the assay individually. Initial standardization of the assay has been satisfactorily performed.

Protocol formulation and standardization of cellular toxicity assay has been performed.

Address for correspondence: R V Pisal, Dept. of Histology and Embryology, Charles University Faculty of Medicine in Hradec Kralove, Simkova 870, Hradec Kralove 500 03, Czech Republic.

Title of the project: Study of cardioprotective effects of ACE-inhibitor, dexrazoxane and its novel derivatives against chronic anthracycline cardiotoxicity in rabbits.

Grant Agency: Charles University ***Project Number:*** 680216

Principal Investigator: Z. Pokorná

Co-investigators: P. Brázdová, E. Jirkovský, M. Štěrba

Starting date: 17.3.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 256

Summary of 2016 results

Title of the presentation: Investigation of cardioprotective effects of perindopril against chronic anthracycline cardiotoxicity on experimental model - an introductory phase

Authors: Z. Pokorná, P. Brázdová, E. Jirkovský, M. Štěrba
Fac. Med., Charles Univ., Hr. Králové: Dept. of Pharmacology

Chronic anthracycline (ANT) cardiotoxicity and associated heart failure are feared complication of cancer chemotherapy. Current options for effective protection of the heart against ANT cardiotoxicity remains extremely limited and majority of patients thus undergo the therapy without any protective intervention. ACE inhibitors are cornerstones of current heart failure treatment, including the dysfunction induced by ANTs. Recently, it has been hypothesized that they could be also used in the prevention of development of ANT cardiotoxicity (when administered throughout the chemotherapy). However, it is still unsure whether they could act as real cardioprotectants truly preventing toxicity induction or they just temporally ameliorate the development of the functional/morphological consequences of the damage induced by ANTs. Hence, one of the aims of this project was to evaluate protective effects of ACE-inhibitor perindopril on an experimental model of chronic ANT cardiotoxicity. In the first year of the project, the cardiotoxicity was induced in rabbits in a well-established schedule (daunorubicin 3 mg/kg, weekly for 10 weeks), while control animals received saline in the same schedule. A week after last administration, the surviving animals were randomized for the termination of the experiment or additional 3 week follow up (without any treatment). Furthermore, in the next set of experiments, the effects of perindopril (administered daily to healthy rabbits for 7 days in drinking water) on arterial blood pressure and load-dependent LV functional parameters was examined in clinically relevant doses previously used in literature in rabbits (0.1 and 0.05 mg/kg). While the higher dose induced slight, but significant effects on the examined parameters in heathy rabbits, the lower dose was yet without significant effect. Thereafter, we initiated study of cardioprotective effects of perindopril against chronic ANT cardiotoxicity. The treatment with lower dose of perindopril (0.05 mg/kg, daily in drinking water) started a week before 1st DAU administration and continued throughout the study. This part of the project is expected to be completed in the next year. Supported by GAUK 680216.

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

Title of the project: Testing of new anticancer therapeutics based on low-molecular-weight inhibitors of DNA-PK

Grant Agency: Charles University ***Project Number:*** 932516

Principal Investigator: M. Pospíšilová

Co-investigators: K. Habartová, M. Seifrtová, M. Řezáčová

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 247

Summary of 2016 results

Title of the presentation: Testing of new anticancer therapeutics based on low-molecular-weight inhibitors of DNA-PK

Authors: Monika Pospíšilová (1), Martina Seifrtová (1), Martin Andrš (2), Jan Korábečný (2), Martina Řezáčová (1)

Dept. of Medical Biochemistry, Fac. Med., Charles Univ., Hr. Králové (1); Biomedical Research Center, Univ. Hospital Hr. Králové (2)

The most sensitive cell structure – a DNA molecule, is the common target of cancer therapy. DNA damage response (controlled by enzymes of phosphatidylinositol 3-kinase-related kinases family, PIKK) presents many encouraging targets for improving both conventional cytotoxic anticancer therapy and individualized monotherapy. DNA-PK is a member of PIKK superfamily and plays an important role in the detection and repair of DNA double-strand breaks via the non homologous end joining pathway. The ability of cancer cells to repair DNA damage is an important element determining their sensitivity to radio- or chemo-therapy. The overactivation of DNA-PK in cancers can result in resistance for anticancer therapy. The inhibition of DNA-PK is a very promising target in the anticancer research. The aim of this study was to analyse chemo-sensitising properties of 18 newly synthetized DNA-PK inhibitors in combination with 0,5 μM doxorubicin. The human colorectal adenocarcinoma cell line HT-29 was exposed to these compounds at interval of 48 hours, and then was the viability tested. All inhibitors themselves have no anti-proliferative effect on HT-29 cell line. Percentage of viable cell varied from 76 - 114 %. However, most of the inhibitors showed chemo-sensitising properties, especially the inhibitors A16 (10 % of cells remained viable) and A17 (8 % of cells remained viable). It is possible to conclude that inhibition of DNA-PK with the new synthetized low molecular-weight inhibitors has chemo-sensitising effect on HT-29 cells and may have therapeutic potential for the treatment of patients with chemo-resistant metastatic colon cancer.

Address for correspondence: M. Pospíšilová, Dept. of Medical Biochemistry, Charles University in Prague, Faculty of Medicine in Hradec Králové, Zborovská 2089, 500 03 Hradec Králové, Czech Republic

Title of the project: Rational design of novel immunomodulators - potential vaccine adjuvans - based on TLR4 ligands

Grant Agency: Czech Republic ***Project Number:*** 15-11776S

Principal Investigator: R. Prymula

Co-investigators: J. Honegr, D. Maliňák, R. Doležal, S. Salajková

Starting date: 1.1.2015 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 3405

Summary of 2016 results

Title of the presentation: Rational design of novel immunomodulators based on TLR4 ligands

Authors: R. Prymula, J. Honegr, D. Malinak, R. Dolezal, S. Salajkova, Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic,

We have undergone in-silico virtual high throughput screening (vHTPS) of 10000 compounds, utilizing the resources of Czech national supercomputing center. 9700 compounds was chosen from Zinc database (a free database of more than 2 000 0000 commercially available drug-like compounds for virtual screening), 200 compounds was chosen from our database of compounds and 100 compounds were specifically designed based on results from previously published works. For narrowing the broad spectrum of available compounds from Zinc database the open source software Screening Assistant 2 was employed. 10000 compounds were subsequently docked into the active spot of TLR4 and bonding energy for each compound were calculated.

From this vHTPS screening we have obtained 60 compounds with high bonding energy to the receptor TLR4. From these sixty compounds obtained from vHTPS we have selected 10 compounds are suitable for alteration of their structures. Some of the compounds were synthesized, some were purchased for evaluation of their in vitro activity on cell line stably expressing human TLR4 receptor. From those compounds we have chosen two lead structures (based both on their physic-chemical properties and on their internal activity). We have designed two batches of ten derivatives of those two lead compounds (total 20 compounds) using basic principles of medicinal chemistry to find more suitable drug candidates (taking into account solubility, π-π interactions, etc) that could act as potential immunomodulators on the innate immunity system. We have found some potentially interesting compounds, that shows in-vitro 50-70% activity of MPLA. In 2016 we have collected PBMC cells from donors, and tested ability of our compounds to elicit production of IL- 2, 6 and 8. We have been able to prove that some of our compounds are able to elicit IL6 and IL8 production. Preliminary data shows that in the first batch of compounds there is some corelation between hTLR4 activity on reporter cell line and production of Interleukin 6 ex vivo on PBMCs. In second batch of compounds we have found no such correlation up to date. We will continue to evaluate ILs production in 2017 and the best compounds will be tested in vivo.

Address for correspondence: R. Prymula Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic,

Title of the project: New methods and approaches in diagnostics and search for predictive and prognostic markers in neoplastic disorders

Grant Agency: Ministry of Education ***Project Number:*** 260289

Principal Investigator: A. Ryška

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

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 635

Summary of 2016 results

Title of the presentation: New methods and approaches in diagnostics and search for predictive and prognostic markers in neoplastic disorders

Authors: Ryška A., Buchta V., Krajina A., Krejsek J., Palička V.

Malignant neoplasms are second major cause of mortality and one of the major causes of morbidity in developed countries. Recently, a dramatic and fast development in understanding of etiology, pathogenesis and molecular mechanisms of malignant neoplasms results in emerging new therapeutic options for many oncologic patients.

For selection of optimal treatment, it is essential not only precise diagnosis encompasing correct typing, grading and staging of the neoplasm. Today, so called molecular rating (identification of specific molecular profile) is an integral part of correct diagnosis. In addition, our better understanding of heterogeneity and plasticity of tumors is crucial. In the recent project, we have focused on search for predictive and prognostic markers from different points of view (imaging, biochemical markers, histologic features, immunologic data, interaction of neoplastic population with the host, etc.). There were altogether 29 individual sub-projects, including among others: GC-MS and HPLC- MS study of new xenobiotic substances abused in toxicomania, changes in tissue perfusion during the perioperative phase of heart surgery, immunopathogenesis of Sjögren syndrome, immunological response in patients after heart surgery; study of intestinal immune system - influence of intestinal microbiome, study of metylation of tumorsupressor genes in hepatocelular carcinoma, molecular subtyping of lung adenocarcinoma, study of serrated lesions of the large intestine, molecular study of colorectal carcinoma cells in vitro, immunophenotypical analysis of atrial amyloid in the heart with focus on IAA form, use of intraarticular synovial haemorrhage sign as an indicator of death due to hypothermia, study of microbial flora shared betweeen humans and their domestic pets (cats, dogs), biochemical analysis of bone in patients with femoral neck fracture, changes of nucleic acids and relative expression of miRNA in sinonasal carcinoma, molecular changes in bone in different metabolic disorders - study in rats, experimental animal study of effect of intestinal microbiome on mucosal immune system, nanoparticles and their interaction with immune system.

Altogether, 6 fulltext papers were published (3 in a journal with IF, cummulative IF 7,92), several additional manuscript are currently under the peer review, 4 doctoral theses are to be defended within next 6 months.

Address for correspondence: Prof. Aleš Ryška, MD, Ph.D.; The Fingerland Department of Pathology; Charles University Medical Faculty Hospital; CZ-500 05 Hradec Králové; CZECH REPUBLIC; ales.ryska@fnhk.cz

Title of the project: Biobanking and Biomolecular Resources Research Infrastructure (BBMRI)

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

Principal Investigator: D. Valík

Co-investigators: A. Ryška, V. Palička, J. Laco, R. Kutová

Starting date: 1.1.2011 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 2900

Summary of 2016 results

Title of the presentation: The cryopreservation of paired tumor and blood samples from patients with selected malignant tumors

Authors: Laco, J., Ryška, A., Kutová, R., Palička, V.

The rapid development of precision of modern histopathological diagnostics of neoplasms and introduction of new molecular markers into routine decision-making in therapy of cancer, there is an increased need for preserved tumorous tissues for future testing. With the means of cryopreservation, i.e. a process where tissues are archived by deep freezing, thereby stopping any biological activity, including cell death, DNA, RNA and proteins are kept intact for further biological and medical research. In this context, the preservation of malignant tumor samples as well as of specimens of patients' serum represents an optimal way of preservation and collection of specimens of extremely valuable biological material and is therefore of utmost importance and interest for both the clinicians and experts in laboratory medicine. During 2016, both participating laboratory departments, i.e. The Fingerland Department of Pathology and Institute of Clinical Biochemistry and Diagnostics, collected, transported and stored both patients´ blood samples and their derivates (serum, plasma, isolated nucleic acids) and fresh tumor tissue samples in a routine manner. According to individual protocols for each tumor diagnosis with defined periods of blood sampling for cryopreservation during the course of the disease and/or during the treatment, the blood samples were collected. Close cooperation with clinical departments is established and routine collection of samples of tumor tissue specimens and of blood serum samples is also performed routinely in selected diagnoses (breast carcinoma, colorectal carcinoma, hepatocellular carcinoma, malignant tumors of head and neck (mainly oropharyngeal and laryngeal carcinomas), gynaecological neoplasms (particularly ovarian carcinomas and borderline tumors) and tumors of the CNS). The laboratories are equipped with consumables for isolation of DNA from both tumor tissue and blood.

During the duration of the entire project, there were collected in total 897 blood/serum samples from 525 patients and 1355 tumor tissue samples. These samples are used both in diagnostic process as well as for various scientific projects. The developed infrastructure will serve as a basis for introduction of additional research projects in the future.

Address for correspondence: Prof. Aleš Ryška, MD, Ph.D.; The Fingerland Department of Pathology; Charles University Medical Faculty Hospital; CZ-500 05 Hradec Králové; CZECH REPUBLIC; ales.ryska@fnhk.cz

Title of the project: Detection of early chemotherapy-induced cardiotoxicity using adwanced echocardiography, proteins cardiomarkers and circulating mikroRNAs

Grant Agency: Charles University ***Project Number:*** 814316

Principal Investigator: M. Skála

Co-investigators: R. Pudil, P. Matoušková, A. Vernerová

Starting date: 8.3.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 219

Summary of 2016 results

Title of the presentation: MicroRNAs as possible sensitive markers for early detection heart tisue damage caused by chemotherapy

Authors: Mikuláš Skála (1), Petra Matoušková (2), Andrea Vernerová (3), Radek Pudil (4)
1st Dept. of Internal Medicine - Cardioangiology, University Hospital Hradec Kralove and Medical Faculty Hradec Králové, Charles University Prague (1,4), Faculty of Pharmacy in Hradec Králové, Charles University Prague, dept. of Biochemical Sciences (2,3)

Cardiotoxicity represents one of the undesirable effects of the treatment of cancer diseases which can negatively affect the patient's life. For that reason, early detection of cardiotoxicity has great importance for its treatment or for modification of cancer therapy. Classical methods for cardiotoxicity detection, which is based on the evaluation of decrease of ejection fraction of left ventricle, are inconvenient because the observed changes are often irreversible. In our study, systolic and diastolic function of both ventricles are evaluated using the advanced echocardiography. Simultaneously, the levels of protein markers of structural and functional damage of myocardial tissue (hsTnT a NT-proBNP) are analyzed in plasma of patients. In addition, the profile of selected circulating micro RNAs in plasma is monitored with aim to find the new markers of early subclinical cardiotoxicity. We just selected some particular miRNAs which can be specific for mycardial damage (miR-208a, miR-208b, miR-367, miR-1, mir-135b, miR-499). For their detection we designed a specific stem-loop primer for reverse transcription and specific forward primer for qPCR assessment. We tested our method for quantification of selected microRNAs in vivo in mice treated with cytostatic drug doxorubicin and preliminary results obtained seem to be promising. However first patients are ranking among to our study and first results are expected next year.

Address for correspondence: MUDr. Mikuláš Skála, 1st Department of Internal Medicine - Cardioangiology, Faculty Hospital Hradec Králové, Sokolská 581, Hradec Králové, 500 05

Title of the project: Development of novel disinfectants against pathogens occuring in the hospital environment

Grant Agency: Ministry of Health ***Project Number:*** 15-31847A

Principal Investigator: O. Soukup

Co-investigators: P. Boštík

Starting date: 1.5.2015 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 7490

Summary of 2016 results

Title of the presentation: Development of novel disinfectants against pathogens occuring in the hospital environment

Authors: Ondrej Soukup (1), David Malinak (1), Jan Korabecny (1), Roman Prymula (1), Lenka Hobzova (1), Lenka Ryskova (1), Pavel Bostik (2), Vanda Bostikova (2), Daniel Jun (2), Jan Marek (2)

Biomedical Research Center, University Hospital Hradec Kralove (1), Faculty of Military Health Sciences, University of Defense (2)

In this project, we would like to develop new compounds based on quaternary ammonium salts with a strong disinfectant potential against nosocomial infections in hospital environment, thus bacterial, fungal and viral pathogens. The project is designed for development of various (3-6) mixtures with strong disinfecting properties and wide spectrum of efficacy by combining individual agents with more specific efficacy. So far during the project solution, 66 analogues of quaternary ammonium salts (QAS) containing quaternary nitrogen and carbon chain of C12, C14, and C16 was prepared by the chemical synthesis. According to the literature and our preliminary data, QAS were found to be effective against both bacteria and fungi. Furthermore, QAS were reported to be effective also against both encapsulated and non-encapsulated viruses. Such findings were confirmed by our pivotal in vitro screening using standard benzalkonium QAS and basic battery of microbes (S. aureus, E. coli, Cl. dificile C. albicans and VZ virus), which serves for the selection of effective concentration for future testing. During the second year in vitro testing has been launched. We have evaluated approximatelly 2/3 of compoounds agains Gramm positive, Gramm negative and anaerobic bacteria and VZ virus. We found out that some of the compounds are better antibacteril agents that standard benzalkonium QAS. The activity against viruses was only weak and only at high concentrations, which were often limited by the solubility of novel drugs. No drug was able to reduce the concentration of the virus by 4 logs as requested by the guidelines. Also, limited activity agains C. difile was found. At high concentrations, some compound showed an interesting effect, at least comparable to standard benzalkonium salts. In the next year testing will continue on fungi and after sumarizing all data, wide-spectrum mixtures will be prepared and tested.

Address for correspondence: O. Soukup, Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, Hradec Kralove 500 05, Czech Republic

Title of the project: Novel hybrid compounds in the cognitive decline caused by neurodegenerationt

Grant Agency: Czech Republic ***Project Number:*** 16-08554S

Principal Investigator: M. Horák

Co-investigators: O. Soukup, J. Říčný

Starting date: 1.1.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 9970

Summary of 2016 results

Title of the presentation: Novel hybrid compounds in the cognitive decline caused by neurodegenerationt

Authors: Ondrej Soukup (1), Jan Korabecny (1), Eugenie Nepovímova (1), Martin Horak (2), Martina Kaniakova (2), Jan Říčný (3), Karel Valeš (3)

Biomedical Research Center, University Hospital Hradec Kralove (1), Insitute of Physology, Czech Academy of Science (2), National Institute of Mental Health (3)

This project follow-up with ending 5-year project focused on hybrid compounds. Based on preliminary data, we would like to deeply investigate newly designed hybrids combining AChEI pharmacophore and NMDA antagonist pharmacophore in a single molecule. We hypothesize that simultaneous inhibition of AChE and NMDA receptors results in amelioration of the cognitive decline associated with neurodegeneration. Using in vitro pharmacological/toxicological profile screening of newly prepared compounds and deep characterization of NMDA and AChE interaction, one candidate will be selected. Toxicity, bioavailability and dosing scheme will be assessed. Subsequently, the compound will undergo in vivo behavioral validation of pro-cognitive properties with subsequent ex vivo biochemical assessment of underlying mechanisms of action. Altogether, the pro-cognitive therapeutic value of such hybrid compounds will be determined.

Address for correspondence: O. Soukup, Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, Hradec Kralove 500 05, Czech Republic

Title of the project: Change of microRNA profile in perifheral blood after methotrexate treatment in patients with rheumatoid arthritis

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-13

Principal Investigator: T. Soukup

Co-investigators: M. Doseděl, J. Nekvindová, P. Bradna, I. Barvík, P. Pávek

Starting date: 1.11.2015 ***Duration (years):*** 1

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

Summary of 2016 results

Title of the presentation:

Change of microRNA profile in perifheral blood after intravenous high dose methylprednisolon treatment in DMARDs naive patients with rheumatoid arthritis

Authors: T. Soukup (1), T. Smutný (2), M. Doseděl (3), J. Nekvindová (4), P. Bradna (1), J.D. Tebbens (2), I. Barvík (5), P. Pávek (2).

Charles Univ., Fac. Med., 2nd Dpt. of Int. Med. GE (1), Fac. of Pharmacy, Dpt. Pharm. Tox. (2), Dpt. of. Soc. and Clin. Pharmacy (3), Fac. Hospital. HK, Inst. Clin. Bioch Dg. (4), Fac. Math and Physic (5)

Background: The microRNA (miR) profile is changed in pathogenetic process of autoimmune diseases and their treatment. The intense research in this area may help to understand antiinflammatory drugs mechanism of action.

Objectives: To identify change of circulating miRs profile in disease modifying antirheumatic drugs naïve patients with rheumatoid arthritis (RA) after i.v. methylprednisolon.

Materials and Methods: There were 7 RA patients with very high clinical activity, 5 RA patients achieving clinical remission enrolled in study. Patients in groups with high clinical activity were exposed to 125mg methylprednisolone i.v., for 5 consecutive days. All patients were subjected to miR microarray analysis and changes in expression profiles were recorded. Using Taqman qPCR, we found activation or suppression of miRs and then we predicted targets microRNA searching in medical databases.

Results: Compared to the pre- and post-glucocorticoid treated RA group, all of the 381 miRs sequences were examined, 1 miR was significantly upregulated and 6 downregulated in the post-treated samples. MiR-149 was the most upregulated (22 fold). MiR-422a, -193a-5p, -125a-3p, -218, -423-5p and -629 were downregulated. When the expression of the miRs in the RA active group was compared to unrelated RA remission group, 6 (miR-548, -579, -483-5p, -125a-3p and 193a-5) showed significant upregulation. In comparison pre- vs. post-tratment, 2 miRs (miR-125a-3p and -193a-5p) of them shared the oposite trend in active vs. inactive RA comparison. These miR were excluded from analysis of miR related to glucocorticoid influence. Further bioinformatic analysis revealed glucocorticoid-related genes, targeted by miRs found.

Conclusions: We demonstrate a link between miRs expression and the effects of methylprednisolone and provide searching genes, which suggests that methylprednisolone acts.

Address for correspondence: Tomas Soukup M.D., Ph.D. 2nd Department of Internal Medicine - Gastroenterology and Rheumatology, University Hospital Hradec Kralove, Sokolska 581, Hradec Kralove, Czech Republic, 500 05. Phone 49 583 4762. e-mail: soukutom@fnhk.cz

Title of the project: Use of Narrow Band Imaging in the diagnosis of mucosal head and neck cancer

Grant Agency: Ministry of Education ***Project Number:*** IP 2016-2018 65

Principal Investigator: J. Šatanková

Co-investigators: K. Smatanová, V. Chrobok

Starting date: 6.4.2016 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 338

Summary of 2016 results

Title of the presentation: Pre-operative diagnostic value of NBI in visualization of laryngeal lesions

Authors: J.Šatanková, V. Chrobok
Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Kralove, Charles University, Faculty of Medicine in Hradec Kralove, Czech republic

NBI (Narrow Band Imaging) is an optical endoscopic method that helps to evaluate pathological neovascularization in early stage of cancerogenesis. It uses a specially filtered light (blue 415 nm and green 540 nm) and this technique is based on the fact that the depth of penetration of light is dependent on its wavelength. These filtered wavelengths penetrate, respectively, the epithelium, thus highlighting the capillary network, and the deeper levels, enhancing the subepithelial vessels. In this way, superficial mucosal lesions that would be missed by standard white light endoscopy, are better identified in view of their neoangiogenic pattern. The method is used ín otorhinolaryngology in the outpatient setting as a screening method, or in the follow-up of patients with a history of cancer treatment (surgical/oncological or combined), as well as during surgical procedures for targeting biopsies and for precise identification of the resection margins during cancer surgery. It is a relatively new method in diagnosis early stage of head and neck cancer .

So this is the reason why we decided to extend the knowledge of students of General Medicine and Dentistry (the 5th year), especially in the field of otorhinolaryngology, to improve teaching in our department. The aim of this project is to create videos with the real findings of tumors (laryngeal, orpharyngeal, hypopharyngeal) in patients who were examined by NBI method. In this time we have completed CD with real videos of 30 laryngeal tumors (benign and malignant). Each video has two parts, the first one is finding in conventional white light endoscopy and the second one in NBI mode. The records will be available not only for students but also for residents to prepare for specialization in ENT. The records are available at our department in local store - Dicompass. The second aim of this project is to improve E-learning section with the inclusion of chapter about using NBI endoscopic method in the diagnosis of head and neck cancer.

Address for correspondence: J. Šatanková, Department of otorhinolaryngology and Head and Neck surgery, University Hospital Hradec Kralove, Charles University, Faculty of Medicine in Hradec Kralove, Czech republic.

Title of the project: Impact of functional polymorphisms influencing inflammation and oxidative stress on outcome and selection of treatment in chronic lymphocytic leukemia

Grant Agency: Ministry of Health ***Project Number:*** 16-32339A

Principal Investigator: T. Papajík

Co-investigators: M. Šimkovič, E. Kriegová, V. Snášel, Y. Brychtová, J. Molinský

Starting date: 1.4.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 20958

Summary of 2016 results

Title of the presentation: Impact of functional polymorphisms influencing inflammation and oxidative stress on outcome and selection of treatment in chronic lymphocytic leukemia

Authors: MUDr. Martin Šimkovič, Ph.D, Dr. Ing. Eva Kriegová, prof. RNDr. Václav Snášel, CSc., MUDr. Yvona Brychtová, Ph.D., MUDr. Jan Molinský, Ph.D.

Chronic lymphocytic leukemia (CLL) is the most common form of leukemia in western countries. Early relaps and refractoriness to initial therapy are associated with poor prognosis and low life-expectancy. Identification of patients with high risk of relapsed/refractory disease may stratify them for on-time personalised treatment using novel BCR-signaling inhibitors and BCL2 antagonists. We aim therefore to study a comprehensive panel of functional polymorphisms influencing the extent/burden of inflammatory reaction and oxidative stress in order to identify risk haplotypes associated with relaps/refractoriness. Moreover, we will develop an electronic tool for estimation of a relapse/refractoriness risk by integrating big data from individual "imunogenetic" signature, somatic genetic variability, clonal heterogeneity and other prognostic markers. Identification of patients with high risk of early relaps/refractoriness may contribute to selection of personalised treatment strategy, thus to improve the prognosis, life-expectancy and lower the treatment costs for these patients.

Aims: To identify "immunogenetic signature" associated with risk of early relaps and refractoriness in chronic lymphocytic leukemia. To develop an electronic application for identification of CLL patients with relaps/refractoriness risk who may profit from on-time personalised treatment strategy.

During 2016 we started the enrolment of patients (continuing till 6/2019), processing of biological material, optimization of genotyping of a comprehensive panel of polymorphisms associated with oxidative stress, collection of clinical and demographic data.

Address for correspondence: Martin Šimkovič, M.D., Ph.D., Charles University and Medical School, 4-th Clinic of Internal Medicine, Haematology Dept., Sokolska street 581, Hradec Kralove 5, 500 05, Czech Republic; martin.simkovic@fnhk.cz

Title of the project: Microscopical analysis of cells, extracellular matrix a new bone formation after MSCs implantation into a bone defect in animal experiment

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-12

Principal Investigator: L. Školoudík

Co-investigators: J. Mokrý, S. Filip, V. Chrobok

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 95

Summary of 2016 results

Title of the presentation: Microscopical analysis of cells, extracellular matrix a new bone formation after MSCs implantation into a bone defect in animal experiment

Authors: L. Školoudík, J. Mokrý, S. Filip, V. Chrobok

Canal wall down mastoidectomy is one of the most effective treatments for chronic otitis media with cholesteatoma. However, it results in anatomical changes in the external and middle ear with negative impact on patient´s quality of life. To provide complete closure of mastoid cavity and normalize the anatomy of the middle and external ear we used human multipotent mesenchymal stromal cells (MSCs), GMP Grade, in a guinea pig model. A method for preparing a biomaterial composed of hMSCs, hydroxyapetite and tissue glue was developed. Animals from a treated group were implanted with biomaterial composed of hydroxyapatite and MSCs, while animals in a control group received hydroxyapatite alone. The group implanted with MSCs showed significantly higher ratio of new bone formation (p=0,00174) and also, the volume percentage of new immature bone was significantly higher (p=0,00166). Angiogenesis was observed in both groups. In experimental group the mean number of small blood vessel lumina was higher but the difference was not significant using 95% confidence interval (p=0,08092). Imunohistochemical examination proved statisticaly signifficant higher distribution of CD3-positive T-lymphocytes in the experimental group (p=0,00368). We observed TRAP-positive cells as the macrophage lineage in all specimens. Migration of T-lymphocytes and macrophages could be the parakrine effect of MSCs that leads to facilitating the osteoin both groups of temporal bones. Inner ear histology in all specimens observed vital neuroepithelium without any abnormalities of inner ear.

Our results proved beneficial effect of MSCs on temporal bone formation and provided a promise to improve the quality of life of patients after canal wall down mastoidectomy by MSCs implantation.

Address for correspondence: L. Školoudík, Department of Otorhinolaryngology and Head and Neck Surgery, University Hospital Hradec Králové, Charles University, Faculty of Medicine in Hradec Králové, Czech Republic

Title of the project: Innovation of cryopreservation method for clinical use of autologous multipotent mesenchymal stem cells in treatment of extensive skeletal defects during revision surgery of total hip arthroplasty

Grant Agency: Czech Republic ***Project Number:*** TG02010020-4

Principal Investigator: P. Šponer

Co-investigators: T. Kučera, S. Filip, P. Měřička

Starting date: 22.2.2016 ***Duration (years):*** 2

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

Summary of 2016 results

Title of the presentation: Clinical use of autologous multipotent mesenchymal stem cells in treatment of skeletal defects during revision surgery of total hip arthroplasty.

Authors: P.Šponer - 1 , T. Kučera - 1, S. Filip - 2, P. Měřička - 3
1 - Dep. of Orthop.Surgery, University Hospital in Hradec Králové
2 - Dep. of Oncology and Radiotherapy, University Hospital in Hradec Králové
3 - Tissue Bank, University Hospital in Hradec Králové

The benefit of the use of mesenchymal stromal cells on β-trikalcium phosphate was found in the healing of the bone defects compared with the use of β-trikalcium phosphate alone in our previous clinical trial (protocol AMSC-BDT-001 - EudraCT number 2012-005599-33). Following the Local Ethics Committee and the State Institute for Drug Control approval, the new clinical study was prepared (protocol AMSC-BDT-002 - EudraCT number 2016-000926-21). When the local situation around the bone defect is jeopardized and impaired, the use of tissue engineered implants should improve the biological potential to allow bone repair. With respect to the recently designated pentaconcept, we will investigate the healing of bone defects after the application of in vitro expanded MSCs combined with ultraporous β-tricalcium phosphate synthetic graft material within 12-month follow-up period in 2017 and contemporary we will study the autologous multipotent mesenchymal stem cells after cryopreservation (without their clinical application).

Address for correspondence: Pavel Šponer, Department of Orthopaedic Surgery, Sokolská 581, Hradec Králové

Title of the project: Vulnerability and reparation possibility of peroperative vas deferens damage in experiment

Grant Agency: Charles University ***Project Number:*** 160315

Principal Investigator: R. Štichhauer

Co-investigators: M. Kaška, J. Koudelka, A. Ryška, K.Petkov, V. Krejčí, T.Čížová

Starting date: 17.4.2015 ***Duration (years):*** 2

Total funds allocated for project - Kč (thousands): 186

Summary of 2016 results

Title of the presentation: Possibilities of lesional vas deferens repair in the experiment

Authors: R. Štichhauer, J. Koudelka, A. Ryška, T.Čížová, M. Kaška

Damage of vas deferens during a surgery of inguinal hernia is one of the most frequent iatrogenic injury in pediatric surgery. There is no guide-line for treatment of that injury. The main idea of our experimental study is a creation of some useful algorithm for injured vas deferens reparation under the conditions of basic paediatric surgery departments with the use of magnifying glasses only. We decided for a prospective experimental study, which was performed on the rat. 70 animals were included into the study and they were divided in seven subgroups according to the method of the vas deferens injury: 1. contusion of the vas deferens by pressing in a pean for 2 sec, 2. anastomosis of the vas deferens by single absorbable stitches (Vicryl R 8/0), 3. joining of both ends each to other with the help of an fibre of absorbable sewing material (PDS 7/0) situated intraluminally, 4. = 3. joining with a non-absorbable fibre of sewing material (Prolen 7/0), 5. anastomosis by absorbable sewing material (Vicryl R 8/0) with a help of the fibre of absorbable sewing material (PDS 7/0) situated inrtraluminally and fixated extraluminally, 6. = 5. but with a use of the fibre of non-absorbable sewing material (Prolen 7/0), 7 = 5. without fixation of the fibre. The vas deferens was checked 3 months after the primary operation and than resected in 20mm lenght with a place of artificial injury. These resected parts of the vas deferens were examined in function by the flow rate of methylene blue solution (μL/min). The pathologist after that performed morphologic evaluation of the resected vas deferens. Findings on the injured vas deferens were compared with those on the second side without surgery. We found a normal liquid flow rate through the resected part of the vas deferens and morphological conditions in this subgroup with the contused vas deferens. The best results in flow rate through the transected vas deferens were evaluated in the subgroup 3. with reparation performed by joining the transected vas deferens with the help of absorbable sewing material. Similar results were found in the subgroups 5. and 7. with use of absorbable sewing material and with absorbable stitch fibre intraluminally aplied.

Address for correspondence: R. Štichhauer, Dept. of Pediatric Surgery, Faculty Hospital, Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove, Czech Republic. e-mail: stiradek@centrum.cz

Title of the project: Interventional left atrial appendage closure vs novel anticoagulation agents in patients with atrial fibrillation indicated for long-term anticoagulation (PRAGUE-17 study)

Grant Agency: Ministry of Health ***Project Number:*** 15-29565A

Principal Investigator: J. Šťásek

Co-investigators: J. Bis, L. Haman

Starting date: 1.5.2015 ***Duration (years):*** 5

Total funds allocated for project - Kč (thousands): 2414

Summary of 2016 results

Title of the presentation: Interventional left atrial appendage closure vs novel anticoagulation agents in patients with atrial fibrillation indicated for long-term anticoagulation (PRAGUE-17 study)

Authors: J. Šťásek, J. Bis, L. Haman

Fac.Med. Charles Univ., Hr. Králové: Dept. of Internal Medicine

Background: Atrial fibrillation (AF), with a prevalence of 1% to 3%, is the most common cardiac arrhythmia. Without antithrombotic treatment, the annual risk of a cardioembolic event is 5% to 6%. The source of a cardioembolic event is a thrombus, which is usually formed in the left atrial appendage (LAA). Prevention of cardioembolic events involves treatment with anticoagulant drugs: either vitamin K antagonists or, novel oral anticoagulants (NOAC). The other nonpharmacologic option for the prevention of a cardioembolic event involves interventional occlusion of the LAA.

Objective: To determine whether percutaneous LAA occlusion is noninferior to treatment with NOAC in AF patients indicated for long-term systemic anticoagulation.

Study design: The trial is a prospective, multicenter, randomized noninferiority trial comparing 2 treatment strategies in moderate to high-risk AF patients (ie, patients with history of significant bleeding, or history of cardiovascular event(s), or a with CHA2DS2VASc ≥3 and HAS-BLED score ≥2). Patients are randomized into a percutaneous LAA occlusion (group A) or a NOAC treatment (group B) in a 1:1 ratio; the randomization is done using web-based randomization software. A total of 396 study participants (198 patients in each group) is planned to be enrolled in the study. The primary end point will be the occurrence of any of the following events within 24 months after randomization: stroke or transient ischemic attack (any type), systemic cardioembolic event, clinically significant bleeding, cardiovascular death, or a significant periprocedural or device-related complications.

Results: 134 patients were included to the study till the end of 2016 in 10 centers. In our center 19 patients were randomized in 2016, 9 to the LAA occlusion group, 10 to the apixaban group. In all patients from the occlusion group was procedure completed successfully, one more implantation was done in patient randomized in 2015. In one patient from the apixaban group is planned crossover to the occlusion group because of minor bleeding on anticoagulation treatment.

Address for correspondence: J. Šťásek, Dept. of Internal Medicine, Charles University in Prague, Faculty of Medicine in Hradec Králové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: The comparison of the efficiency of colon capsule endoscopy and optical colonoscopy in patients

Grant Agency: Ministry of Health ***Project Number:*** 16-29614A

Principal Investigator: Š. Suchánek

Co-investigators: I. Tachecí, J. Špičák, O. Málek

Starting date: 1.4.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 14708

Summary of 2016 results

Title of the presentation: The comparison of the efficiency of colon capsule endoscopy and optical colonoscopy

Authors: Tacheci I.

In the Czech Republic, there is one of the highest colorectal cancer (CRC) incidence and mortality worldwide. In 2000, the National CRC screening program was introduced, focusing on individuals at average CRC risk older than 50 years. The program is based on the immunochemical fecal occult blood tests (FIT) and/or colonoscopy. The main problems are limited coverage of the target population (fear of colonoscopy procedure) and colonoscopy capacity. The solution can be based on modern minimally invasive test (colon capsule endoscopy - CCE) incorporated into the program as filter test between the FIT and colonoscopy or as alternative for individuals unwilling or unable to undergo the usual program procedures. The objective of the project is to show that the negative predictive value of the CCE applied in patients with positive result of FIT is sufficient (> 85 %) to safely spare the patients the optical colonoscopy examination. 230 individuals with average colorectal cancer risk (asymptomatic, aged 50 – 75 years, with negative personal medical history of CRC and adenomas and negative family history of CRC) will be included. All individuals will be invited to the study after the positive semiquantitative FIT performed within the regular National CRC screening program. All patients included in the study will have CCE afterwards, followed by optical colonoscopy. We started the methodical, material and software preparation at all participating centres and target group identification during the first year of the project.

Address for correspondence: MUDr. Ilja Tachecí, Ph.D., 2nd Dpt of Internal Medicine – Gastroenterology, University Hospital and Charles University Faculty of Medicine, Sokolská 581, Hradec Králové

Title of the project: Role of oxidative stress in the interplay between cellular senescence and apoptosis

Grant Agency: Czech Republic ***Project Number:*** 15-03379S

Principal Investigator: Z. Hodný

Co-investigators: V. Tambor, K. Pimková, B. Šalovská

Starting date: 1.1.2015 ***Duration (years):*** 3

Total funds allocated for project - Kč (thousands): 8832

Summary of 2016 results

Title of the presentation: Development of a redox proteomics quantitative assay for a cellular senescence and apoptosis model

Authors: V. Tambor (1), K. Pimková (1), B. Šalovská (2), Z. Hodný (2)

Biomedical Research Center, University Hospital Hradec Kralove (1), Institute of Molecular Gentetics, Academy of Sciences Czech Republic (2)

Cellular senescence, an essentially permanent cell cycle arrest, represents an essential anticancer barrier. Yet there is evidence that accumulation of senescent cells in normal and cancer tissues can promote proinflammatory milieu, which may result in enhancement of tumorigenic or metastatic potential of tumour cells. Besides mitochondrial energy metabolism, NADPH oxidases, the key enzymes involved in redox signalling, represent a second major cellular source of free oxygen and nitrogen radicals. Uncontrolled activity of these enzymes play an important role in several pathological processes associated with onset of cancer. The current project aims at elucidating the redox balance in a cellular model of senescence using proteomics by assessing reduced vs. oxidized protein forms at a whole-proteome level. In order to distinguish native reduced protein forms, free thiol groups are tagged using a particular flavor of iodo tandem mass tags labels (Thermo Fisher Scientific, Germany). The oxidized cysteine residues are in turn reduced in vitro and the newly formed thiol groups are then tagged by another variant of the iodoTMT labels. The sample is then analyzed using liquid LCMS in order to obtain information on a) the protein content of the sample and b) the ratio between the reduced and oxidized forms of individual proteins. During the second year, we focused at method optimization. Our results show that we have increased the cysteine peptide coverage ~two fold from 2600 peptides to more than 5500. This provides sufficient coverage for subsequent evaluation of the redox changes ocurring in the celluar senescence model. Currently, work is in progress in preparing senescent cells, which will be analyzed using the newly developed and optimized method.

Address for correspondence: V. Tambor, Biomedical Research Center, University Hospital Hradec Kralove, Sokolska 581, 500 05 Hradec Kralove

Title of the project: "Frailty patient" - effects of strong ions supplementation

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-14

Principal Investigator: A. Tichá

Co-investigators: R. Hyšpler, S. Filip, Z. Zadák

Starting date: 1.1.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 225

Summary of 2016 results

Title of the presentation: "Frailty patient" - effects of strong ions supplementation

Authors: RNDr. Alena Tichá, PhD., MUDr. Radomír Hyšpler, Ph.D., prof. MUDr. Stanislav Filip, Ph.D., DSc, prof. MUDr. Zdeněk Zadák, CSc.,

Palliative treatment and related nursing care are currently a major problem in the organization of healthcare. Outpatient palliative cancer care helps to verify the clinical status of cancer patients and helps to promote the best treatment options. Malnutrition and cachexia occur frequently in cancer patients and are indicators of poor prognosis (i.e. decreased survival, increased complications). Alkalization therapy has positive effects on nitrogen metabolism in oncology patients, where metabolic acidosis is often present. The aim of our study was to verify the effectiveness of a daily dose of 90 mmol strong ion cationts (Na+, K+ and fully metabolized organic aniont) supplementation.

Methods:
Patients for our study were recruited from the palliative oncology clinic of the Complex oncology centre in University Hospital Hradec Kralove. Inclusion criterion was supportive care treatment only. The exclusion criteria were preterminal status of disease and chemotherapy treatment in the last month. 14 patients were assessed (average 67,7 year). The patients were divided into 2 groups – without (7 patients) and with (7 patients) strong ion supplemntation. Acid base parameters and biochemical markers of nitrogen metabolism were determined in blood and urine.

Discussion and Conclusion:
Blood pH above 7,4 and urine pH above 6,5 is suitable for muscle protein synthesis and biochemical markers of nitrogen metabolism.

Literature:
Mazzucco, S., Agostini, F., Frings-Meuthen, P., Svetlic, S., De Giori, S., Mangogna, A., Buehlmeier, J., Heer, M., Biolo, G. Long-term alkalinization decreases protein catabolism and erythrocyte glutathione utilization leading to increased antioxidant capacity during experimental bed rest in humans. Clinical Nutrition Supplements, 2011, vol. 6, no. S1, p. 1.

Address for correspondence: Alena Tichá, ScD, PhD, Dept. of Research and Development, University Hospital Hradec Králové, Sokolska 581, 500 05 Hradec Králové, Czech Republic, e-mail: alena.ticha@fnhk.cz

Title of the project: Deceleration Injury of Thoracic Aorta: Descriptive study

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-6

Principal Investigator: J. Trlica

Co-investigators: T. Dědek, V. Závalová

Starting date: 1.4.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 20

Summary of 2016 results

Title of the presentation: Mortality of Traumatic Injury of Thoracic Aorta in Region of Traumacenter of Univerity Hospital Hradec Kralove.

Authors: J. Trlica (1), V. Závalová (2), Š. Kučerová (3), E. Kočová (5), J. Kočí (6), P. Habal (4), J. Raupach (5), I. Guňka (1), T. Dědek (1)

Fac. Med., Charles Univ., Hr. Králové: Dept of Surgery (1), Dept. of Forensic Medicine (3), Dept. of Radilogy, Dept. of Emergency (6), Dept. of Kardiology

Fac. Nat. Scient., Univ. of Hr. Králové (2)

Aim: The objective of this study was the retrospective analysis of deceleration thoracic aortic injury focused on mechanism of injury, type and range of injury considering the mortality.

Methods. A total of 175 patients with deceleration thoracic aortic injury were identified over the six-year period in University Hospital in Hradec Králové. Diagnosis deceleration injury of the thoracic aorta was defined as a fall of 6 meters or more and the emergence of the trauma in a traffic accident. All cases were analyzed from age, gender, type and localization of aortic rupture, mechanism of injury and degree of atherosclerosis. We were further evaluated the probability of survival and other data about the hospitalization.

Results: From total number 175, 150 patients underwent pathological-anatomical section. 139 individuals died at the accident site and 11 people transported to University Hospital HK were followed by dead. A total of 36 patients were received, of which 29 primary and 7 secondary. Of these, primarily received 11 patients died. There was no death in the group hospitalized secondarily. The above figures show that, died on the spot and 79% of the injured to the hospital for 6 % and 31 % of hospitalized patients. Injury survived 15 % of the total number of patients and 69 % of hospitalized patients.When evaluating the probability of survival (PS) There were no unexpected deaths (PS> 0.5) in patients who died due to injuries of the thoracic aorta. Mean Injury Severity Score (ISS) in the group of died was 64 and 38 in survived group, respectively. All died patient due to thoracic aortic injury had probability of survival less than 7 %. No patient died because unrecognized thoracic aortic injury.

Conclusion: On the basis of our new data analyzed from different perspectives and the compared with the previous period is a noticeable progress in the direction, diagnosis and treatment of this injury with the increase in survival and associations of seriously injured patients. Minimizing lethality resulting in injury of the thoracic aorta can achieve the primary direction of triage positive patients to trauma centers.

Key words: thoracic injury, deceleration injury, aortic injury

Address for correspondence: J. Trlica, Dept. of Surgery, Charles University in Prague, Faculty of Medicine in Hradec Kralové, Šimkova 870, 500 38 Hradec Králové, Czech Republic

Title of the project: Concentration of Donepezil in the Cerebrospinal Fluid of AD Patients: Evaluation of Dosage Sufficiency in Standard Treatment Strategy

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-15

Principal Investigator: M. Vališ

Co-investigators: J. Masopust, O. Vyšata, J. Hort, R. Doležal, J. Tomek, J. Misík, K. Kuča, J. Žďárová Karasová

Starting date: 1.1.2014 ***Duration (years):*** 2

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

Summary of 2016 results

Title of the presentation: Concentration of Donepezil in the Cerebrospinal Fluid of AD Patients: Evaluation of Dosage Sufficiency in Standard Treatment Strategy

Authors: M. Vališ

Although some studies have described the pharmacokinetics and pharmacodynamics of donepezil in the peripheral compartment, studies focused on drug transport across the blood–brain barrier are still very rare. To our knowledge, the fluctuation in the cerebrospinal fluid concentration of donepezil after administration of the drug has not been described in the literature so far. We recruited 16 patients regularly taking a standard therapeutic dose of donepezil (10 mg per day). All patients (Caucasian race) were treated for at least three months with a stable dose of 10 mg per day prior to sample collection. Patients were divided into two groups depending on the time of plasma and cerebrospinal fluid sampling: 12 h (n = 9; 4 M/5F aged 78.68 ± 7.35 years) and 24 h (n = 7; 3 M/4F aged 77.14 ± 5.87 years) after donepezil administration. The cerebrospinal fluid sample was collected by standard lumbar puncture technique using a single-use traumatic needle. The samples were analysed on an Agilent 1260 Series liquid chromatograph comprising a degasser, a quaternary pump, a light-tight autosampler unit set, a thermostated column compartment, and a UV/VIS detector. Agilent ChemStation software, the statistical software Prism4, version 5.0 (GraphPad Software, USA), and IBM SPSS Statistics were used for the analysis of the results. The difference in plasma concentration of donepezil after 12 h (mean ± SEM; 39.99 ± 5.90 ng/ml) and after 24 h (29.38 ± 1.71 ng/ml) was nonsignificant. In contrast, the donepezil concentration in the cerebrospinal fluid was significantly higher in the 24-h interval (7.54 ± 0.55 ng/ml) compared with the 12-h interval (5.19 ± 0.83 ng/ml, which is *70 % based on mean cerebrospinal fluid values). Based on these data, it is plausible to predict that donepezil might produce a stronger AChE inhibition in the brain at 24 h compared with 12 h following the administration. This information may help physicians individually adjust the time of drug administration in the patients according to time course of the disease symptoms.

Address for correspondence: M. Vališ, Department of Neurology, University Hospital, Sokolská 581, 50005 Hradec Králové, martin.valis@fnhk.cz

Title of the project: New analytical methods for effective monitoring of biomarkers

Grant Agency: Czech Republic ***Project Number:*** TA04010954

Principal Investigator: Z. Zadák

Co-investigators: R. Hyšpler, A. Tichá, I. Svobodová, M. Vacková

Starting date: 1.7.2014 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 16756

Summary of 2016 results

Title of the presentation: New analytical methods for effective monitoring of biomarkers

Authors: Z. Zadák, A. Tichá, R. Hyšpler, I. Svobodová, M. Vacková
Dept. of Research and Development, University Hospital Hradec Králové

Within monitored research period were completed the following particular topics of research project:

1. Determination of statin metabolites in urine for monitoring of the correct drug use. Test for simvastatin and atorvastatin determination has been developed. Method of urine analysis for statins monitoring was accepted as a “Certified Method”.

2. Determination of gut failure biomarkers.

2.1 Indoxyl sulfate was considered in the past as a biomarker of intestine damage. Method: Urine samples has been collected from patients in whom a disorder of intestinal passage has been diagnosed and where the finding of 3-indoxyl sulfate is expected as an indicator. The collection of urine samples from the patients concerned suffering from disorders of passage is stored for analytical experiments and as the substrate for the search for an advantageous analytical method, which would enable not only a rapid recognition of the existing disorder of passage but also the development of its risk.

2.2 New method of indoxyl sulfate determination in urine was validated and accepted “Certified Method”.

2.3 Another method for the monitoring of intestinal damage is quantification of the modified structure of albumin by means of copper ions. According to quite recent items of knowledge in the site of insufficient blood supply due to hypoxia of the tissue an increase in the concentration of simple aldehydes, above all methylglyoxal, is observed. These aldehydes are capable, by means of the process of the so-called glycation, to covalently bind to several last N-terminal amino acids, which results in the loss of the ability of the albumin molecule to bind copper ions Cu2+. Examination of this modification is especially valuable because analytical determination of methylglyoxal in ex vivo samples is, due to its high reactivity, very difficult and in routine practice nearly impossible. Our aim is application of IMA (ischemia modified albumin) to predict dehiscence of anastomosis, which according to our research seems to be very promising.
Method was validated and developed diagnostic kit accepted officially as a "Utility Model".

Address for correspondence: Prof. Zdeněk Zadák, M.D., Ph.D., Dept. of Research and Development, University Hospital Hradec Králové, Sokolská 581, 500 05, Hradec Králové, Czech Republic, e-mail: zdenek.zadak@fnhk.cz

Title of the project: The innovation of infusion solutions according to up-to-date knowledge about glycocalyx protection

Grant Agency: Ministry of Commerce ***Project Number:*** FV10454

Principal Investigator: Z. Zadák

Co-investigators: R. Hyšpler, A. Tichá

Starting date: 1.11.2016 ***Duration (years):*** 4

Total funds allocated for project - Kč (thousands): 28000

Summary of 2016 results

Title of the presentation: The innovation of infusion solutions according to up-to-date knowledge about glycocalyx protection

Authors: Z. Zadák, A. Tichá, R. Hyšpler
Dept. of Research and Development, University Hospital Hradec Králové

This project started 01.11.2016, so that results of research are limited.
The investigation team in the year 2016 was focused on the development of electrolyte formulations of infusion solution by means of computer methods “in silico”.

1. First part of research activity resulted in several pilot balanced isotonic infusion electrolyte formulas designated first of all for the replacement of circulating volume.

2. Second aim of the research team exertion was theoretical formulation of the balanced infusion solution containing organic metabolizable anions. This approach is convenient in term of energy contribution in the course of volume resuscitation and replacement. At present three formulas proposed.

3. Third newest aspect of the electrolyte infusion solution development is based on the role of glycocalyx (GCX) in fluid replacement kinetics. The syndecan 1 determination has been introduced and tested at present as a marker of GCX insult.

Literature:
1. Donati, A., Damiani, E., Domizi, R., et al. Alteration of the sublingual microvascular glycocalyx in critically ill patients. Microvascular Research, 2013, 86-89.
2. Raghunathan, K., Nailer, P., Konoske, R. What is the ideal crystalloid? Current Opinion Critical Care, 2015, 21, 309-314.

Address for correspondence: Prof. Zdeněk Zadák, M.D., Ph.D., Dept. of Research and Development, University Hospital Hradec Králové, Sokolská 581, 500 05, Hradec Králové, Czech Republic, e-mail: zdenek.zadak@fnhk.cz

Title of the project: Thromboembolic complications in relation to a selected antithrombotic therapy in patients with atrial fibrillation.

Grant Agency: Ministry of Health ***Project Number:*** RVO-FNHK/2016-21

Principal Investigator: V. Závalová

Co-investigators: A. Dvořáková, M. Kuneš

Starting date: 1.6.2015 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 30

Summary of 2016 results

Title of the presentation: Thromboembolic complications in relation to a selected antithrombotic therapy in patients with atrial fibrillation.

Authors: V. Závalová1,2, A. Dvořáková3, M. Kuneš1,2

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

2 Department of Biology, Faculty of Science, University of Hradec Králové

3 2nd Department of Surgery, Center for Vascular Disease, St. Anne´s University Hospital Brno, Brno, Czech Republic

Atrial fibrillation is one of the most frequent clinically significant disorders of the heart rhythm. The prevalence of this disease has been growing, and correlates with an increasing polymorbidity, age and sex of patients. To date, the number of patients reaches about 2 % of population and because this figure does not include asymptomatic arrhythmias, the actual number of patients with atrial fibrillation is probably much higher. The main cause of the increased morbidity and mortality in these patients results from up to 6-fold higher risk of thromboembolic complications especially stroke as well as transient ischemic attacks, and embolism in peripheral lower limbs or the visceral artery1.

The aim of this project was to find and characterize the correlation between the development of peripheral arterial thromboembolism and the selected medication for the atrial fibrillation-induced coagulopathy.

The evaluated set included 478 patients admitted to the 2nd Department of Surgery, St. Anne's University Hospital in Brno, during a period of 40 months. Patients were divided into individual groups on the basis of chronic medication of antithrombotic drugs (warfarin at 1.5 – 10 mg/dose, acetylsalicylic acid at 100 mg/dose, acetylsalicylic acid 100 mg + clopidogrel 75 mg/dose, 1 group of patients without indicated antiplatelet/anticoagulation medication), and the therapy effectiveness was evaluated on the basis of the thromboembolia prevalence. According to criteria were excluded 34 patients. Out of the total number of 444 patients with atrial fibrillation in the investigated group, thromboembolic complications occurred in 117 patients, i.e. 26,4 % cases. Detailed statistical analysis and its interpretation will be presented at the poster.

Address for correspondence: V. Závalová, Dept. of Cardiosurgery, University Hospital Hradec Králové, Sokolská 581, 500 05 Hradec Králové Czech Republic

Title of the project: Specific methods for detection of antibiotic resistance mechanisms in the clinical microbiology laboratory

Grant Agency: Ministry of Education ***Project Number:*** NF-CZ07-MOP-4-254-2015

Principal Investigator: H. Žemličková

Co-investigators: G. Skov Simonsen

Starting date: 2.5.2016 ***Duration (years):*** 1

Total funds allocated for project - Kč (thousands): 301

Summary of 2016 results

Title of the presentation: Summary of mobility project

Authors: Helena Zemlickova

Due to the project implementation we had a possibility to visit a host organisation (Arctic University of Norway - Faculty of Health Sciences, Tromso) and to have a view on the routine activities of the Department of Biology/Microbiology at university hospital. A part of the project was devoted to training of our staff to perform specific laboratory methods/diagnostic practices in which a host institution is highly experienced. We could compare our activities in the field of medical microbiology teaching with future implementation of helpful techniques in order to better motivate our students to be closely involved in education proces. Secondly, the visit gave us an opportunity to present mutually our scientific activities and create a link for future bilateral scientific projects. The overal objective of the project is an increased teaching and scientific skills of academic staff. We had a possibility to learn a part of complex methodology on molecular analysis of antibiotic resistance, including the special lectures on bioinformatic. During the week period we have been able to visit Department of clinical microbiology of University hospital Tromso and discuss all topics we have been interested in (clinical practise, ATB stewardship programme in the hospital, infection control, epidemiology of antibiotic resistance). We could take a part by ourselves in daily routine work on antibiotic reference centre, and process our own microbiological samples. We also have an opportunity to be actually present on the practicals for students on Faculty of Health Science and we could compare the differencies and similarities on contents of microbiology lectures. We have established a closer relationship with research group focused on antibiotic resistence and we would like to publish the results of analysis of our strains which was realised in cooperation with norwegian partners.

Address for correspondence: Helena Zemlickova, Department of Clinical Microbiology, University hospital, Sokolská 581, 500 05 Hradec Králové and Faculty of Medicine in Hradec Králové, Charles University, Prague, Czech Republic