Bridges in Life Sciences 9th Annual Scientific Conference

Split, Croatia

May 27 – June 1, 2014

RECOOP HST ASSOCIATION

Table of Contents

Organizing Committee	**3**
Conference Venue	**4**
RECOOP HST Association	**5**
Cedars – RECOOP Research Centers	**8**
Croatian Medical Journal	**12**
Preconference Workshop – "Protect and Publish"	**13**
Agenda of the Bridges in Life Sciences 9th Conference	**20**
Abstracts	**34**
Plenary Session May 30: 9:00 – 10:30	**35**
Poster Session May 30: 10:30 - 12:00	**36**
Plenary Session May 30: 11:00 – 12:30	**42**
Plenary Session May 30: 14:00 – 16:00	**50**
Poster Session May 30: 16:00 - 18:00	**57**
RECOOP HST Association General Assembly	**72**
Plenary Session May 30: 18:00 – 19:30	**73**
Plenary Session May 31: 8:30 – 10:15	**80**
Poster Session May 31: 10:30 - 11:30	**87**
Plenary Session May 31: 11:30 – 12:30	**97**
Plenary Session – RECOOP REVIEW May 31: 14:00 – 15:00	**103**
Research Networks' Breakaway sessions May 31: 15:00 - 16:00	**117**
Closing Poster Session May 31: 16:00 - 18:30	**118**
Plenary Session – Closing Remarks May 31: 18:30 - 19:30	**133**
5th RECOOP TriNet Meeting	**134**
RECOOP Visegrad Scholarship Program	**135**
Split Information Package	**137**
Conference Participants	**143**


Organizing Committee

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Preconference Workshop – „Protect and Publish“

Venue: HOTEL DUJAM

May 27 – 29, 2014

Bridges in Life Sciences 9th Annual Scientific Conference in Split, Croatia

Venue: ATRIUM HOTEL

May 30 – 31, 2014

Hotel Accommodation

HOTEL DUJAM *** & YOUTH HOSTEL, Velebitska 27, 21000 Split – Croatia
Phone: +385 (0) 21/273-080; www.hoteldujam.com

BEST WESTERN Hotel Art ****, UlicaSlobode 41, 21000 Split, Croatia
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ATRIUM HOTEL ****** Domovinskog rata 49a, 21000 Split, Croatia
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The Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST)

Short Name: **RECOOP HST Association**

1. Introduction

In 2006 Cedars–Sinai Medical Center (CSMC) with eleven CEE universities and academic organizations from six countries (Croatia, Czech Republic, Hungary, Romania, Slovak Republic and Ukraine) formed the Regional Cooperation for Health, Science and Technology (RECOOP HST) Association.

In 2012 CSMC converted the Association into the Association for Regional Cooperation in the Fields of Health, Science and Technology which was registered by the Court of Debrecen in Hungary under the registry number 4160 on May 10, 2012, TAX ID: 18299140-1-09.

Members of the Association constituted from 8 countries (Croatia, Czech Republic, Denmark, Hungary, Romania, Slovak Republic, Ukraine and USA) and 14 higher education or research organizations.

Members of the RECOOP HST Association**:**

1. Cedars-Sinai Medical Center, Los Angeles, USA
2. Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
3. IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
4. Institute of Macromolecular Chemistry, Academy of Sciences, Czech Republic
5. Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic
6. Faculty of Pharmacy, University of Copenhagen, Denmark
7. University of Debrecen, Hungary
8. University of Pecs, Hungary
9. University of Szeged, Hungary
10. Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
11. Slovak Medical University, Bratislava, Slovak Republic
12. Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine
13. Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
14. Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

2. International Partnerships of CSMC RECOOP HST Association

World Health Organization (WHO)
RECOOP HST Mother and Child Health Network was one of the partners of the International *PRE*term *BI*rth *C*ollaborative (PREBIC; www.prebic.net) of the World Health Organization, Department of Reproductive Health and Research (http://www.who.int/reproductive-health/index.htm).

World Intellectual Property Organization (WIPO)
Beside research opportunities the RECOOP Association inspires young scientists and clinical researchers for creative thinking, and helps to learn how to make decision on "publish and disclose" or "protect and publish". The Association is working with the World Intellectual Property Organization (WIPO; http://www.wipo.int) to train faculty members, managers, young scientists, clinicians and PhD students for innovation management and technology transfer from the RECOOP HST Association. The members of the participating organizations are taking the WIPO World Wide Academy (WWA) Distance Learning (DL) courses and until 2011 more than 500 faculty members were trained from the Association.

National Institutes of Health (NIH)
The first "Bridges in Life Sciences" regional networking meeting was organized in October 2003 by Cedars-Sinai Medical Center (CSMC) with four Hungarian Universities along with the Fogarty International Center (FIC) at the National Institutes of Health (NIH) in Budapest, Hungary.

On June 4, 2008 the representative of the RECOOP Association met with the representatives of the Division of International Relations, John E. Fogarty International Center for Advanced Study in Health Sciences, NIH, DHHS, USA and agreed to continue the "Bridges in Life Sciences" regional networking meeting organized in October 2003.

The 4th Annual Scientific Meeting took place in Debrecen, Hungary on April 4 – 5, 2009 with the Visegrad Group's (V4) academic organizations and NIH funded PIs from the USA with robust collaborative works in the Central and Eastern Europe (CEE) Countries. The host organization University of Debrecen on the behalf of the Association applied for a Standard Grant and CSMC provided the matching fund. The Bridges in Life Sciences US - CEE Regional Networking Meeting provided opportunity for 60 CEE RECOOP young scientists selected from the submitted 100 abstracts by the Scientific Advisory Board of the RECOOP Association and additional 20 young Ukrainian scientists were selected for the Young Scientists Forum with the support of California Association to Aid Ukraine (CAAU).

The U.S. Government Global Health Initiative
Ms. Lois Quam, Executive Director of U.S. Government Global Health Initiative, U.S. Department of State in her letter sent to the Bridges of Life Sciences 7th Annual Conference recognized the RECOOP HST Association Networks' research activities as valuable assets of Global Health Initiative.

Visegrád Group (V4)
From 2008 the members of the RECOOP HST Association with the financial support of Cedars – Sinai Medical Center applied for 20 Standard Grants and won 14, also one Strategic Grant.

During the last four years the RECOOP HST Association won 14 Standard Grants (20810243, 20820016, 20820040, 20820041, 20910138, 20920001, 20920002, 20920023, 20920024, 20910138, 21010083, 21010070, 21020052**,** and 21110096) to support the formation and management of research networks and multinational - multidisciplinary research projects.

Also the IVF Standard Grants helped to build support networks for Biosafety and Biosecurity, Animal use in Research, Clinical Research Management, Research and Innovation Management Training.

Creation of the RECOOP Life Science Research Platform was sponsored by **the International Visegrad Fund Strategic Grant** (31110035) "Future of Visegrad Four Families Depends on Healthy Women and Children".

The last five years IVF and CSMC-RECOOP extended activities toward the EU Eastern Partnership, the Visegrad + Western Balkans and the RECOOP HST Association is building bridges to United States Global Health Initiative.

The IVF grants won by CSMC – RECOOP also helped the International Visegrad Fund to move toward to the 21st Century and broaden the scope of funded activities in the Visegrad Group to involve more young scientists into the new biotechnology developments, could create innovative ideas, new medical treatment modalities and jobs in the region.

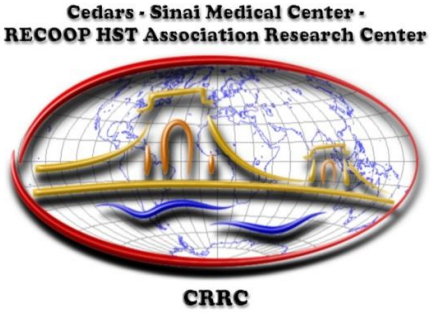
3. Research Capacity Building

The RECOOP HST Association's main goal is to enhance research collaboration and provide platforms for scientific networking in life sciences within the Visegrad Four Countries, Central - Eastern Europe, and Western Balkan. The research priority of the Association is women's and children's health in the Visegrad Four European Macro-Region (Czech Republic, Hungary, Poland and Slovak Republic) and the neighboring countries (Belarus, Bosnia and Herzegovina, Croatia, Moldova, Macedonia, Montenegro, Romania, Serbia and Ukraine). The Association paves the way toward GLOBAL Research Programs of the National Institutes of Health, the World Health Organization and private funds (Gates, Clinton).

In the last six years the RECOOP HST Association implemented translational and retro-/prospective clinical research in the Mother and Child Health (M&CH), the Women's Health and Cardiovascular Diseases (WH&CVD) and in the NanoBioTechnology (NBT) Research Networks.

The Mother and Child Health and the Women's Health and Cardiovascular Diseases retro and prospective clinical research studies are using web based electronic data entry forms (EDEF; http://www.flexiform.eu/).

Beside the management of research networks for multinational - multidisciplinary research projects, the RECOOP HST Association also built support networks for Biosafety and Biosecurity, Animal use in Research, Clinical Research Management, Research and Innovation Management Training.



In the RECOOP Research Networks 147 scientists are working in 18 research projects in clinical, basic and translational research studies to investigate biological pathways leading to gender differences in cardiovascular diseases, preterm birth, breast, cervical and brain tumors.

In 2012 the RECOOP HST Association integrated the multidisciplinary, multicenter research studies of the **RECOOP Research Networks** into the RECOOP Life Science Research Platform and formed 18 **CSMC RECOOP Research Centers (CRRC)** from 7 countries (Croatia, Czech Republic, Hungary, Poland, Romania, Slovak Republic, and Ukraine) working on translational and clinical research in the field of Genomics - Proteomics, Epigenetics, Metagenomics, Molecular Biology, Metabolomics and NanoBioTechnology.

The CSMC - RECOOP Research Centers (CRRC):

Cardiovascular Disease and Women's Health:

Lifestyle intervention in women's cardiovascular health with different reproductive and risk factors
Jan Pitha MD, PhD, Head of Laboratory for Atherosclerosis Research Department of Cardiology, IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Myocardial contractile dysfunction
Attila Borbely, MD, PhD, Cardiologist, Institute of Cardiology, Medical and Health Science Center, University of Debrecen, Hungary

The role of myofilamentary protein changes in diastolic dysfunction of newborns
Zoltan Papp, MD, PhD, DSc, Institute of Cardiology, Clinical Physiology Department, University of Debrecen, Hungary

Obesity, bone density and cardiovascular diseases
Prof. Martin Gajdoš, MD, PhD and Dr. Zora Krivosikova, PhD Medical Faculty, Department. of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, Slovak Republic

Obesity, stress and cardiovascular function
Prof. Marija Heffer MD, PhD, Department of Medical Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Oxidative stress markers for metabolic and cardiovascular diseases
Prof. Elizabeta Has-Schön, PhD, Department of Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Role of semicarbazide sensitive amine oxidase (SSAO) in Cardiovascular Diseases (endothelial cells and/or adipocytes)
Prof. Éva Szökő, PhD, DSc, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University

Vitamin D deficiency and effect on neurotransmitters in diabetes, ischemic heart disease and preterm birth
Tatiana Borisova, Department of Neurochemistry, Palladin Institute of Biochemistry NAS of Ukraine

Glycosylation and Bioseparation
Prof. András Guttman, PhD, DSc, MHAS, Research Centre for Molecular Medicine, Horváth Laboratory of Bioseparation Sciences, Medical and Health Science Center, University of Debrecen, Hungary

Mother and Child Health:

Iron intake and preterm birth
Chander P. Arora, PhD, Cedars-Sinai Medical Center International Research and Innovation Management Program, Los Angeles, CA, USA

Cervical fluid IL-6 - a possible predictor of early onset sepsis in pregnancies complicated by late PPROM
Marian Kacerovsky, MD, PhD, Department of Obstetrics and Gynecology, University Hospital in Hradec Kralove, Czech Republic

Animal models to study preterm birth
Prof. Gyorgy Falkay, PhD, DSc. & Robert Gaspar, PhD Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary

Enterovirus infection in mother, neonate, infants and follow up in child development
Prof. Shubhada Bopegamage, PhD, Entervirus Laboratory, Virology Department, Slovak Medical University, Bratislava, Slovak Republic

Screening mothers and newborns for cytomegalovirus infection
William J. Britt, MD, University of Alabama- Birmingham, Alabama, USA and Iuliana Ceausu, MD, PhD, the Department of Obstetrics and Gynecology of “Dr. I. Cantacuzino” Hospital, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

NanoBioTech and Cancer:

Iron nanoparticles for monitoring different diseases, (regenerative medicine, cardiovascular and cancer)
Prof. Daniel Horák, PhD Department of Polymer Particles, Institute of Macromolecular Chemistry, Academy of Sciences, Prague, Czech Republic
Tatiana Borisova, PhD, DSc, Head, Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiyv, Ukraine

Biofunctionalization of nanoparticles and targeted drug delivery studies,
Prof. Rostyslav Stoika, PhD, DSc, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Targeted drug delivery for cancer treatment
Professor Roman Bogdanovych Lesyk, PhD, D. Sc, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv Ukraine

Tunable nanocrystals for biomedical imaging
Artur Podhorodecki, PhD Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland

In vitro and in vivo immune assays for evaluation of immunotoxic effect of nanoparticles
Jana Tulinska, MD, PhD. Laboratory of Immunotoxicology, Slovak Medical University, Bratislava, Slovak Republic

The participating CRRCs from the member universities and research organizations implemented research programs for Medical and PhD Students.

RECOOP Life Science Research Platform already realized short term (1-2 years) pilot research studies. The Networks continue the translational and clinical research studies in midterm (5 years), and the RECOOP HST Life Science Research Platform shall plan to continue the clinical research studies for minimum 20 - 30 years and follow up the women, men and newborns registered in the Electronic Data Entry Forms (EDEF) started in 2011.

The Association inspires young scientists and clinical researchers for creative thinking, and helps to learn how to make decision on “publish and disclose” or “protect and publish”. RECOOP provides training for young scientists to learn proper and scientifically sound communication of their research results in their manuscripts and presentation. The Association provides practical training on the presentation of data in the manuscript from the “Introduction, Methods, Results and Discussion” to the “Conclusion” sections of the manuscript and guides the young scientists how to organize data in tables and graphs, presenting results of statistical analysis.

The RECOOP HST Association organizes annually the Bridges in Life Sciences Conferences to review the scientific progress in the Association. During the Bridges in Life Sciences Annual Meeting the Scientific Advisory Board selects the top ten young scientists.

The RECOOP scientific works were published in the Annual Scientific Review Journal the Biopolymers and Cell (www.biopolymers.org.ua) 2010. Vol. 26. N 2 & N 2 supplementary; 2011. Vol. 27. N 2 & N 2 Supplementary; 2012. Vol. 28. N 2 supplementary and 2013.Vol. 29. N2 supplementary.

The winner gets the Cedars-Sinai RECOOP Scholar Grant to visit Los Angeles for two weeks with her/his supervisor. They are visiting research groups and laboratories and presenting their scientific work during research seminars.

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences have the opportunity to apply for International Visegrad Fund (IVF) Scholarship and receive the RECOOP Young Scientists Matching Fund. The Visegrad Scholarship is the Visegrad Four European Macro-Region's Fulbright Program. Therefore it could be important to link the Visegrad Scholarship and the Fulbright Foreign Student Program.

CSMC – RECOOP Research Centers (CRRC) are the Center of Excellences of the RECOOP HST Association. They host young scientists, PhD students with CSMC – RECOOP (IVF – CSMC - RECOOP) Scholarship. The RECOOP HST Association Scientific Advisory Board selects the young scientists could apply for IVF – CSMC - RECOOP Scholarship.

The selected young scientists (preferably PhD students) will spend maximum four semesters and receive: €2,300 / semester and the corresponding host universities/institutes receive €1,500/semester/scholar. The host CRRC will get €1,000 for laboratory expense and consumables from CSMC – RECOOP HST Association.

RECOOP activities compliment the U.S. Government's commitment to the Global Health Initiative "the way U.S. Government agencies conduct global health activities, building on successful bipartisan leadership in global health and expanding their impact for sustainable results around the world."

"The RECOOP HST Association explores and enhances LOCAL scientific outputs of the partner organizations, creates critical mass of scientifically sound innovative research at REGIONAL level and exploits the research outcomes at GLOBAL level to improve the prevention and treatment of major public health problems."™

The RECOOP Research Networks could be a model for geographical regions have lingual, cultural, historical, economic and political common ground worldwide in regions in Africa, Asia, North and South America to improve women's and children's health.

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CROATIAN MEDICAL JOURNAL

The Croatian Medical Journal (June 2014, www.cmj.hr) published thematic issue dedicated to the RECOOP Bridges 9th Annual Scientific Conference in Split, Croatia.

The mission of Croatian Medical Journal (CMJ) was from the very beginning to support the biomedical research in emerging countries. CMJ first aimed to help Croatian research community to publish their high quality papers in a more friendly way, but very soon, the scope has widened to the neighboring countries, South East Europe, Asia and Africa.

The major tool that CMJ is offering to researchers of emerging countries was specific Author-Helpful Policy. The submitted articles were judged according to their potential and the dedicated team of professionals was there to assist the authors. The team consisted of the members of CMJ editorial group or CMJ friends were engaged to help authors to present their research according to the high standards of international publishing. Such policy had many benefits. The first were for the Journal itself, as it helped to establish a core of knowledge under CMJ umbrella which although offered internationally was indeed a national asset. Croatian experts in statistics, manuscript editing, scientific language, and manuscript production were established. The criteria of manuscript quality subsequently raised and the CMJ Impact Factor increased to its maximum of 1.8, which is a remarkable success for a general medical journal.

The effects on global community were as well visible. Many young researchers were able to publish their first contributions in CMJ, many other national journals followed the suite and the strength of the previously unrecognized research became obvious and accessible to the global scientific community.

CMJ offered and shared those virtues with the RECOOP scientists during the preparation of this thematic issue.

We at CMJ strongly believe that RECOOP and CMJ share their basic mission. By increasing the across border collaboration in the emerging part of the world the undiscovered opportunities start to shine and bring benefits to the national, regional (Central and Eastern Europe) and global community. Indeed, the scientific community should continuously discoverer multinational and multidisciplinary collaborations, and should be nourished, developed and properly presented.

The whole venture to create this thematic issue was an excellent international team effort, were many small pieces were arranged together to identify, develop and present the excellence growing under RECOOP umbrella. Therefore, we hope that the published papers in this thematic issue are not only a snapshot of current RECOOP activities, but also ~~a~~ long-term establishment of regional excellence, excellence that CMJ wants to be part of.

Prof. Srecko Gajovic
Editor-in-Chief
Croatian Medical Journal

The **Croatian Medical Journal (**CMJ) is a *Diamond Open Access* journal with impact factor 1.28 (2013). CMJ has no author's fees, no processing fees and no fees to download the full text.
The manuscripts of the RECOOP scientists published in CMJ Thematic Issue are included in PubMed Central full text database and as full text articles can be downloaded directly from PubMed Central.
The RECOOP thematic issue is as well fully accessible at journal web page www.cmj.hr

Preconference Workshop – „Protect and Publish“

Communication is not something added onto science - it is the essence of science.

“Clear Thinking = Clear Writing”

Significance, Impact, Innovation are vital to scientific and medical writing!

Capacity building for innovation, science communication and grant writing for young scientists in Central and Eastern Europe (CEE)

Preconference Workshop on May 28 and 29 in HOTEL DUJAM *** & YOUTH HOSTEL web: www.hoteldujam.com.

For the Workshop arrival on May 27 (Tuesday), 2014 and the workshop will be on May 28 and 29. The participating young scientists will stay in that hotel during the Bridges Conference since they are invited to attend the RECOOP 9th Annual Scientific Conference.

The Bridges in Life Sciences 9th Annual Conference will follow on May 30 (Friday) from 8:30 to 1:00 pm and May 31 (Saturday) from 9:00 to 6:00 pm in Hotel Atrium, Domovinskog rata 49a, 21000 Split, Croatia, web: http://www.hotel-atrium.hr/en/

1. Aims

RECOOP HST Association implements Science and Innovation Capacity Building (SCICAB) program as part of the RECOOP Protect and Publish (P&P) Strategy for innovation management, science communication and grant writing in the Central and Eastern European Countries. The objectives of SCICAB are to increase the number of young scientists capable to make decision in protect and publish, able to write good quality manuscripts in peer review journals and competitive grants.

2. Goals

RECOOP HST Association’s main goal is to build multinational, multidisciplinary collaboration and to establish long term life sciences research and public health program in seven Central and Eastern European (CEE) countries; Croatia, Czech, Hungary, Poland, Romania, Slovak, and Ukraine. Research results need to be published to become accessible and contribute to the progress of research and patient care. Publishing of the scientific outcomes it is necessary but protecting the intellectual property rights is crucial to convert the results into products: laboratory tests, diagnostic and treatment modalities. Inventors should reinvest the patent income into the innovators’ research facilities and reward scientists too. The ability to write well is critical for healthcare professionals and researchers to precisely convey their ideas and research results.

3., Methods

The participating young scientists with help of the tutors will convert their abstracts that were written to present their research at the 9th Bridges in Life Sciences Conference in Split, Croatia, May 2014 into a manuscript and those will be scrutinized and discuss to study the most important aspects of writing research articles. The same method will be used for the grant writing. Tutors will help them to learn how to make decision on protect & publish.

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Sandor G. Vari, MD
Director, International Research and Innovation Management Program
Cedars-Sinai Medical Center, Los Angeles, CA, USA &
President of the RECOOP HST Association

Workshop – Protect and Publish

May 28, 2014 Day 1 – Wednesday – Publish - Manuscript Writing

8:30 Welcome and introductions – Sandor G. Vari

8:45 Communicating results of scientific research – Ana Marušić

9:30 Are there differences in communicating science among different scientific fields? – Ana Marušić

10:15 Coffee break

10:30 Structure of the scientific article: Writing articles in Life Sciences – Team of Ana Marušić

11:15 Structure of the scientific article: Introduction and Discussion - Team of Ana Marušić

12:00 Structure of the scientific article: Methods and Results - Ana Marušić

13:00 Lunch break

14:45 Around IMRAD: title, abstract, acknowledgments, disclosures, references – Ana Marušić

15:30 Ethics in scientific publications, case studies in publication ethics - Team of Ana Marušić

16:00 Coffee break

16:15 Preselected young scientists (two) who published their work in the Croatian Medical Journal (April 2014) RECOOP Special issue will present their work in brief (max 15 minutes) oral presentation.

Senka Blažetić, Department of Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
Rostyslav Panchuk, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology NAS of Ukraine, Lviv, Ukraine

17:00 Lecturers and students will discuss the structure and delivery of the presentation – Team of Ana Marušić

18:30 Close of the day: Questions and answers - Ana Marušić

19:00 Dinner Round Table: Scientific article – is it difficult to write a scientific paper? Moderator: Team of Ana Marušić

May 29, 2014 Day 2 – Thursday – Protect

8:30 Welcome and introductions – Sandor G. Vari

8:45 Reasons and ways to protect research results before publication – Tamas Bene

9:30 The importance of intellectual property (IP) management at universities and public research organizations (PRO)– Tamas Bene

10:15 Coffee break

10:30 Patent Cooperation Treaty as an umbrella for patenting – Vladimir Yossifov

11:15 Market analysis, planning – Baker Group

12:00 Market-based IP valuation – Baker Group

13:00 Lunch break

14:45 Licensing, product development direction - Baker Group

15:30 Case study – Baker Group

16:00 Coffee break

16:15 Funding opportunities in Central and Eastern Europe – Sandor G. Vari

17:00 EU – FP8 – Horizon 2020 – Sandor G. Vari

18:30 NIH Grant types – Kristin Martinez

19:00 Dinner Round Table: Who could write grants – Sandor G. Vari

Summary of RECOOP Protect and Publish Workshop

PUBLISH

COMMUNICATING RESULTS OF SCIENTIFIC RESEARCH – Ana Marušić
Scientific and medical journal editors and peer reviewers continually point out that unclear writing is one of the most important reasons for article rejection. Stated another way, anything that gets between the writer's scientific message and the reader's comprehension of that message, derails the paper and upsets the reviewers.

Unclear writing signals unclear thinking. To write clearly, authors need to understand all the nuances and aspects of the subject before they begin wordsmithing the article. Writing down the ideas even in an outline form helps to clarify thinking. Scientists tend to imitate the academic, obscure writing that is seen in some scientific journals and in grants, attempting to impress the reader with complex, long and unclear phrases and paragraphs. If authors want to write more clearly, let go of this style and develop your own clear way of communicating. They should always think of the reader who needs to understand what authors are intending to communicate.

The workshop designed to help young scientists and medical professionals to understand the root of poor writing, identify red flags within their own writing style, and design clear, concise, powerful pieces that meet the requirements of the most demanding audiences within their professional careers.

The training includes:

Discussion of the differences in science communication in different scientific fields.
The basic writing principles that authors actually know but forget during manuscript writing.
Underline the power of conciseness, saying more with less.
Redundancies in writing: why repetition is *not* always a good thing.
How to keep reviewers' focus on science, and not on the grammar

The faculty and participating young scientists will have writing exercises to get authors on the path to becoming a "Successful Author":

The structure of articles in Life Sciences
Introduction and Discussion
Methods and Results
Around IMRAD: title, abstract, acknowledgments, disclosures, references

The faculty also will discuss "Ethics in Scientific Publications" and will have case studies in publication ethics.

PROTECT

REASONS AND WAYS TO PROTECT RESEARCH RESULTS BEFORE PUBLICATION – Tamas Bene

Dissemination of scientific knowledge through publication is one of the most common and rapid instruments. Publishing, however, is not always as timely as it may appear as the peer review process can delay the final article publication. The protection granted by the IP system to an article or publication is copyright, which arises automatically when the researcher writes and publishes it. However, copyright is not the right tool to exploit inventions commercially. If the research activities result in a promising practical innovation, researchers should consider applying for a patent in order to commercially exploit the invention and have in return revenues. Patenting and publishing do not exclude each other, but the right order of these activities is critical. Patenting always should be done first. The lecture will give an overview on various IP rights and explain the basics of the patenting procedure.

THE IMPORTANCE OF INTELLECTUAL PROPERTY (IP) MANAGEMENT AT UNIVERSITIES AND PUBLIC RESEARCH ORGANIZATIONS (PRO) – Tamas Bene

More and more universities and public research organizations recognize the need for encouraging the practical application and economic use of the results of research carried out at the institution for the benefit of the general public. To achieve this goal, these institutions need to define intellectual property broadly, and their policies need to be comprehensive in their coverage. Depending on the direction of R&D activities, each institution should decide on the type of intellectual property (IP) to be covered by its IP policy. A good IP policy deals with all aspects of IP management: it provides legal certainty in research activities and technology-based relationships with third parties and sets out the procedures on the identification, ownership, protection and commercialization of Intellectual Property. It also ensures that economic benefits arising from the commercialization of the IP rights distributed in a fair and equitable manner that recognizes the contributions of all stakeholders. The session will give an overview on university IP management and explain the basics of technology transfer activities.

PATENT COOPERATION TREATY AS AN UMBRELLA FOR PATENTING – Vladimir Yossifov

The Patent Cooperation Treaty or PCT is an international agreement for filing patent applications having effect in up to 148 countries.

Although the PCT system does not provide for the grant of an international patent, the system:

- simplifies the process of filing patent applications
- delays the expenses associated with applying for patent protection in other countries
- allows the inventor more time to assess the commercial viability of his/her invention.

Under the PCT, inventors can file a single international patent application in one language with one patent office and simultaneously seek protection for an invention in 148 countries throughout the world.

The session will give an overview of the PCT systems and of other regional patent systems (EPO, EAPO, ARIPO, OAPI, and GCC)

MARKET ANALYSIS AND PLANNING – Baker Group
Early market research is key to determining whether an innovation can and should be translated from scientific study to a commercial product. In addition to understanding the technology, researchers interested in creating a viable business opportunity must be able to identify, anticipate and satisfy consumer needs – all while making a profit. External market validation through stakeholder input and published data about products, markets and brands, are key data points in the decision making process.

MARKET-BASED IP VALUATION – Baker Group
Estimating the potential for IP to produce future revenue is at the core of determining the value of a patent. Market-based valuation of intellectual property heavily considers competitive products, consumer need and cost. Within the medical sectors, the ability to reduce healthcare costs and improve patient outcomes are key value considerations.

LICENSING & PRODUCT DEVELOPMENT – Baker Group
By understanding the key tenants and terms of a licensing agreement, one can determine how commercial development may be positively or negatively impacted. With this knowledge, inventors can identify licenses that will enhance innovation and create viable business opportunities.

CASE STUDY – Baker Group
The team at Cedars-Sinai Regenerative Medicine Institute is developing a minimally invasive spinal injection device for the treatment of Amyotrophic Lateral Sclerosis (ALS). Current surgical spinal devices are difficult to use, have an excessive part count and have problems related to precise positioning. The device being developed at Cedars-Sinai is intended to safely, accurately and securely deliver therapeutic agents into the spinal cord, brain, and/or joints. The first prototype is being developed for use in pre-clinical trials this year and is expected to be commercialized for future use for this and other spinal injection applications.

PROTECT – IP PROTECTION, MARKET VALUE & TECHNOLOGY TRANSFER – Baker Group
Prior to publishing or publicly disclosing information researchers must protect intellectual property in order for IP to have commercial value. Within the business sector, only well-protected IP is considered for future development. Adhering to an organized, intentional technology transfer process is of benefit to all involved in the process of commercializing IP.

FUNDING OPPORTUNITIES IN CENTRAL AND EASTERN EUROPE – Sandor G. Vari
The RECOOP HST Association's main goal is to enhance research collaboration and provide platforms for scientific networking in life sciences within the Visegrad Four Countries, Central - Eastern Europe, and Western Balkan. The research priority of the Association is women's and children's health in the Visegrad Four European Macro-Region (Czech Republic, Hungary, Poland and Slovak Republic) and the neighboring countries (Belarus, Bosnia and Herzegovina, Croatia, Moldova, Macedonia, Montenegro, Romania, Serbia and Ukraine). The RECOOP HST Association is using grant opportunities of International Visegrad Fund (IVF; http://visegradfund.org/) to organize scientific meetings and workshops. The IVF is the only institution of the Visegrad co-operation established in 1999 and headquartered in Bratislava. The budget of IVF made by the Council of Ministers formed from the prime ministers of the Visegrád Group, also called the Visegrád Four or V4 (Czech Republic, Hungary, Poland and Slovak Republic). The grant opportunities are Small, Standard and Strategic Grants, scholarships and artist residencies.

EU – FP8 – Horizon 2020 – Sandor G. Vari

The research collaborations become increasingly complex and requiring integration of knowledge, data and resources by way of transnational collaboration. In the next years RECOOP Association will apply for selected EU Horizon 2020 calls therefore, it is important to provide information for the RECOOP members about the legal basis and rules of participation, the Horizon 2020, the Health Programme 2014-2020 and the upcoming programs in 2014-2015:

PHC – 1 Understanding health, ageing and disease

PHC-03-2015 Understanding common mechanisms of diseases and their relevance in co-morbidities

PHC-11-2015 Development of new diagnostic tools and technologies: in vivo medical imaging technologies

NIH GRANT TYPES – Kristin Martinez

The National Institutes of Health (NIH), a part of the US Department of Health and Human Services, is the medical research agency for the US. Made up of 27 Institutes and Centers, each with their own area of concentration, the NIH uses different grant types to focus and fund research. By understanding the common types of research grants, international investigators will be able to plan and organize their research application whether they apply for NIH funding directly or as part of collaboration (subcontract) with a US research team.

Agenda of the Bridges in Life Sciences 9th Annual Scientific Conference

May 29 – June 1, 2014

Partially sponsored by the International Visegrad Fund Standard Grant: ID 21320181

Conference Agenda on May 29 (Thursday), 2014

Venue: ATRIUM HOTEL

16:00 – 19:30 Registration and poster installation in the following sessions:

Mother and Child Health

Cardiovascular Diseases

Stress, Obesity and Metabolic Diseases

Neurological Disorders and Brain Research

Cancer Research

NanoBioTechnology

Translational Research

Poster size is the following: width 90 cm x height 120 cm.

The posters can be reviewed and discussed throughout the Conference and poster room will be open:

May 30 (Friday) from 8:30 to 19:00

May 31 (Saturday) from 9:00 to 19:00

The posters have to be removed on May 31 (Saturday) after 19:00

20:00 Buffet Dinner

Conference Agenda on May 30 (Friday), 2014

9:00 to 10:30 Plenary Session

9:00 Opening Remarks
Edward Prunchunas
Senior Vice President for Finance and Chief Financial Officer, Cedars-Sinai Medical Center, Los Angeles, CA, USA & Chairman of the Supervisory Board of the RECOOP HST Association

9:15 Review of the Research Activities in the RECOOP
Sandor G. Vari, MD
Director, International Research and Innovation Management Program
Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of the RECOOP HST Association

9:30 Keynote Speaker: Biomedical Innovation at Cedars – Sinai Medical Center
Shlomo Melmed, MD
Senior Vice President, Academic Affairs, Dean of the Medical Faculty, Cedars-Sinai Medical Center, Los Angeles, CA, USA

10:00 Protect – IP Protection, Market Value and Technology Transfer
Michael Baker
Baker Group LLP, A Technology Advancement Company, Portland, OR, USA

10:30 Coffee break

10:30 – 12:00 Poster Session
Poster presentation: 5 minutes explanation and 3 minutes discussion

Session Chairs
Calvin J. Hobel
Marija Heffer
Daniel Horak

Ratio of cord to maternal serum PCB concentrations in relation to their congener-specific physicochemical properties
Kinga Lancz
Department of Environmental Medicine, Slovak Medical University, Faculty of Public Health, Bratislava, Slovak Republic

Electromyographic investigation on pregnant rat uterus in vivo
Kálmán Szűcs
Department of Pharmacodynamics and Biopharmacy, University of Szeged, Szeged, Hungary

Cardiosphere-derived cell exosomes stimulate cardiomyocyte proliferation and angiogenesis in vitro, and improve functional recovery post myocardial infarction in mice
Ahmed Ibrahim
Heart Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Cell-based quantum dot labeling of RANK-mediated signaling mediators predicts survival of patients with prostate cancer
Haiyen E. Zhau
Louis Warschaw Prostate Cancer Center, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Chelidonine interferes with IL-6R/STAT3 signaling in uveal melanoma cells
Eniko Nizsaloczki
Department of Biophysics and Cell Biology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

11:00 – 12:30 Plenary session

Oral presentation timed strictly for 12 minutes!

Session Chairs
Livia Puljak
Chander P. Arora
Artur Podhorodecki

Antenatal Maternal and Fetal Steroid Treatment: Neonatal Outcome
Tibor Ertl
Department of Neonatology, School of Medicine, University of Pécs, Hungary

Acute stress and female hormones alter cardiomyocyte contractile function in ovariectomized rats
Attila Borbély
University of Debrecen, Faculty of Medicine, Institute of Cardiology, Division of Clinical Physiology, Debrecen, Hungary

Influence of body weight and body composition on bone mineral content and density in female rats with and without ovariectomy
Patrícia Kramárová
Department of Toxicology, Slovak Medical University in Bratislava & Slovak Medical University, Bratislava, Slovak Republic

GAP-43 as a potential marker of neuronal stress - in vivo imaging and immunofluorescence study applied on ischemic brain lesion
Srećko Gajović
Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

Polymeric nanobioconjugates for cancer treatment
Julia Y. Ljubimova
Nanomedicine Research Center, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Section-specific determination of smooth muscle activity by smooth muscle electromyography in normal and immobilized rats
Robert Gaspar
Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

Q&A and Panel Discussion (15 minutes)

12:30 – 14:00 Buffet Lunch

14:00 – 16:00 Plenary session

Oral presentation timed strictly for 12 minutes!

Session Chairs
Rostyslav Stoika
Jan Pitha
Leland Chung

Perinatal hypoxia: Different effects of the inhibitors of GABA transporters GAT1 and GAT3 on the initial velocity of [^{3}H]GABA uptake by cortical, hippocampal and thalamic nerve terminals
Tatiana Borisova
Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine,

Contribution of myofilament protein carbonylation to systolic and diastolic dysfunction
Zoltán Papp
University of Debrecen, Faculty of Medicine, Institute of Cardiology, Division of Clinical Physiology, Debrecen, Hungary

Impact of ovariectomy, high fat diet and lifestyle modifications on oxidative/antioxidative status in rat liver
Rosemary Vuković
Department of Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Alginate a stabile and biocompatible stem cells-seeded hydrogel carrier for the targeted delivery in the stroke lesion
Lejla Ferhatović Hamzić
Croatian Institute for Brain Research, University of Zagreb School of Medicine, Zagreb, Croatia

RANK- and c-Met-mediated feed forward signal network promotes metastasis by recruiting dormant cells and predicts the survival of prostate cancer patients
Leland WK Chung Zhau,
Departments of Medicine, and Surgery, Uro-Oncology Research Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA

A pilot study applicability of standardized modified method of dry (throat/buccal) **swabs in PCR diagnosis of enteroviral infections**
Shubhada Bopegamage
Enterovirus Laboratory, Slovak Medical University, Bratislava, Slovakia

Q&A and Panel Discussion (15 minutes)

16:00 – 16:30 Coffee Break

16:00 – 18:00 Poster Session

Poster presentation: 5 minutes explanation and 3 minutes discussion

Session Chairs
Julia Y. Ljubimova
Shubhada Bopegamage
Robert Gaspar

Characteristics of autoimmune diseases in patients from Osijek-Baranja County (Croatia) – a population study
Martina Mihalj
Department of Physiology and Immunology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Androgenic effect of honeybee drone milk in castrated rats
Adrienn Seres
Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

Cardiovascular and cerebrovascular complications in rheumatoid arthritis patients at Osijek Baranja County (Croatia)
Sanja Novak
Department of Physiology and Immunology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Lifestyle intervention in general practice for physical activity, smoking, alcohol consumption and diet in elderly
Davorka Vrdoljak
Department of Family Medicine, University of Split School of Medicine, Split, Croatia

Impact of adipose tissue inflammation in the pathogenesis of atherosclerosis
Anna Králová
Laboratory for Atherosclerosis Research, Institute of Clinical & Experimental Medicine (IKEM), Prague, Czech Republic

Prevalence of cardiovascular complications in three age groups of patients with Systemic Lupus Erythematosus (SLE)
Zrinka Mihaljević
Department of Physiology and Immunology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Cutaneous expression of calcium/calmodulin-dependent protein kinase II following diabetes induction
Antonia Jeličić Kadić
Laboratory for Pain Research, University of Split School of Medicine, Split, Croatia

The differentiation of SH-SY5Y neuroblastoma cells – the effect of the antidepressants
Zsófia Ulakcsai
Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

The expression of calcium/calmodulin-dependent protein kinase II in spinal cord in rat models of type 1 and type 2 diabetes
Matija Borić
Laboratory for Pain Research, University of Split School of Medicine, Split, Croatia

Validation of an aminonaphthalene trisulfonate labeled N-glycan database by CGE
Csaba Váradi carries for Márta Kerékgyártó
Horváth Laboratory of Bioseparation Sciences, MMKK, University of Debrecen, Debrecen, Hungary

In vivo bioluminescent imaging of inflammation dynamics after different MCAO periods
Dora Polšek
University of Zagreb School of Medicine, Zagreb, Croatia

Functionalized core-shell porous silica/maghemite nanoparticles: Design, synthesis and immunotoxicity
Beata Zasonska
Department of Polymer Particles, Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

RECOOP consortium – promising platform for new grant opportunities
Jana Tulinská
Department of Immunology and Immunotoxicology, Department of Biology, Slovak Medical University, Bratislava, Slovak Republic

Functionalization of quantum dots with PEG dervatives – a versatile approach for obtaining water soluble fluorescent nanomarkers
Anna Żelazo
Institute of Physics, Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland

RECOOP HST Association General Assembly 16:30 – 18:00

Cedars-Sinai Medical Center, Los Angeles, USA
Edward Prunchunas, CSMC and Sandor G. Vari, CSMC - RECOOP

Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
Authorized Representative

IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Jan Pitha

Institute of Macromolecular Chemistry Academy of Sciences, Czech Republic
Daniel Horák

Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic Vanda Bostik

Faculty of Pharmacy, University of Copenhagen, Denmark
S. Moein Moghimi

University of Debrecen, Hungary
Laszlo Matyus

University of Pecs, Hungary
Tibor Ertl

University of Szeged, Hungary
Gyorgy Falkay

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
Iuliana Ceausu

Slovak Medical University, Bratislava, Slovak Republic
Authorized Representative

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine Tatiana Borisova, Palladin Institute of Biochemistry NAS of Ukraine

Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Rostyslav Stoika

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Roman Lesyk and/or Oleh Pinyazhko

Invited: Elizabeta Has-Schön, Department of Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
Ivančica Pavličević, Department of Family Medicine, University of Split School of Medicine, Split, Croatia
Artur Podhorodecki, Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland
William J. Britt, MD, University of Alabama- Birmingham, Alabama, USA

18:00 – 19:30 Plenary session

Oral presentation timed strictly for 12 minutes!

Session Chairs
Tatiana Borisova
Gyorgy Falkay
Roman Lesyk

Allostatic Load Score Defines the Percent of Women who are Biochemically & Physiologically "Unhealthy" during the Postpartum Period. Implications for reducing the Risk of Cardiovascular Disease.
Calvin Hobel
Department of Obstetrics, Gynecology, Cedars Sinai Medical Center, Los Angeles, CA, United States

Nanoparticle-Mediated Tumour Growth: Cause for Reflection
S. Moein Moghimi
Centre for Pharmaceutical Nanotechnology and Nanotoxicology, Department of Pharmacy, University of Copenhagen, Copenhagen Denmark

Changes in Cardiovascular Risk Profile in Women after Menopause (Prague Pre- and Post-Menopausal Female Study)
Jan Pitha
Laboratory for Atherosclerosis Research, Institute of Clinical & Experimental Medicine, Prague, Czech Republic

Effects of high fat diet, ovariectomy and physical activity on leptin receptor expression in rat brain and white fat tissue
Senka Blažetić
Department of Biology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

Specific antioxidant compounds differentially modulate cytotoxic activity of doxorubicin and cisplatin: *in vitro and in vivo study*
Rostyslav Panchuk
Institute of Cell Biology National Academy of Sciemces of Ukraine, Lviv, Ukraine

A comparative study of neurotoxic potential of synthesized polysaccharide-coated and native ferritin-based magnetic nanoparticles
Arsenii Borysov
Palladin Institute of Biochemistry National Academy of Sciemces of Ukraine, Kiev, Ukraine

Q&A and Panel Discussion (15 minutes)

19:30 Buffet Dinner

Conference Agenda on May 31 (Saturday), 2014

8:30 - 10:15 **Plenary session**

Oral presentation timed strictly for 12 minutes!

Session Chairs
S. Moein Moghimi
Srećko Gajović
Zoltan Papp

Enhanced anticancer activity and circumvention of drug resistance mechanisms by novel polymeric/phospholipidic nanocarrier
Rostyslav Stoika
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Preterm Birth and its consequences in rural and urban communities of India
Chander P. Arora
International Research and Innovation Management, Cedars-Sinai Medical Center, Los Angeles, CA, USA

The influence of selected factors of life style on bone health – the experimental study
Zora Krivošíková
Department of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, Slovak Republic

SSAO enzyme activity in adjuvant arthritis and anti-inflammatory effect of a new SSAO inhibitor
Tamás Tábi
Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

Transcatheter Patent Ductus Arteriosus Closure in VLBW Infants
Charles F. Simmons, Jr.
Department of Pediatrics, Cedars – Sinai Medical Center, Los Angeles, CA, USA

N-Glycan mapping by capillary electrophoresis
Andras Guttman
MTA-PE Translational Glycomics Research Group, University of Pannonia, Veszprem, Hungary

Q&A and Panel Discussion (15 minutes)

10:15 **Coffee break**

10:30 – 11:30 Poster Session

Poster presentation: 5 minutes explanation and 3 minutes discussion

Session Chairs
Iuliana Ceausu
Jana Tulinska
Charles F. Simmons

Research Achievements (2012-2014) of the Department of Regulation of Cell Proliferation and Apoptosis at the Institute of Cell Biology (NAS of Ukraine) Due to Collaboration Network Within the RECOOP-HST Association and Collaboration with Other None-RECOOP Institutions
Rostyslav R. Stoika
Institute of Cell Biology National Academy of Sciences of Ukraine, Lviv, Ukraine

Experimental esophagitis research: new insight on animal models and translational aspects
Oksana Zayachkivska
Physiology Department, Lviv National Medical University, Lviv, Ukraine

Cervical fluid IL-6 and IL-8 levels in pregnancies complicated by preterm prelabor rupture of membranes
Daniel Lesko carries Ivana Musilova
Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, Czech Republic

How does maternal smoking influence the early neurobehavioral development of rat pups?
Barbara Mammel
Department of Obsterics and Gynecology and Department of Anatomy, Medical Faculty, University of Pécs, Pécs ,Hungary

The renin release in ischemia/reperfusion kidney injury; gender differences
Anna Kosik
1st Department of Pediatrics, Semmelweis University Budapest, Budapest, Hungary

Microalbuminuria and cardiometabolic risk factors in general population of high school students
Radana Kollarova
Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovak Republic

Diabetes-induced impairments of the exocytosis process and the effect of gabapentin: the link with cholesterol level in neuronal plasma membranes
Irene Trikash
Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

The role of *musashi* in *Caenorhabditis elegans* – the gene of forgetting?
Gábor Bánk Fenyves
Department of Medical Chemistry, Semmelweis University, Budapest, Hungary

11:30 - 12:30 Plenary session

Session Chairs
András Guttman
Shubhada Bopegamage
William J. Britt

Value of hysteroscopy for endometrium evaluation in postmenopause
Iuliana Ceausu
"Carol Davila" University of Medicine and Pharmacy, "Dr. I. Cantacuzino" Department of Obstetrics and Gynecology, Bucharest, Romania

Gender differences in expression of estrogen receptor β and leptin receptor in adrenal gland after chronic and acute stress
Marta Balog
Department of Medical Biology, Laboratory of Neurobiology, Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia

5-Ylidene-4-thiazolidinones anticancer agents' source: problem solution
Roman Lesyk
Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Visualization of melanoma tumor with lectin-conjugated rare-earth nanocrystals
Artur Podhorodecki
Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland

Q&A and Panel Discussion (15 minutes)

12:30 – 14:00 Buffet Lunch

14:00 - 15:00 Plenary session – RECOOP Review

14:00 – 14:30 **Standard Operating Procedures for CRSMN at the RECOOP HST Association**
Linn Defensor RN, MSHS-CRA, CCRP
Office of Research Compliance and Quality Improvement, Cedars-Sinai Medical Center, Los Angeles, CA, USA & Global Clinical Trial Leader, CRSMN, RECOOP HST Association

14:30 – 15:00 RECOOP Future Activities

Grants – NIH, EU Horizon 2020 and IVF

Meetings: 5th TriNet Meeting, 10th Anniversary Bridges Scientific Conference

Q&A and Panel Discussion (15 minutes)

15:00 - 16:00 Breakaway sessions (The sessions will be in different rooms)

Mother and Child Health

Chander P. Arora

Women's Health and Cardiovascular Diseases

Zoltan Papp

NanoBioTechnology

Rostyslav Stoika

16:00 – 16:30 Coffee Break

16:00 – 18:30 Closing Poster session

Poster presentation: 5 minutes explanation and 3 minutes discussion

Chairs
Éva Szökő
Zora Krivosikova
Tibor Ertl

Risk factors for preterm birth in Southern Croatia: a case control study at University Hospital
Tanja Vukušić-Pušić
Department of Obstetrics and Gynaecology, University Hospital Split, Split, Croatia

Detection of novel auto-antigens in patients with recurrent miscarriage: Description of an approach and preliminary findings
Rostyslav Stoika carries for Yuriy Y. Kit
Institute of Cell Biology National Academy of Sciemces of Ukraine, Lviv, Ukraine

Cervical fluid IL-6 and IL-8 levels in pregnancies complicated by preterm prelabor rupture of membranes
Martin Stepan
Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, Czech Republic

Risk factors and clinical follow-up of patients with preterm births in a tertiary referral maternity unit in Bucharest, Romania
Cristian Poalelungi
”Carol Davila” University of Medicine and Pharmacy, Bucharest, ”Dr. I. Cantacuzino” Hospital, Department of Obstetrics and Gynecology, Bucharest, Romania

The Role of PACAP on Retinal Vascular Changes in the Rodent Model of Retinopathy of Prematurity
Timea Kvarik
Dept of Anatomy MTA PTE “Lendulet” PACAP Research Team and Dept of Obstetrics and Gynecology, University of Pecs, Pecs, Hungary

Shared decision making in life style and nutrition for intervention in women with risk factors in cardiovascular health
Ivančica Pavličević
Department of Family Medicine, University of Split School of Medicine, Split, Croatia

Astrocyte toxicity to motor neurons is dependent on age
Melanie M. Das
The Regenerative Medicine Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Synthesis and surface functionalization of $NaGdF_4:Yb^{3+},Er^{3+}$ nanocrystals for their biomedical applications
Agnieszka Noculak
Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland

Haptoglobin N-glycome Alteration Analysis of Inflammatory and Malignant Lung Diseases by Capillary Electrophoresis
Csaba Váradi
Horváth Laboratory of Bioseparation Sciences, University of Debrecen, Hungary

Study protocol for the project Coxsackievirus infections during pregnancy
Shubhada Bopegamage
Enterovirus Laboratory, Slovak Medical University, Bratislava, Slovak Republic

Genotyping and characterization of Varicella zoster clinical isolates from Hradec Kralove, Czech Republic
Monika Schmidt
Centre of Advanced Studies, FMHS, University of Defence, Hradec Kralove, Czech Republic

Entero-salivary nitrites recirculation is an endogenous modulator of leukocyte-mediated inflammation in experimental oesophagitis
Irena Pshyk-Titko
Physiology Department, Lviv National Medical University, Lviv, Ukraine

18:30 – 19:30 Plenary session – Closing Remarks

RECOOP Research Networks – project plan

Mother and Child Health

Chander P. Arora

Women's Health and Cardiovascular Diseases

Zoltan Papp

NanoBioTechnology

Rostyslav Stoika

Overview of the RECOOP Association's General Assembly

Sandor G. Vari

19: 30 - 22:00 Buffet Dinner

June 1, 2014 (Sunday)

Departure

Abstracts

May 30 (Friday), 2014

Plenary Session

9:00 - 10:30

Protection, Market Value and Technology Transfer

Brenda Edin and Michael Baker

Baker Group LLP, A Technology Advancement Company, Portland, OR, USA

www.bakergroupllp.com

Baker Group Background:

Since our inception in 1999, the Baker Group has taken several products "*from sketch to launch*". Based on over 25 years of product development history with Philips Medical, GE Medical and Lockheed-Martin, we have developed a programmatic, phased approach to commercialization. With critical roles in bringing several first-in-class products to market, many of which required FDA 510K clearance, we are most proud of those that improve the health of people and the planet. Our passion is to create solutions that improve wellbeing and enhance lifestyle control in a manner that is cost-effective and able to reach a broad adoption base.

Expertise and Offerings:

The Baker Group is focused specifically on the medical device, medical informatics and advanced energy markets, commercializing technology in one of two ways: by licensing, acquiring or developing technology within the Baker Group, or by participating with other companies and institutions that seek innovation support from our team. The Baker Group offers:

- Business case development and validation
- Financial viability modeling and forecasting
- Monetization strategy
- Commercialization strategy and management
- Market analysis and planning
- Product development and launch
- Market-based IP valuation
- Investor readiness and diligence preparation
- Fundraising guidance or oversight
- Regulatory and quality management

We place confidentiality and best practices in high regard in order to maintain professional long-term relationships with our clients. For your protection and ours, we offer a Non-Disclosure Agreement for those wishing to share proprietary information with the Baker Group.

Advanced Innovation:

Adept at transferring technology from government and academic arenas to the business sector, we are uniquely skilled at identifying new markets for existing technology. Hired by corporations like General Electric and Hewlett Packard, we are equally skilled in the academic and hospital settings with clients like Cedars-Sinai Medical Center, Samaritan Health Systems and Oregon State University. Members of the Baker Group have advanced over fifty products over the course of our careers. A few that we are most proud of include:

- Home hemodialysis machine
- Picture archive and communication system
- Microscale water purification system
- Holographic ultrasound imaging
- High efficiency electric motor
- Hemostatic wound dressing

May 30 (Friday), 2014

Poster Session

10:30 – 12:00

Session Chairs

Calvin J. Hobel

Marija Heffer

Daniel Horak

Ratio of cord to maternal serum PCB concentrations in relation to their congener-specific physicochemical properties

Lancz K[1], Palkovičová Ľ[1], Patayová H[1], Drobná B[1], Wimmerová S[1], Šovčíková E[1], Kováč J[2], Hertz-Picciotto I[3], Jusko TA[4], Trnovec T[1]

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[2]Department of Stomatology and Maxillofacial Surgery, Comenius University, Faculty of Medicine in Bratislava, Špitálska 24, 813 72 Bratislava, Slovak Republic
[3]Division of Environmental and Occupational Health, Department of Public Health Sciences, School of Medicine, University of California Davis, One Shields Avenue, Med-Sci 1C, Davis, California, USA
[4]Department of Public Health Sciences, University of Rochester School of Medicine and Dentistry, 265 Crittenden Blvd, CU 420644, Rochester, NY 14642, USA

Corresponding author: Kinga Lancz ✉ kinga.lancz@szu.sk

Key words: PCBs, placental transfer, partition coefficient octanol water; physicochemical parameters

Introduction: Polychlorinated biphenyls (PCBs) have been detected in fetuses where they can exert adverse effects. To reach the fetus they must cross the placenta. However PCBs in the environment are a mixture of congeners, each of which is characterized by its own physicochemical properties and toxicity.

Aim: The aim of this study was to characterize the placental transfer of some congeners of polychlorinated biphenyls (PCBs) and to explore the possibility of predicting in utero exposure to these pollutants from their physicochemical properties.

Methods: We included in the study 1134 births during the period 2002-2003 from two districts in eastern Slovak Republic highly contaminated by PCBs. Concentrations of 15 PCB congeners (IUPAC No. 28, 52, 101, 123+149, 118, 114, 153, 105, 138+163, 167, 156+171, 157, 180, 170, and 189) in the umbilical cord (C) and maternal serum (M) were determined.

Discussion and conclusion: Results for congeners with sufficient values above the limit of detection showed that the C/M ratios were significantly related, either positively or inversely depending on parameter, to the logarithm of partition coefficient octanol-water (K_{OW}), fusion enthalpy at the melting point, molecular weight, water solubility, total surface area of the molecule, solvent accessible surface area, melting point, molar volume, and molecular electronegativity distance vector. We found an inverse association between log K_{OW} and lipid adjusted log C/M ($\beta = -0.128$, $p < 0.001$, $R2 = 0.033$). Parameters evaluated were interrelated except fusion enthalpy at the melting point and electron affinity vs. solubility. Additionally we discuss the possible role of cholesterol as a transplacental transporter of PCBs. That PCB congener specific C/M values are related to several physicochemical parameters was expected as many physicochemical parameters of PCB congeners are interrelated.

Electromyographic investigation on pregnant rat uterus in vivo

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Key words: pregnancy, smooth muscle, electromyography

Introduction: The pregnant rat uterus contractions are generated by electrical processes. This electrical regulation is not known completely. It is assumed there are some specific cells which are responsible for the generation of electrical slow waves that control movements as in gastrointestinal tract.

Aim: We have a few possibilities to measure myometrial contractions and diagnose premature labor. Therefore we developed smooth muscle electromyography equipment containing sophisticated filters, electrodes and recording techniques for more precise analysis of smooth muscle electrical signals separating them from cardiac, brain and other electrical interferences.

Methods: We inserted silver filament electrodes into the cecum and myometrium of the anaesthetized rat. A subcutaneous electrode was placed above the uterus. Additionally, we also placed a mechanical strain gauge electrode on the surface of the uterus. Electromyographic activity was recorded by an online amplifier-computer system. We were able to use a new analysis method: we can characterize and separate electrical signs from primary curves by fast Fourier transformation.

Results: During preterm labor i.v. administrated oxytocin (1 μg/kg) increased, while i.v. terbutaline (50 μg/kg) inhibited uterine contractility. These mechanical changes were in a very close correlation with measured signs from filament electrode, and also correlated with the signals from subcutaneous electrode. The registered gastrointestinal smooth muscle signals were well separated from the myometrial signals using a special digital filter.

Conclusion: Smooth muscle electromyography seems to be a useful method both for invasive and non-invasive detection of smooth muscle function even in alert animals. This technique may have great importance as method of integrative pharmacology and a potential tool for human diagnostic.

Sources of funding: This work was supported by the project TÁMOP-4.2.2.A-11/1/KONV-2012-0035.

Cardiosphere-derived cell exosomes stimulate cardiomyocyte proliferation and angiogenesis in vitro, and improve functional recovery post myocardial infarction in mice

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Key words: MicroRNA, Exosomes, Myocardial Infarction, Cardiac Stem Cells, Regeneration

Introduction: Exosomes are nano-sized bilayer vesicles that are secreted by most cell types. Exosomes are rich in microRNAs (mirs) which may function in a paracrine fashion. Cardiosphere-derived cells (CDCs) have been shown to regenerate heart after myocardial infarction (MI) in animal models and in the CADUCEUS clinical trial. However, most of the regenerated muscle is of innate origin, which suggests indirect pathways.

Methods and results: *In vitro* and *in vivo*, we compared three treatment groups: vehicle (control), CDC-derived exosomes and normal human dermal fibroblast (NHDF)-derived exosomes. Neonatal rat cardiomyocytes (NRCMs) incubated with CDC exosomes expressed higher levels of Ki67 (CDC: 42.7 ± 0.04%, NHDF: 22.5 ± 0.04%, control: 9.1% ± 0.03, n=4, $p<0.001$) and lower expression of TUNEL (CDC: 25.2 ± 0.04%, NHDF: 45.1 ± 0.05, control: 41.4 ± 0.05%, n=4, $p<0.01$). CDC exosomes also stimulated tube formation in HUVEC cells compared to NHDF exosomes or control (CDC: 9393 ± 689; NHDF: 2813 ± 494.5; control: 1097 ± 116.1, n=3, $p<0.05$). SCID mice injected with exosomes from CDCs during acute MI showed higher LVEF at two weeks (CDC: 40.8 ± 2.33 NHDF: 32.34 ± 2.0, control: 31.31 ± 3.2, n=6, $p<0.05$) and four weeks (CDC: 44.03 ± 1.5 NHDF: 31.8 ± 1.7, control: 31.5 ± 2.7, n=6, $p<0.05$) post MI, as well as increased viable mass. Mir microarray analysis identified mir-146a among the most highly-upregulated mirs in CDC-exosomes compared to NHDF (262, fold). Mir-146a-treated NRCMs were more resistant to H_2O_2-induced stress compared to a mimic control. Array analysis of NRCMs treated showed suppression of IRAK1 and TRAF6 transcripts (1.9 and 2.0 folds lower than control respectively, n=4).

Conclusion: Mir-containing exosomes secreted by CDCs exhibit multiple beneficial effects on injured myocardium, suggesting that exosomes may mediate some of the therapeutic effects of CDCs. Most notably mir-146a provides cardioprotection in an acute model of MI.

Sources of funding: Institutional Cedars- Sinai Medical Center

Cell-based quantum dot labeling of RANK-mediated signaling mediators predicts survival of patients with prostate cancer

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Key Words: RANKL, p-c-Met, neuropilin-1, prostate cancer, survival prediction

Introduction: Metastatic spread of prostate cancer (PCa) causes significant morbidity and mortality in patients. The need for biomarkers to predict PCa lethality is acute.

Methods: Multispectral quantum dot labeling (mQDL) was used to label RANK and c-Met convergent pathway mediators simultaneously on formalin fixed paraffin embedded (FFPE) PCa tissue specimens from PCa patients with documented overall survival. The labeling intensity of tissues and cells after mQDL was quantified at the sub-cellular level, and subjected to statistical analyses.

Results: We found that: 1) Cell-based RANKL, phosphorylated-c-Met (p-c-Met) and neuropilin-1 (NRP-1) expression in cytoplasm **(C)** or nucleus (N) alone or as combination (C+N) was highly correlated with the overall survival of PCa patients ($p<0.05$). 2) RANKL, p-c-Met and NRP-1 expression intensity positively correlated with the development of castration resistant PCa (CRPC). 3) A racial disparity was found in the expression of these biomarkers, suggesting that genetic background influences PCa survival (Hu, et al. PLoS one, 2011). The overall survival of PCa patients did not correlate with patient age or pathological Gleason score.

Discussion: Our results demonstrated that activation of RANK signaling increases the expression of RANKL, p-c-Met, and NRP-1 and confers castration resistance and lethality in clinical PCa.

Conclusion: The mQDL of RANK pathway-associated biomarkers in primary PCa specimens may serve as predictors for PCa cancer progression and overall patient survival.

Sources of funding: NIH2 P01CA 98912, NIH1 R01 CA122602 and PCF Challenge Award and BOG Endowed Cancer Research Chair to LWKC.

Acknowledgements: We dedicate this study to the late Dr. John A. Arcadi for his inspirational devotion to basic and clinical urologic research.

Chelidonine interferes with IL-6R/STAT3 signaling in uveal melanoma cells

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Key words: interleukin-6, chelidonine, cell death, STAT3 activation, Bcl-2

Introduction: There is increasing evidence suggesting the importance of IL-6 in tumorigenesis. Among others, IL-6 can promote persistence of tumor cells through the inhibition of apoptosis. The major mechanism responsible for its anti-apoptotic effect is thought to be the up-regulation of Bcl-2 expression. Chelidonine was reported previously to provoke cell death in a variety of tumor cells. Herein we studied the interference of chelidonine with the IL-6R/STAT3 signaling pathway in human uveal melanoma (UM) cells.

Methods: Cell proliferation was measured using DDAO as a proliferation tracer. The apoptotic potential of chelidonine was followed by the DNA fragmentation and PI exclusion/annexin V binding assays. Expression of STAT3, Bcl-2 and IL-6Rα as well as the efficiency of STAT3 activation was assessed by immunofluorescence. Experiments were performed on FACSAria or FACSArray flow cytometers.

Results: Chelidonine inhibited the proliferation of UM cells on a dose-dependent manner and induced apoptotic (and necrotic) cell death. We could observe – even at sublethal doses of chelidonine – the appearance of cells exhibiting abrogated STAT3 phosphorylation upon IL-6 stimulation and reduced level of Bcl-2. Cells with reduced expression of STAT3 and IL-6Rα could also be detected; however the amount of these cells was significantly lower than that of cells with abolished STAT3 signaling, indicating that down-regulation of these proteins is secondary to inhibition of STAT3 activation.

Discussion: According to our results, chelidonine exerts its effect via a STAT3-dependent mechanism. Inhibition of STAT3 activation leads to down-regulation of Bcl-2 and – as a consequence – causes cell death. IL-6Rα and STAT3 itself are also down-regulated amplifying further the effect of chelidonine.

Conclusion: Our findings imply the possible use of chelidonine in the therapy of UM: it can either provoke cell death or – even at lower doses – weaken the anti-apoptotic machinery of tumor cells fueled by IL-6, therefore sensitizing them for apoptosis with other agents.

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Acknowledgement: We thank Adrienn Bagosi and Edina Nagy for their excellent technical assistance.

May 30 (Friday), 2014

Plenary session

11:00 – 12:30

Session Chairs

Livia Puljak
Chander P. Arora
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Antenatal Maternal and Fetal Steroid Treatment: Neonatal Outcome

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Key words: antenatal steroid, fetal treatment, respiratory distress syndrome, fetal growth restriction, maternal hypertension

Introduction: The utilization of antenatal corticosteroids (ANCS) in women at risk of preterm delivery has increased worldwide. Recent data suggest that in pregnancies complicated with preeclampsia (PE) and/or fetal growth restriction (FGR) the incidence of respiratory distress syndrome (RDS) is not decreased. Animal studies demonstrated that ANCS therapy resulted in impaired fetal growth; however, direct fetal glucocorticoid administration induced lung maturation without apparent effect on growth.

Methods: Ultrasonography-guided single direct fetal intramuscular injection of betamethasone (0.5 mg/kg-estimated fetal weight) for the prophylaxis of RDS was applied in pregnancies complicated with preterm premature rupture of membranes, PE, and FGR. We analyzed the perinatal data of neonates born to mothers with severe PE and/or FRG who received fetal steroid prophylaxis at our department. Infants who received fetal steroid treatment were divided into two subgroups according to the presence of maternal hypertension (maternal hypertension group: n=77, gestational age: 29.0±2.4 weeks, birth weight: 1085±361.5 g; without hypertension group: n=25, gestational age: 30.4±2.4 weeks, birth weight: 1022±260 g; mean±SD). Growth restricted infants were further analyzed and divided according to the route of steroid prevention (fetal steroid group: n=41, gestational age: 29.5±2.1 weeks, birth weight: 923±252 g; maternal steroid group: n=46, gestational age: 28.9.±2.2 weeks, birth weight: 939±220 g; no steroid group: n=27, gestational age: 28.4±2.4 weeks, birth weight: 823±240 g). Maternal steroid group included infants only after complete course of betamethasone administration. The Apgar scores, incidences of RDS, intraventricular hemorrhage (IVH), bronchopulmonary dysplasia (BPD), retinopathy (ROP), surfactant treatment, the need and duration of ventilation as well as the surviving rate were monitored. Demographic characteristics and clinical variables were compared across study subgroups using χ^2, ANOVA and Student t-tests. Linear-correlation and logistic regression analysis were employed to assess correlates of steroid prevention with clinical variables. SPSS 14.0 for Windows was used for statistical analysis. The study protocol was approved by the Regional Research Ethical Committee.

Results: The incidences of RDS, IVH, surfactant treatment, BPD, the need of ventilation, mortality rate were similar after fetal or maternal steroid prevention. The occurrence of retinopathy was less frequent after fetal treatment ($p<0.02$). When the infants born after fetal steroid treatment were stratified by the presence of PE and compared to those without hypertension the mortality was not significantly different.

However, the incidences of RDS ($p<0.001$), surfactant treatment ($p<0.01$), IVH ($p<0.03$), and the need of ventilation ($p<0.003$) were higher when the pregnancy was complicated with hypertension. In growth restricted preterm infants fetal steroid treatment resulted in significantly higher Apgar scores ($p<0.01$) and better surviving rate ($p<0.02$) compared to those who did not receive any prenatal steroid prophylaxis.

Clinical data of growth restricted infants born between the 24^{th}-32^{nd} weeks of gestation after fetal, maternal, and without steroid treatment.

Clinical characteristics	**Fetal steroid (n=41)** **I**	**Maternal steroid (n=46)** **II**	**No steroid (n=27)** **III**	**P value** **I vs II**	**P value** **I vs III**	**P value** **II vs III**
Maternal age (years)	29.2 ± 6.0	28.8 ± 5.9	28.5 ± 5.9	ns	ns	ns
Gestational age (weeks)	29.5 ± 2.1	28.9 ± 2.2	28.4 ± 2.4	ns	ns	ns
Caesarean section	41 (100%)	35 (76.1%)	23 (85.2%)	p<0.01	ns	ns
Male/Female	25/16	25/21	13/14	ns	ns	ns
Birth weight (g)	923.4 ± 252.8	939.6 ± 219.8	822.6 ± 233.9	ns	ns	ns
Apgar score 1 min	8 (7-9)	7 (6-8)	7 (4-8)	ns	p<0.005	ns
Apgar score 5 min	9 (9-10)	9 (8-9)	9 (6.5-9)	ns	p<0.012	ns
RDS	25 (60.9%)	34 (73.9%)	14 (51.8%)	ns	ns	ns
Surfactant treatment	21 (51.2%)	28 (60.9%)	12 (44.4)	ns	ns	ns
Ventilation	22 (53.6%)	32 (69.6%)	15 (55.5%)	ns	ns	ns
Infection	22 (53.6%)	21 (45.6%)	10 (37%)	ns	ns	ns
IVH	15 (36.6%)	18 (39.1%)	9 (33.5%)	ns	ns	ns
ROP	7 (17%)	19 (41.3%)	75 (18.5%)	p<0.019	ns	ns
BPD	7 (17%)	6 (13%)	1 (5.9%)	ns	ns	ns
28 day survival	36 (87.8%)	41 (89.1%)	17 (63%)	ns	p<0.012	p<0.014

Conclusion: The fetal route of steroid administration is similarly effective as maternal treatment to improve neonatal survival rate in FGR preterm infants. We also found that the occurrence of ROP was less frequent after fetal compared to maternal steroid treatment. Our results suggest that fetal betamethasone treatment between 24^{th}-32^{nd} weeks of gestation in FGR may be an alternative method to improve neonatal outcome. However, we found no apparent benefit of the use of fetal steroid in maternal hypertension with respect to RDS and IVH.

Acute stress and female hormones alter cardiomyocyte contractile function in ovariectomized rats

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Aim: To investigate the effects of acute stress on mechanical and biochemical properties of cardiomyocytes derived from control and ovariectomized female rats.

Methods: Non-ovariectomized (Control) and ovariectomized (OVX) female rats were kept under normal conditions or exposed to restraint cold stress (Control-S and OVX-S). Serum progesterone levels were measured using a chemiluminescent immunoassay. Left ventricular myocardial samples were used for isometric force measurements and protein analysis. Ca^{2+}-dependent active force (F_{active}), Ca^{2+}-independent passive force ($F_{passive}$) and Ca^{2+}-sensitivity of force production (pCa_{50}) were determined in single, mechanically isolated, permeabilized cardiomyocytes. Stress- and ovariectomy-induced myofilament protein alterations were analyzed by SDS gel electrophoresis using protein and phosphoprotein stainings.

Results: Serum progesterone levels were significantly increased in stressed rats (Control-S, 35.6±4.8 ng/ml and OVX-S, 21.9±4.0 ng/ml) compared to Control (10±2.9 ng/ml) and OVX (2.8±0.5 ng/ml) groups. F_{active} was higher in the ovariectomized groups (OVX, 25.9±3.4 kN/m^2 and OVX-S, 26.3±3.0 kN/m^2) compared to the values measured in rats with functional ovaries (Control, 16.4±1.2 kN/m^2 and Control-S, 14.4±0.9 kN/m^2). Regarding the potential molecular mechanisms, F_{active} correlated with myosin-binding protein C (MyBP-C) phosphorylation while myofilament Ca^{2+}-sensitivity inversely correlated with serum progesterone levels. $F_{passive}$ was unaffected by the treatments.

Conclusion: Stress significantly increases ovary-independent synthesis and release of progesterone, which may regulate Ca^{2+}-sensitivity of force production. Acute stress and female hormones differently alter Ca^{2+}-dependent cardiomyocyte contractile force production.

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Influence of body weight and body composition on bone mineral content and density in female rats with and without ovariectomy

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Key words: osteoporosis, high fat diet, ovariectomy, bone mass density, bone mineral content

Introduction: Obesity and osteoporosis are both multi-factorial disorders with a number of common genetic and environmental risk factors and regulatory pathways. The exact role of fat tissue in bone metabolism is still unclear. The aim of our study was to evaluate the influence of body weight (BW) changes induced by high fat diet (HFD) on bone mineral content (BMC) and bone mineral density (BMD) in rats with/without ovariectomy.

Methods: Thirty-two female rats were divided into two groups and fed with standard diet (SD) or HFD for 8 weeks. Then half number of rats from each group underwent ovariectomy and sham surgery was performed on the rest. Feeding continued with the previous diet for another 10 weeks. Data obtained from densitometry and blood analysis were compared among the following groups: sham operated rats fed with SD (Sham-SD), sham operated rats fed with HFD (Sham-HFD), ovariectomized rats fed with SD (Ovx-SD) and ovariectomized rats fed with HFD (Ovx-HFD).

Results: Final BW differed significantly among the studied groups as followed: 294.3±8.4 g in Sham-SD, 356.3±9.4 g in Ovx-SD ($p<0.05$), 390.7±22 g in Sham-HFD ($p<0.01$) and 443.1±19.9 g in Ovx-HFD ($p<0.001$). The observed BW gain corresponded with abdominal circumference (AC), body fat and abdominal fat rise as well as total bone area and total BMC in the same order respectively. On the contrary, total BMD decreased in mentioned order. The same trends were observed in the case of solo evaluation for femur, tibia and lumbar vertebra. BMD negatively and BMC positively correlated with AC, BW and fat predominantly.

Discussion and conclusion: This study demonstrates that BW increase induced by HFD, leads in young rats during their maturation to increased bone size. Simultaneous balanced acceleration of bone mineralization is sufficient to keep useful quality of bone. On the contrary, simultaneous higher bone mineralization rate in adult rats is not sufficient for production of bone with required quality. Further evaluation of selected parameters of bone remodeling in this study is prepared.

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GAP-43 as a potential marker of neuronal stress - in vivo imaging and immunofluorescence study applied on ischemic brain lesion

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Introduction: Growth Associated Protein 43 (GAP-43) is neuron specific phosphoprotein involved in neurites growth and plasticity-associated processes. It is present in the developing nervous system, but its expression is downregulated in the adult brain. The transgenic mouse model Gap-43 fluc/gfp, where luciferase was coupled to GAP-43 was created. Through in vivo imaging strong up-regulation of GAP-43 was observed in adult neurons following transient cerebral ischemia.

Methods: In order to clarify the role of GAP-43 during acute phase after transient cerebral ischemia, Gap-43 fluc/gfp reporter mice were used. The ischemic brain lesion was induced by 1 hour transient middle cerebral artery occlusion (tMCAO). The spatial and temporal dynamics of the GAP-43 were monitored in vivo using biophotonic/bioluminescence signals. GAP-43 was significantly induced 24 hours after MCAO and remained upregulated during first 4 days of acute phase that were examined. To address the question whether GAP-43 was associated with early neuronal stress, VivoGlo™ Caspase-3/7 substrate was administered to Gap-43 fluc-gfp mice 1 and 4 days after tMCAO. Upon activation of caspase-3 or -7, DEVD peptide was intracellularly cleaved and then liberated, after which aminoluciferin reacted with luciferase and generated light.

Results: This innovative application demonstrated an increase in bioluminescence signal following VivoGlo administration after tMCAO, suggesting a caspase-3 activity in the cells expressing the GAP-43 driven luciferase. Immunohistological analysis revealed localization of GAP-43 in NeuN positive cells - neurons. Furthermore, GAP-43 staining was co-localizing with cleaved-caspase-3 and ATF-3 markers, suggesting that early GAP-43 induction was associated with the early neuronal stress/apoptosis response to transient ischemic injury.

Conclusion: Based on our findings, early induction of the GAP-43 signal in ischemic neurons may be associated with the initial, ischemia-induced neuronal stress.

Sources of funding: EU FP7 project GlowBrain and CIHR (Canadain Institutes of Health Research)

Polymeric nanobioconjugates for cancer treatment

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Key words: Nanobiopolymers, multi-targeting, molecular markers inhibition, nanodrugs against brain and breast cancer

Introduction: Nanopolymers are promising vehicles for multi-targeted combined personalized therapy. A natural nanobiopolymer, polymalic acid (PMLA), was used here as a nanoplatform for the family of Polycefin™ drugs for imaging and treatment of primary and metastatic tumors.

Results: Molecular mechanisms of the nanodrug delivery into cancer cells and escape from endosomes were determined. To demonstrate therapeutic efficiency in several preclinical models we used Polycefin™ with covalently attached antisense oligonucleotides (AON) to cancer molecular markers α4 and β1 laminin chains, triple negative breast cancer (TNBC) marker EGFR, and HER2-positive breast cancer marker HER2, as well as tumor-specific monoclonal antibodies (mAb) to either EGFR (Cetuximab) or HER2 (Herceptin). Transferrin receptor (TfR) antibody was also attached for delivery through mouse endothelial system including brain blood and tumor barriers (BBB/BTB).

In brain tumors treated with Polycefin™ bearing AON to chains of tumor vascular protein, laminin-411, the vascular area was decreased and tumor size was reduced 10-fold. For HER2-positive primary breast cancer, more than 90% growth inhibition was achieved *in vivo* versus commercial therapeutic mAb Herceptin that reduced tumor size by 50%. Treatment of primary TNBC with anti-EGFR AON on Polycefin™ also significantly reduced tumor size.

Polycefin™ drugs were successfully used to treat brain metastases that are not curable by the drugs used to treat corresponding primary cancers. Animal survival after Polycefin™ treatment of lung metastasis, HER2-positive breast cancer and TNBC was significantly higher than in control animals: 65% increase for lung cancer, 47% for HER2-positive breast cancer, and 97% for TNBC.

Conclusion: We have developed versatile biodegradable, non-immunogenic and non-toxic nanoconjugates that specifically targeted brain, lung, and breast primary and metastatic tumors, inhibited the expression of multiple tumor-specific markers, caused tumor cell apoptosis, blocked their growth, and significantly increased tumor-bearing animal survival.

Acknowledgements: This work was supported by grants from NIH/NCI (R01 CA123495, R01 CA136841 and U01 CA151815 to JYL), Winnick Family Foundation Clinical Scholar award (to JYL), and Martz Breast Cancer Research Fund (to EH).

Section-specific determination of smooth muscle activity by smooth muscle electromyography in normal and immobilized rats

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Key words: SEMG, smooth muscle, electric activity, stress

Introduction: Smooth muscle electromyography (SEMG) is a method to detect and follow the activity of smooth muscles in vivo. During the last 5 years cooperating with Experimetria Ltd., Hungary we have developed new SEMG equipment proper for fine detection and analysis of changes in smooth muscle electric activity. The recent aim of our study was to characterize the SEMG signals of different sections of gastrointestinal tract (GI). Our other aim was to determine the stress-induced alteration of SEMG signals in GI tract.

Methods: For the SEMG signal characterization male SPRD rats were anesthetized with the combination of ketamine and xylazine. Only the stomach or small intestine (ileum) or large intestine (coecum) was left, the rest parts of the GI tract were removed from the abdomen. Mechanical sensor (strain gauge) and filament electrode have been inserted into the target organ, while subcutaneous electrode has been placed in the abdominal area. The mechanical and the electric signals have been recorded in parallel. In the next series of experiment we used non-anesthetized, gastrointestinally intact rats with subcutaneous electrode. For half an hour the rats were recorded without restriction in their moves, then they were immobilized for another 30 min.

Results: The fast Fourier transformation revealed that the characteristic signals for stomach, ileum and coecum can be found at 3-5, 20-25 and 0-3 cycle per minute (CPM), respectively. Although we found some discrepancy between the signals of the implanted and subcutaneous electrodes in the ileum, we suppose that some parts of the ileal signal may be lost on the abdominal surface.
The results were analyzed by the CPM values gained from the previous experiment. We found that immobilization enhanced the electric activity of the given GI tract sections. The signal intensities in the coecum, ileum and stomach were increased by 140, 100 and 90%, respectively.

Discussion and Conclusion: We can conclude that the SEMG signals from the different sections of the GI tract can be separated and the most sensitive organ to immobilization-induced stress is the large intestine. Our method may be useful for the measurement of stress level in vivo.

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May 30 (Friday), 2014

Plenary session

14:00 – 16:00

Session Chairs
Rostyslav Stoika
Jan Pitha
Leland Chung

Perinatal hypoxia: Different effects of the inhibitors of GABA transporters GAT1 and GAT3 on the initial velocity of [^{3}H]GABA uptake by cortical, hippocampal and thalamic nerve terminals

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Key words: perinatal hypoxia, β-alanine, NO-711, plasma membrane GABA transporters, synaptosomes, rat brain cortex, hippocampus, thalamus

Introduction: Perinatal hypoxia leads to multiple chronic neurological deficits including mental retardation, learning and memory disability, behavioral abnormalities and epilepsy. The effects of highly selective blocker GAT1, NO-711, and substrate inhibitor GAT3, β-alanine, on the initial velocity of [^{3}H]GABA uptake by cortical, hippocampal and thalamic nerve terminals (synaptosomes) were analyzed after perinatal hypoxia.

Methods: Rats were underwent to hypoxia and seizures (airtight chamber, 4% O_2 and 96% N_2) at the age of 10-12 postnatal days and used in the experiments 8-9 weeks after hypoxia.

Results: The effects of NO-711 (30 μM) and β-alanine (100 μM) on [^{3}H]GABA uptake by cortical synaptosomes were similar in control and hypoxia. In hippocampal synaptosomes, NO-711 inhibited 84.3% of the initial velocity of [^{3}H]GABA uptake in norm and 80.1% after hypoxia, whereas the effect of β-alanine was increased after hypoxia from 14.4% to 22.1%. In thalamic synaptosomes, the inhibitory capacity of NO-711 was decreased from 79.6% in control to 70.9% after hypoxia, whereas the effect of β-alanine was increased after hypoxia from 20.2% to 30.2%.

Conclusion: Therefore, the effectiveness of β-alanine to influence GABA uptake was increased in hippocampal and thalamic nerve terminals because of perinatal hypoxia, whereas the capacity of NO-711 in thalamic nerve terminals was decreased. These results may indicate changes in the ratio of active GAT1/GAT3 expressed in the plasma membrane of nerve terminals after perinatal hypoxia. A principal possibility of non-GAT1-targeting modulation of GABA transporter activity in different brain regions by exogenous and endogenous β-alanine was suggested.

Contribution of myofilament protein carbonylation to systolic and diastolic dysfunction

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Introduction and Methods: The role of oxidative modifications of myofilament proteins in systolic and diastolic dysfunctions were investigated in left ventricular (LV) murine cardiomyocytes 10 weeks after ligation of the left anterior descending (LAD) coronary artery, and in human cardiomyocytes after in vitro exposures to Fenton reagents. Mechanical and biochemical characteristics of cardiomyocytes were analyzed in detail.

Results: In murine hearts: Ca^{2+} sensitivity of force production was significantly lower in the anterior wall (pCa50: 5.81 ± 0.03, means ± SEM, at 2.3 µm sarcomere length) than that in the controls (pCa50: 5.91 ± 0.02) or in the MI inferior area (pCa_{50}: 5.88 ± 0.02), and thus these changes implicated a contribution to systolic myocardial dysfunction. In addition, significantly higher carbonylation levels, [e.g. in myosin heavy chain (MHC) and actin] were observed in the MI anterior wall [carbonylation index (CI), CI_{MHC}: 2.06 ± 0.46, CI_{actin}: 1.46 ± 0.18] than in the controls (CI: 1). In vitro Fenton-based myofilament carbonylation in the control murine and human cardiomyocytes also decreased the Ca^{2+} sensitivity of force production, and had the potential to increase passive force ($F_{passive}$) suggestive for a role in diastolic dysfunction. Furthermore, the Ca^{2+} sensitivity correlated strongly with myofilament carbonylation levels in all investigated samples.

Conclusion: Post-MI myocardial remodelling involves increased myofibrillar protein carbonylation and decreased Ca^{2+} sensitivity of force production, leading potentially to systolic contractile dysfunction in the remaining cardiomyocytes of the infarcted area.

Acknowledgements: This research was supported by the Hungarian Scientific Research Fund (OTKA K 109083), co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 "National Excellence Program" (A.B.) and by the Social Renewal Operational Programme (TÁMOP-4.2.2.A-11/1/KONV-2012-0045). The support of the Cedars Sinai Medical Center's International Research and Innovation Management Program and the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) is greatly appreciated.

Impact of ovariectomy, high fat diet and lifestyle modifications on oxidative/antioxidative status in rat liver

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Key words: liver, oxidative damage, ovariectomy, high-fat diet, physical activity

Aim: The aim of this study was to estimate the impact of high fat diet (HFD) and estrogen deficiency on the oxidative and antioxidative status in liver of the ovariectomized (OVX) rats, and to investigate possible ameliorating effect of lifestyle modifications, such as physical activity or consumption of functional food containing bioactive compounds with antioxidative properties, on oxidative damage in rat liver.

Materials and methods: As an indicator of liver oxidative damage, lipid peroxidation (LPO) levels expressed in terms of thiobarbituric acid reactive substances (TBARS) were determined, while liver antioxidative status was determined by catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR) activities and glutathione (GSH) content. Sixty-four female Wistar rats divided into eight groups were tested: sham operated (SH) and OVX rats that received standard diet (SD) or HFD, SH and OVX rats that received HFD and were subjected to physical activity, and SH and OVX rats received HFD supplemented with cereal selenized onion biscuits (SOB).

Results: Results showed that HFD significantly increased TBARS content in liver relative to control groups that received SD. Furthermore, HFD decreased antioxidant defense capacities, as evaluated by the significant decrease in the activities of CAT, GR and GST as well as in content of GSH. GPx activity remained unchanged in all groups tested. Physical activity and SOB showed protective effect through increased GR activity in SH rats, while in OVX rats CAT activity was increased in rats that additionally received SOB.

Discussion and Conclusion: Feeding rats with HFD was accompanied by decreased antioxidative enzyme activities and increased LPO, in both OVX and SH rats. Decreased antioxidant defense suggests lowered oxidative stress resistance, which could be reflected in oxidative damage of rat liver and metabolic disorders. Bioactive compounds of SOB showed potential in attenuating adverse impact of HFD on antioxidative status.

Sources of funding: "Center of excellence of environmental health" project, ITMS No.26240120033, based on the supporting operational Research and development program financed from the European Regional
Acknowledgement: Women's Health and Cardiovascular Diseases Research Network of Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium formed by Cedars-Sinai Medical Center (CSMC), Los Angeles, CA, USA.

Alginate a stabile and biocompatible stem cells-seeded hydrogel carrier for the targeted delivery in the stroke lesion

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Key words: neural stem cells, alginate, stroke, mouse

Introduction: Stroke-induced lesion site is characterized by necrotic loose tissue which can accept relatively high volume injections without damaging brain tissue, and thus it is appealing site for therapeutic actions. Stem cell therapy for tissue regeneration and replacement of the lost cells after stroke can be directed to lesion site by using different hydrogels as a physical and biological support for cells. Natural polysaccharide alginate was proved to improve biological fate of cells in the three dimensional cultures. We hypothesised that encapsulation of neural stem cells (NSCs) within alginate matrix will enhance their viability, proliferation, differentiation *in vitro* and provide a stabile physical support for the cells *in vivo*.

Methods: NSCs were isolated from the brains of 14 days old mouse fetuses and cell encapsulation was achieved by mixing the cell slurry with alginate and dropping into a 0.1M calcium chloride solution for 10 min. Soft alginate bead size was standardized to 3 mm in diameter. After 9 days following encapsulation, immunocytochemistry, live/dead test and DNA quantification tests were performed. Encapsulated NSCs were stained with PKH26 stain and then injected stereotaxically to the mouse brain. After the injection on day 1 and day 3 Nissl staining and light and fluorescence microscopy were performed.

Results: NSCs were viable 9 days after encapsulation within alginate beads and the number of cells increased by five folds. They also expressed Map 2 marker, confirming the differentiation to neurons. PKH26 stain and Nissl staining showed alginate hydrogel- seeded NSC population at the injection site 1 and 3 days after the injection.

Discussion: Our results proved efficacy of alginate hydrogel in improving NSCs biological outcome *in vitro.* Moreover, it also provides a stabile physiological structure to entrap NSCs at the injection site, where their therapeutic activity is needed.

Conclusion: Alginate hydrogel may serve as stabile and biocompatible physical support of NSCs in the lesion site-directed stem cell therapy after stroke.

Sources of funding: The study was supported by EU FP7 grant GlowBrain (REGPOT–2012–CT2012–316120).

RANK- and c-Met-mediated feed forward signal network promotes metastasis by recruiting dormant cells and predicts the survival of prostate cancer patients

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Key Words: RANKL, RANK, c-Met, Prostate Cancer, Metastasis

Introduction: Prostate cancer (PCa) metastasis to bone is lethal and there is no adequate model to study the mechanisms underlying the metastatic process and the predictor for the progression and survival of PCa patients.

Methods: Cells with activated receptor activator of NF-κB ligand (RANKL, or called MICS, the Metastasis-Initiating Cells) were co-inoculated with cells devoid of RANKL (the bystander or dormant cells) and the formation of bone and soft tissue metastases were determined and characterized.

Results: Activated RANK-mediated signaling network promotes a feed forward loop, involving the induction of RANKL and c-Met, but repression of androgen receptor (AR) expression and AR signaling pathways. Site-directed mutagenesis and transcription factor deletion/interference assays identified common transcription factor complexes (TFs), c-Myc/Max and AP4, as critical regulatory nodes. RANKL-RANK signaling activated a number of master regulator TFs that control the epithelial-mesenchymal transition (EMT) (Twist1, Slug, Zeb1, Zeb2), stem cell properties (Sox2, Myc, Oct3/4 and Nanog), neuroendocrine differentiation (Sox 9, HIF-1α and FoxA2) and osteomimicry (c-Myc/Max, Sox2, Sox9, HIF1α and Runx2). Abrogating RANK or its downstream c-Myc/Max or c-Met signaling network, abolished PCa skeletal metastasis in mice. RANKL-expressing LNCaP cells recruited and induced neighboring dormant cells to express RANKL, c-Met, p-c-Met, while downregulated AR expression. These initially non-tumorigenic dormant cells, once retrieved from the tumors, acquired the potential to colonize and grow in bone. In clinical PCa tissues, we found activated RANK-mediated signal network predicts the survival of PCa patients.

Discussion: RANK-mediated signal network confers cancer metastasis through a well-orchestrated and coordinated feed forward action and can be further developed as valuable biomarkers predicting the progression and survival of cancer patients.

Conclusion: We propose a novel mechanism in which recruitment and induction of dormant cells by MICs via a feed forward action is involved in the development of cancer metastasis in PCa patients.

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A pilot study applicability of standardized modified method of dry (throat/buccal) swabs in PCR diagnosis of enteroviral infections

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Key words: enteroviruses, throat swabs, buccal swabs, diagnosis, PCR

Introduction: Enteroviruses (EVs) spread via the fecal-oral route. They infect the oro-pharyngeal mucosal cells and tonsils. After replicating at these sites, they pass on to the alimentary tract. We standardized modified methodology of dry throat swabs for EV diagnosis by PCR. Among the commonly collected material for diagnosis besides the stool and blood samples are throat swabs, used for isolations and identifications and PCR diagnosis. The EVs are relatively stable. Commonly for diagnosis the throat swabs are collected in transport medium. We recently standardized the dry swab method and checked the applicability in 10 random volunteers. The rationale of the present study is to standardize the sample collection for diagnosis/screening of EV infections, and to make the sample collection as convenient the patient, staff, and transportation.
Methods: Thirty seven volunteers (RECOOP HST Association 4th TriNet Meeting participants) from Croatia, Czech Republic, Hungary, Poland, Romania, Slovak Republic, Ukraine and USA attended the pilot screening study. The sample processed in the Enterovirus laboratory, Slovak Medical University (SMU), Bratislava. As buccal swabs are more convenient to obtain, we collected buccal swabs (two swabs per person for diagnosis of EVs) were from volunteers. Swab-A inserted in sterile test tube and immediately frozen at -80°C and transported from Split to Bratislava on dry ice. Swab-B allowed drying for 1 hour at ambient temperature and processed (vortexed in RNAse-free water) after that the Swab B samples were transferred from Split to Bratislava on dry ice. The resulting suspension was frozen at -80°C. RNA isolation and reverse transcriptase and nested PCR were performed in Bratislava at SMU.
Results: Viral RNA was detectable in 6/37 (16.22%) volunteers as compared to 7/10 (70%) throat swabs collected during standardization. In the present study positive samples were random in different countries. Evaluation on terms of intensity of the PCR reaction showed that Swab-A (immediately frozen at -80°C) methodology was more effective for screening or diagnosis.
Conclusion: In the samples collected during the RECOOP HST Association meeting, we did not observe a high incidence of EV infections. We conclude the dry swab- method is efficient. We recommend that prior to transport to the Virological laboratory 1.the swabs should be dried at ambient temperatures (if in absence of transport medium), inserted into the sterile containers, 2.frozen at -20°C to -80°C or storage at 4°C depending on availability of freezers and quickness of transport to the Virological laboratory, 3. Considering the viral pathogenesis and our comparative results of the throat swabs (without ruling out the possibility that the throat swabs could have been collected when the virus was endemic) vs. buccal swabs we suggest the throat swabs to be the choice for collection of samples though they maybe less convenient for the patient and staff.

Sources of funding: The Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association); NRC for Enterovirus Laboratory from Ministry of Health Slovak; Norwegian Financial Mechanism, Mechanism EEA and Slovak Government and the State Budget of the Slovak Republic (SK 0082), "Center of excellence of environmental health", ITMS No.24240120033.
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May 30 (Friday), 2014

Poster Session

16:00 – 18:00

Session Chairs
Julia Y. Ljubimova
Shubhada Bopegamage
Robert Gaspar

Characteristics of autoimmune diseases in patients from Osijek- Baranja County (Croatia) – a population study

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Key words: autoimmune diseases, rheumatoid arthritis, population Study

Introduction: The aim of this study was to define characteristics of autoimmune diseases (AID) in population of Osijek – Baranja county (Croatia).
Methods: More than 10,000 medical histories taken at Department of Rheumatology, Clinical Immunology and Allergology at the Clinical Hospital Centre Osijek (KBC Osijek) were analysed and resulted in a data basis of more than 1000 AID patients.

Results: The average age of AID patients included in this study was 58 years. Most frequent AID in the studied population was Rheumatoid Arthritis (RA), followed by SLE, while the least common AID was vasculitis. All AID are more common in women. Consistent to general prevalence of AID, the most frequent AID in both sexes is RA, followed by SLE, whereas Sjogren's syndrome was the least common in men, and PM/DM and vasculitis in women. Patients in the youngest age group suffer from Systemic sclerosis (SSc), SLE and RA. Autoantibody profile found in patients treated at KBC Osijek is consistent with the data available from scientific literature and other epidemiological studies. Joint pain is present in approximately 50% of patients regardless of disease, while the general fatigue was present in between 15% (SSc) and 54% of patients (vasculitis). The most common complications in our patients are cardiovascular diseases and osteoporosis. All diseases are more frequent in older age groups (55-64 years and > 65 years).

Discussion: The highest incidence of complications in higher age groups could be explained by the highest incidence of AID in middle age groups and chronic course of the diseases. Osteoporosis is caused apart of the age and reduced physical activity also by the corticosteroid drugs.

Conclusion: Clinical findings on AID population in Osijek – Baranja are consistent to previous reports in other countries; however, there are also some discrepancies that should be considered when planning AID treatment. This databasis presents a valuable tool for further investigations on AID.

Acknowledgements: This project was supported by IPA Hungary – Croatia Cross-border Co-operation Programme, HEALTH IMPULSE project (HUHR/1001/2.1.3/0006).

Androgenic effect of honeybee drone milk in castrated rats

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Key words: androgen, prostate, fatty acids, rat

Introduction: Numerous honeybee *(Apis mellifera)* products have been used in traditional medicine to treat infertility and to increase vitality in both men and women. Drone milk (DM) is a relatively little-known honeybee product with a putative sexual hormone effect. The oestrogenic effect of a fraction of DM has recently been reported in rats. However, no information is available on the androgenic effects of DM. Aim. The purpose of the present study was to determine the androgen-like effect of DM in male rats and to identify effective compounds.

Methods: A modified Hershberger assay was used to investigate the androgenic effect of crude DM, and the plasma level of testosterone was measured. The prostatic mRNA and protein expression of Spot14-like androgen-inducible protein (SLAP) were also examined with real-time PCR and Western blot techniques. GC-MS and NMR spectroscopic investigations were performed to identify the active components gained by bioactivity-guided fractionation.

Results: The crude DM increased the relative weights of the androgen-dependent organs and the plasma testosterone level in castrated rats and these actions were flutamide-sensitive. DM increased the tissue mRNA and protein level of SLAP, providing further evidence of its androgen-like character. After bioactivity-guided fractionation, two fatty acid esters, methyl palmitate (MP) and methyl oleate (MO), were identified as active compounds. MP alone showed an androgenic effect, whereas MO increased the weight of androgen-sensitive tissues and the plasma testosterone level only in combination.

Conclusion: The experimental data of DM and its active compounds (MO and MP) show androgenic activity confirming the traditional usage of DM. DM or MP or/and MO treatments may project a natural mode for the therapy of male infertility.

Cardiovascular and cerebrovascular complications in rheumatoid arthritis patients at Osijek Baranja County (Croatia)

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Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease affecting joints, but also other visceral organs. It is three times more common in women than men. It has been shown that persistent inflammation can cause endothelial dysfunction which leads to vascular disease. The objective of this study was to investigate the occurrence of cardiovascular and cerebrovascular complications in patients in different stages of rheumatoid arthritis.

Methods: A retrospective analysis of data obtained from the records of RA patients (N=647) from Clinical Hospital Centre Osijek, Croatia was performed. The study was approved by the Ethics Committee of the Faculty of Medicine University of Osijek. SPSS software package (Statistics for Windows 17.0. Chicago: SPSS Inc.) was used for data analysis and $p<0.05$ was considered statistically significant. Given the type of data chi square test ($\chi 2$) and Fisher's exact test was used.

Results: Patients' average age was 64.1 years. 523 (80.8%) RA patients were females. Most of patients had disseminated disease affecting small, medium and large joints (29.1%) Incidence of cardiovascular and cerebrovascular complications was higher in men than women (5.6% and 11.3% compared to 2,6% and 7, 1%, respectively). Of cardiovascular complications the most frequent in women was arrhythmia, while men presented with arrhythmia and heart attack.

Discussion: Inflammation present in RA can be important trigger causing atherosclerosis. Literature shows that incidence of deaths due to cardiovascular disease is 50% higher than in general population. This has practical importance in treating RA patients, who should be considered a high risk group similar to diabetic patients.

Conclusion: Our results showed a statistically significant difference in frequency of cardiovascular and cerebrovascular complications between male and female patients suffering from RA.

Acknowledgement: This project was supported by IPA Hungary – Croatia Cross-border Co-operation Programme, HEALTH IMPULSE project (HUHR/1001/2.1.3/0006).

Lifestyle intervention in general practice for physical activity, smoking, alcohol consumption and diet in elderly

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Key words: physical activity; smoking; alcohol; diet; elderly; general practice

Introduction: Physical inactivity and sedentary lifestyle are the hallmarks of modern human behavior worldwide. Smoking is one of the most important risk factors for atherosclerosis, cardiovascular (CV) disease and many cancers. Modern diet is rich in calories, saturated fat and refined carbohydrates. Many studies have shown that Mediterranean diet, based on olive oil, nuts, fish and poultry, fruits and vegetables, brown bread and complex carbohydrates has a protective effect on CV system. Longitudinal studies of elderly European populations have shown that the health behavior pattern that includes a healthy diet, non-smoking and moderate physical activity was associated with lower risk of morbidity and overall mortality. The aim was to compare the effectiveness of programmed and intensified intervention on lifestyle changes, including physical activity, cigarette smoking, alcohol consumption and diet, in patients aged ≥ 65 with the usual care of general practitioners (GP).

Methods: In this multicenter randomized controlled trial, 738 patients aged ≥ 65 were randomly assigned to receive intensified intervention (N=371) or usual care (N=367) of a GP for lifestyle changes, with 18-month follow-up. The main outcome measures were physical activity, smoking, alcohol consumption and diet. The study was conducted in 59 general practices in Croatia between May 2008 and May 2010.

Results: The patients' mean age was 72.3 ± 5.2 years. Significant diet correction was achieved after 18-month follow-up in the intervention group, comparing to controls. More patients followed strictly Mediterranean diet and consumed healthy foods more frequently. There was no significant difference between the groups in physical activity, tobacco smoking and alcohol consumption or diet after the intervention.

Conclusion: An 18-month intensified GP's intervention had limited effect on lifestyle habits. GP intervention managed to change dietary habits in elderly population, which is encouraging since elderly population is very resistant regarding lifestyle habit changes.

Clinical trial registration number: ISRCTN31857696.

Acknowledgements: We are grateful to patients and physicians who participated in the study.

Impact of adipose tissue inflammation in the pathogenesis of atherosclerosis

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Key words: atherosclerosis – adipose tissue – inflammation – macrophages

Introduction: Atherosclerosis and its clinical complications are still the most common cause of death in developed countries. In addition to hypercholesterolemia, it is driven by inflammatory cells, mainly by macrophages. We focused on identifying specific phenotypes of macrophages connected with proinflammatory status in adipose tissue of living kidney donors.

Methods: Anthropometric characteristics including body mass index and clinical data were collected from all subjects. Samples of 2,5 grams of subcutaneous, perirenal and perivascular (surrounding a.renalis) adipose tissue were exposed to collagenase and then repeatedly filtered and purified and stroma vascular fraction (SVF) was eluted from the sample. SVF was then labeled with monoclonal antibodies conjugated with fluorochromes (CD14, CD16, CD36, CD163, CD68 and calprotectin) and subsequently analyzed by flow cytometry. The same surface markers were determined in blood samples of the subjects.

Result: High expression of calprotectin and CD16 marker in adipose tissue macrophages of living kidney donors was found, whereas blood monocytes did not express calprotectin with only a minor population expressing CD16. Although number of macrophages in subcutaneous adipose tissue was lower compared to perirenal and perivascular adipose tissue, only in this tissue we observed a positive correlation ($p<0,05$) of number of CD14+ monocytes and CD14+calprotectin+monocytes with body mass index.

Conclusion: The presented method allowed to detect several types of monocyte/macrophage populations. We found fundamental differences between characteristic markers of blood monocytes and adipose tissue macrophages. Furthermore we demonstrated positive correlation of BMI with number of CD14+ monocytes and CD14+calprotectin+ monocytes in subcutaneous adipose tissue of kidney donors.

Sources of funding: Supported by the project (Ministry of Health, Czech Republic) for the development of research organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support.

Acknowledgements: We thank the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association).

Prevalence of cardiovascular complications in three age groups of patients with Systemic Lupus Erythematosus (SLE)

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Key words: systemic lupus erythematosus, cardiovascular complications

Introduction: Systemic lupus erythematosus (SLE) is an autoimmune chronic generalized connective tissue disease. Vascular disease is the leading cause of death in patients with SLE. Vascular complications in patients with SLE can affect arteries of all sizes, but usually a small or medium-sized arteries are affected which leads from mild skin changes to severe organ failure, including renal failure, myocardial infarction, cerebrovascular accident and antiphospholipid syndrome.

Methods: A retrospective analysis of data collected from 143 medical records of male (12) and female (131) SLE patients (<34 (N=50), 35-49 (N=57) and> 50 years of age (N=31)) and a cross-sectional study of microcirculatory flow measured using Laser Doppler Flowmetry (LDF) were conducted (N=4 per each age group). All data were analyzed in IBM SPSS Statistics 20.

Results: Correlation between age when SLE was diagnosed and cardiovascular complications is significant at the risk level of 6% ($r = 0.15$, $p = 0.055$). Similar to previous findings, cardiovascular complications are more frequent in older patients. In contrast, there is no significant correlation between the duration of disease and the prevalence of complications and co-morbidities. In addition, there was no statistically significant difference in microcirculatory flow among the age groups.

Discussion: Previous studies by LUMINA showed that patients diagnosed SLE diagnosed in adolescence, have a longer and more frequent periods of active disease, results more frequently inkidney disease. Our study did not show such results, possibly due to limitations which include non-systematised database and lack of information on the age when the disease occurred for all patients and the period of taking medications.

Conclusion: In order to obtain valid results, further analysis should be carried out, that would include a larger number of laboratory results monitored over a longer period of time, the duration of the disease, and other parameters that could affect the cardiovascular system, including the incidence of cardiovascular complications.

Acknowledgements: This project was supported by IPA Hungary – Croatia Cross-border Co-operation Programme, HEALTH IMPULSE project (HUHR/1001/2.1.3/0006).

Cutaneous expression of calcium/calmodulin-dependent protein kinase II following diabetes induction

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Key words: diabetes mellitus, CaMKII, skin

Introduction: Diabetes mellitus (DM) is the leading cause of peripheral neuropathy in the Western world. Calcium/calmodulin-dependent protein kinase II (CaMKII) is one of the key intracellular signaling molecules for neuropathy development. We analyzed the expression of total CaMKII (tCaMKII) and its alpha isoform in the foot pad skin of animal models of diabetes type 1 (DM1) and type 2 (DM2).

Methods: Male Sprague-Dawley rats were used (n=40). DM1 was induced with intraperitoneal injection of streptozotocin (STZ) and DM2 with a combination of STZ and high-fat diet. Control rats for both DM1and DM2 were injected with pure citrate buffer solution. Two months after induction of diabetes rats were sacrificed; skin samples from plantar surface of the both hind paws were removed. Immunohistochemistry was performed for detection of tCaMKII and its alpha isoform. For detection of peripheral nerve fibers we used polyclonal antiserum against protein gene product 9.5 (PGP 9.5).

Results: The results showed that CaMKII was expressed in the skin of both diabetic models. Total CaMKII was uniformly distributed throughout the epidermis and pCaMKIIα was limited to stratum granulosum. Both tCaMKII and pCaMKIIα were not visible in neurofibers. Two months after induction of diabetes in rats, significant increase in epidermal expression of tCaMKII and pCaMKIIα was observed in DM1 animals compared to controls, but not in DM2 animals.

Discussion: CaMKII was previously found in subepithelial and intraepithelial nerve fibers of facial skin, nasal mucosa and palate. This study is the first description of cutaneous CaMKII expression pattern in a diabetic model. CaMKII could play a role in transformation of skin layers and contribute to cutaneous diabetic changes.

Conclusion: These results indicate potential role of CaMKII in diabetic neuropathy development. Further research on physiological role of CaMKII in skin and its role in cutaneous diabetic complications should be undertaken in order to elucidate its function in epidermis.

Sources of funding: The study was funded by the Croatian Foundation for Science (HRZZ) grant no. 02.05./28 awarded to Livia Puljak.

The differentiation of SH-SY5Y neuroblastoma cells – the effect of the antidepressants

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Key words: neuroblastoma, CREB transcription factor, depression, antidepressants

Aims: In this work, we aimed to study the differentiation inducing effect of various antidepressants in human SH-SY5Y neuroblastoma cell line. The activation of CREB pathway and expression of BDNF are also to be examined.

Background: Depression is a common mental disorder that affects millions of people worldwide. Its pathomechanism is still not clear, however recent studies have suggested that the inappropriate production of the neurotrophic factor BDNF and the impaired activation of the CREB transcription factor may play a role in the development of depression. The activation of the BDNF-CREB pathways can have neuroprotective effect and may stimulate the neurogenesis and neuronal differentiation.

Methods: Human SH-SY5Y neuroblastoma cells were used to investigate the effect of citalopram, venlafaxine and imipramine on differentiation, CREB expression and phosphorylation and BDNF synthesis. Phorbol 12-myristate 13-acetate and retinoic acid were used as positive control. Differentiation of the cells was detected by immunocytochemistry staining of neuron specific beta-3 tubulin. The protein expression and phosphorylation of CREB were evaluated by using western blot technique. BDNF expression was studied on the mRNA level by real time PCR method.

Results: The antidepressant-treated cells, especially the citalopram-treated ones have shown morphological changes similar to the positive control cells. The BDNF expression was increased in the antidepressant treated cells. On the other hand, CREB expression and phosphorylation have not been altered significantly.

Conclusion: The effect of the antidepressants on the neuronal differentiation and the enhancement of BDNF expression have been demonstrated. This process however seems rather unrelated to the cAMP-PKA-CREB pathway.

The expression of calcium/calmodulin-dependent protein kinase II in spinal cord in rat models of type 1 and type 2 diabetes

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Key words: diabetic neuropathy, CaMKII, spinal cord, dorsal horn

Introduction: Diabetic neuropathy is one of the most common complications of the diabetes. The activation of calcium/calmodulin-dependent protein kinase II (CaMKII) has been suggested as a key player in chronic pain development. Therefore, the aim of this study was to investigate the expression of CaMKII in the dorsal horn of spinal cord in early model of DM type 1 and type 2.

Methods: Sprague-Dawley rats (n=40) were used. DM1 model was induced after overnight fasting with 55 mg/kg of streptozotocin (STZ) and DM2 model using combination of high-fat diet and low-dose STZ. Pure citrate buffer solution was intraperitoneally injected to control rats for DM1and DM2. Rats were sacrificed two weeks and two months after diabetes induction. The expression of total CaMKII and its alpha isoform in dorsal horn was quantified using immunohistochemistry.

Results: Increased expression of tCaMKII and pCaMKIIα was seen in dorsal horn of DM1 animals 2 weeks and 2 months after diabetes induction. In DM2 animal model, similar change in the expression of tCaMKII and pCaMKIIα was visible after 2 weeks, but not after two months. The expression of pCaMKIIα was pronounced the most in laminae I-III. Difference in the IB4 expression was not observed between groups.

Discussion: Studies has shown increased expression of CaMKII in dorsal root ganglia in rat models of DM1 and DM2 two weeks and two months after diabetes induction. Changes in the CaMKII expression were also observed in DRG and dorsal horn six months and one year following induction of DM1. However, the expression pattern of CaMKII in dorsal horn in early diabetes has not been reported yet.

Conclusion: The observed changes in the expression of CaMKII and its alpha isoform may be involved in the neuropathic pain development early in DM1, but not in DM2. CaMKII may be a suitable target for diabetic pain treatment.

Key words: diabetic neuropathy, CaMKII, spinal cord, dorsal horn

Sources of funding: The study was funded by the Croatian Foundation for Science (HRZZ) grant no. 02.05./28 awarded to Livia Puljak.

Validation of an aminonaphthalene trisulfonate labeled N-glycan database by CGE

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Key words: aminonaphthalene trisulfonate; glycan database; capillary gel electrophoresis

Introduction: In the post-genomic area the next major challenge will be identification and understanding of post-translational modifications, especially glycosylation. Nowadays high-performance analytical techniques play a crucial role in the structural elucidation of carbohydrates in biomedical and biopharmaceutical fields. The frequently used techniques for sugar analysis are high performance liquid chromatography (HPLC), capillary electrophoresis (CE) and mass spectrometry (MS) or some combinations of those.

Methods: The glycan database for 8-aminonaphtalene-1,3,6-trisulfonic acid labeled carbohydrates was generated by a novel capillary gel electrophoresis system with light-emitting diode induced fluorescent detection (CGE-LedIF).

Results: At this stage, information about 25 oligosaccharide standards are in the glycan database including glucose unit values (GU) with corresponding standard deviations, the abbreviated names of the carbohydrates with their structural formula as well as molecular masses. Validation of the generated GU values was clarified with N-glycans released from immunoglobulin G and bovine ribonuclease B containing core fucosylated biantennary structures with sialic acid residues as well as high-mannose structures, respectively. Nevertheless, this novel CGE system offers a rapid carbohydrate investigation tool (<220 sec) for data interpretation.

Discussion and conclusion: Research on these topics also involves exoglycosidase digestion of the individual sugar structures that is used for further structural elucidation in our laboratories. In the future we envision this novel application to provide a broadly applicable bio-analytical toolset for glycan analysis in biotechnology and clinical samples.

Acknowledgements: This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP-4.2.4.A/ 2-11/1-2012-0001 ‘National Excellence Program’ and the MTA-PE Translation Glycomics Grant (#97101).

In vivo bioluminescent imaging of inflammation dynamics after different MCAO periods

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Key words: inflammation, brain, blood flow, occlusion, bioluminescent imaging

Introduction: The TLR2 pathway is activated during inflammation after stroke playing an important role in the development of edema and subsequent lesion size. Bioluminescent imaging is a novel approach to visualizing the dynamics of in vivo processes on a same mouse through time.

Methods: To determine the effect of length of ischemia on inflammation in the brain, we used TLR2-GFP-luc transgenic mice and detected the bioluminescent signal through 15 days post middle cerebral artery occlusion (MCAO). During the operation, decrease of cerebral blood flow was monitored by LASER Doppler and the filament occlusion was held during 30, 60 and 90 minutes. TLR2 expression was visualized on experimental and sham operated mice by detecting luciferase generated light by using Xenogen IVIS imaging system in sequential time points from acute to the chronic phase of reperfusion.

Results: A strong induction of TLR2 signal was shown in the first 5 days following stroke in all experimental groups. Preliminary results confirm a correlation between occlusion time and the intensity of inflammation. There was a high individual animal variability regardless of occlusion time, showing the importance of collateral vasculature variations and increasing the need for animals in each group.

Conclusion: Intensity of inflammation after the stroke shown by TLR2-GFP-luc transgenic mice was dependent on occlusion time.

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Functionalized core-shell porous silica/maghemite nanoparticles: Design, synthesis and immunotoxicity

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Key words: maghemite, nanoparticles, core-shell, phagocytic activity

Introduction: Surface modified superparamagnetic iron oxide nanoparticles (NPs) are becoming increasingly important in a wide range of applications in medicine and biotechnology. Aim of this study was to synthesize such silica-coated magnetic NPs and investigate their possible effect on phagocytic activity and respiratory burst of human peripheral blood leukocytes.

Methods: Maghemite (γ-Fe_2O_3) NPs were obtained by the coprecipitation of Fe^{+2} and Fe^{+3} salts with ammonium hydroxide followed by controlled oxidation with NaClO. The γ-Fe_2O_3 NPs were modified by tetramethyl orthosilicate, (3-aminopropyl)triethoxysilane and 3-(2-aminoethylamino)propylmethyldimethoxysilane. The coated γ-Fe_2O_3 NPs were characterized by scanning and transmission electron microscopy, dynamic light scattering, FT IR spectroscopy, elemental analysis, BET surface area and EDAX.

Results and Discussion: The average size of the NPs increased from 9 to 13 and 30 nm after the modification with silica. Also the zeta potential increased from -41 mV to +30 mV with increasing amounts of silica in the shell. NPs formed very stable aqueous colloids. Preliminary results showed no marked effect of low NP concentration on phagocytic activity of granulocytes, monocytes and respiratory burst of phagocytic cells exposed to concentration range 0.12–75 □g/cm^2. High NP dose suppressed phagocytic activity of granulocytes and respiratory burst of phagocytic cells.

Conclusion: The developed particles can be useful for biomedical applications, such as cell labelling and magnetic resonance imaging, or in drug delivery systems.

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RECOOP consortium – promising platform for new grant opportunities

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The nanotechnology brings benefits to broad range of applications including new medical technologies. The unknown hazards and risks of nanomaterials raise concerns about the reliability of current testing approaches. European research consortium on nanosafety involved in QualityNano project of 7RP EC: "A pan-European infrastructure for quality in nanomaterials safety testing" creates a scientific and technical space in which scientists can engage, develop, and share the scientific best practices in the field.

NanoBioTechResearch Network developed within RECOOP consortium used this opportunity of access to scientific infrastructures. Five scientists and PhD students successfully applied for grant support. Scientists from research institutes of RECOOP consortium used/will use research infrastructure of Slovak Medical University involved in QualityNano project. First topic of research is dedicated to assessment of immunosafety of nanocrystals doped with lanthanide ions. Second area of interest is to study *in vivo* possibilities of imaging using semiconducting optically and magnetically active gadolinium nanocrystals. Another aim is to study cellular uptake and immunotoxicity of newly synthesized iron oxide nanoparticles with different coating. Next topic is dedicated to the possible effects of endotoxins contamination on testing of cytotoxicity of nanoparticles.

QualityNano project of 7RP EC brought new opportunities for cooperation within RECOOP consortium On the other hand, RECOOP consortium created promising platform for new grant opportunities.

Acknowledgements: The authors also wish to thank the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC) for support.
We acknowledge the support of the European Commission 7th Framework Programme for the QualityNano project, INFRA-2010-1.1.31, Contract no: 214547-2, NanoTEST project, Health-2007-1.3-4, Contract no: 201335 and support of the ITMS project no.26240120033, Operational research and development program financed from the European Regional Development Fund.

Functionalization of quantum dots with PEG dervatives – a versatile approach for obtaining water soluble fluorescent nanomarkers

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Key words: semiconductor nanocrystals, ligand exchange, optical markers, poly(ethylene glycol)

Introduction: Semiconductor nanocrystals, so – called quantum dots (QDs) have attracted considerable attention, due to their unique properties. Good colloidal stability, small hydrodynamic sizes, reduced photobleaching rates (in comparison with the most common presently used organic dyes) and tunable spectroscopic properties makes them promising optical markers in applications such as *in vivo* imaging, *in vitro* clinical diagnostics and cellular labeling. One of the main disadvantages characterized these nanoparticles is hydrophobic nature of their surface.

Aim: The aim of our work was adaptation surface of different types of semiconductor nanocrystals (CdS, CdSe, CdSeS, PbS) to water environment.

Methods: To obtain this goal, we have developed efficient surface modification by ligand-exchange approach. The novel PEG-derivative ligands have been synthesized and characterized. They consist of dithiol (lipoic acid) anchoring group, which bounds the surface of QDs, a hydrophilic poly(ethyleneoxy) chain which ensures water solubility, and the functional terminating group (OH, NH_2) which allows to further bio-functionalization. To confirm cap exchange, we used absorption and emission experiments, FT–IR spectroscopy, NMR method and emission lifetime measurements.

Results: We succeed with the ligand exchange approach and obtain water soluble semiconductor nanocrystals. Moreover, we have observed significant changes in emission spectra of functionalized QDs. The reason behind this phenomenon is also discussed.

Conclusion: Ligand exchange approach by using ligands based on dihydrolipoic acid and poly(ethylene glycol) is efficient method to provide hydrophilic nature of QDs' surface.

16:30 – 18:00 RECOOP HST Association General Assembly

Cedars – Sinai Medical Center, Los Angeles, USA
Edward Prunchunas, CSMC and Sandor G. Vari, CSMC - RECOOP

Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
Authorized Representative

IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Jan Pitha

Institute of Macromolecular Chemistry Academy of Sciences, Czech Republic
Daniel Horák

Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic Vanda Bostik

Faculty of Pharmacy, University of Copenhagen, Denmark
S. Moein Moghimi

University of Debrecen, Hungary
Laszlo Matyus

University of Pecs, Hungary
Tibor Ertl

University of Szeged, Hungary
Gyorgy Falkay

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
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Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine Tatiana Borisova, Palladin Institute of Biochemistry NAS of Ukraine

Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Rostyslav Stoika

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Roman Lesyk and/or Oleh Pinyazhko

Invited:
Elizabeta Has-Schön, Department of Biology, University J.J. Strossmayer
Ivančica Pavličević, Department of Family Medicine, University of Split School of Medicine, Split, Croatia
Artur Podhorodecki, Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland
William J Britt: Departments of Pediatrics Universityof Alabama at Birmingham, Birmingham, USA

May 30 (Friday), 2014

Plenary session

18:00 – 19:30

Session Chairs

Tatiana Borisova

Gyorgy Falkay

Roman Lesyk

Allostatic Load Score Defines the Percent of Women who are Biochemically & Physiologically "Unhealthy" during the Postpartum Period. Implications for reducing the Risk of Cardiovascular Disease

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Key words: allostatic markers, metabolic dysregulation, post partum, cardiovascular disease

Introduction: Approximately 10-25% of older overweight (OW) and obese (O) women are characterized as metabolically "healthy" (Bluher M Current Opinion in Lipidology, 2010), while the remainder 80-75% are "unhealthy" and are at risk for hypertension, insulin resistance, diabetes and cardiovascular disease. Healthy and unhealthy OW and O women during the postpartum period have not been characterized. The LA-site, one of the 5 Community Child Health Network (CCHN) sites participated in an NICHD study to assess multiple factors during the interconception period thought to contribute to the maternal and child risk.

Methods: The CCHN-LA site selected 4 biomarkers & 2 physical risk profiles known to characterize major biological regulatory systems. We performed a nested study of 83 normal (BMI=18.5-24.9) compared to 55 OW (BMI=25-29.9) and 56 O women (BMI=>30) to study 4 profiles of risk. These profiles are: 1) Vitamin D (Brain/Neuro); 2) Blood Pressure (Cardiovascular); 3) C-Reactive Protein (Inflammation); 4), HgA1c, HDL cholesterol & waist circumference (Metabolic Dysregulation). Each parameter was given a score of 0(normal), 1(borderline), 2(abnormal) to calculate an Allostatic Risk Score. All biomarkers were determined on blood spots using a Cleo Reference Laboratory ZRT (info@zrtlab.com). Standard definitions were used to define scores of 0 to 2 according to accepted ranges recommend by the American Heart Association.

Results: Using ANOVA there were significant differences for vitamin D & BP (NvsOW & NvsO) [p=<0.0001); CPR & Waist C (all 3 groups [p=<0.0001], HgA1c (NvsO) [p=<0.002] and HDL [p=0.132]. Four cut point were used to define a threshold to define "Healthy" vs. "Unhealthy" score across the three groups. The best clinical profile was profile 4 (indicating 59% of Normal women as "healthy" compared to 16.1% in the OW group and 3.6% in the O group. This profile fits into the "healthy profile" defined by Bluher (2). However, during the post partum period 23% of women changes from (N) weight group to (OW) group and 43% changed from (OW) group to (O) group and showed a significant increased risk score, therefore the threshold was changed so that the incidence of "Healthy" increased in the (N) to 80.7 %, to 32.1% (OW) and 16.4% in the (O). The Risk Score means significantly differed across the 3 groups and revealed an increasing trend (p=<0.0001).

Conclusion: This is the first study using vitamin D plus standard regulatory Allostatic markers to identify the degree of metabolic dysregulation and the first to identify the low per cent of OW & O women during the postpartum period with a normal risk profile. Intervention to reduce risk of metabolic dysregulation at this critical period for women during the interconception period or post partum period is important.

Nanoparticle-Mediated Tumour Growth: Cause for Reflection

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Introduction: A wide variety of nanocarriers and particularly cancer nanomedicines activate the complement system, which is the first line of the innate immune defence mechanism. Complement activation may induce inflammatory responses, but such responses arising from uncontrolled complement activation could be life threatening [1]. Accordingly, the role of complement in initiation of adverse reactions to particulate and polymer therapeutics is receiving increasing attention. Furthermore, the involvement of complement-activation products in promoting tumor growth has also been indicated [2]. This could be of serious concern for development of cancer nanomedicines and cancer nanotechnology initiatives, and tested here.

Methods: Three types of poloxamine 908-coated nanoparticles with different complement activating and circulation half-life profiles were engineered [3]. Nanoparticles were injected intravenously into syngeneic male immunocompetent C57BL/6 mice as well as C5 and C5a receptor knock-out mice bearing TC-1 tumor, and tumor growth was monitored.

Results: On tumor accumulation long-circulating nanoparticles enhanced tumour growth, which was complement-dependent.

Discussion: Nanoparticle-mediated intra-tumoral accumulation enhances tumour growth through recruitment of C5a receptor bearing suppressive immune cells. Nanoparticles are expected to play important roles in development of functional and safe cancer medicines. These advances, however, must be based on detailed understanding of integrated biological processes, including those at the interface of tumor biology-immune system, and their rational translation, and should not be influenced by market forces and accelerated demands [4,5].

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Changes in Cardiovascular Risk Profile in Women after Menopause (Prague Pre- and Post-Menopausal Female Study)

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Key words: menopausal transition – cardiovascular risk factors – plasma lipids – longitudinal study

Introduction: Atherosclerosis is the main cause of mortality in the Czech Republic. In our previous cross-sectional studies, we detected a high prevalence of metabolic cardiovascular risk factors in women before and after menopause and found menopausal transition to be critical period for atherosclerosis acceleration. In the present longitudinal study, we studied changes of main cardiovascular risk factors in women after transition to menopause.

Methods: During 6 year period we analysed data of 195 women which became menopausal and 292 women who stayed in menopause. The cardiovascular risk factors under study were as follows: smoking, body mass index, waist circumference, blood pressure, plasma lipids including apolipoprotein B and A1 and fasting glycemia.

Results and Discussion: The most striking differences between newly and steadily menopausal women were found in changes of plasma lipids. With the exception of HDL cholesterol all changes were less favorable in newly menopausal women and were not associated with treatment with statins. No significant differences between both groups were found for changes in body mass index, waist circumference, blood pressure and fasting glycemia.

Conclusion: In longitudinal study we confirmed that time around menopausal transition is one of the most dynamic periods regarding changes of cardiovascular factors, mainly plasma lipids.

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Effects of high fat diet, ovariectomy and physical activity on leptin receptor expression in rat brain and white fat tissue

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Key words: obesity, physical activity, leptin receptor, brain, adipose tissue

Aim: To evaluate whether ovariectomy, high fat diet and physical activity in the form of running compared to sham surgery, standard diet and sedentary conditions affect leptin receptor (Ob-R) distribution in the brain and white fat tissue in rat animal model.

Methods: Study included 48 female laboratory Wistar rats (4 weeks old). Following eight weeks of feeding with standard or high fat diet, rats were subjected to either ovariectomy or sham surgery. After surgery all animals continued their standard or high fat diet for the next 10 weeks. Free-floating immunohistochemistry and Western blot methods were carried out to detect Ob-R in the brain and adipose tissue.

Results: In lateral hypothalamic nuclei standard diet-ovariectomy-sedentary group showed higher number of Ob-R positive neurons in comparation with standard diet-sham-sedentary group. There was no difference in Ob-R positive neurons in arcuatus nuclei in all groups. Ob-R distribution is increased in high fat diet group in comparation to standard diet group in barrel cortex. Piriform cortex region was most variable in regard to Ob-R positive neurons. Ob-R expression was decreased at standard diet ovariectomised group in perirenal and subcutaneous fat.

Conclusion: This is the first report about regulation of Ob-R positive neurons by combining high fat diet, ovariectomy and physical activity to continue the effort to explore correlation between central obesity and development of cardiovascular disease. Observed changes in regions related to feeding behaviour might be possible neurological bases for long-term changes in complex behaviours linked to obesity phenotype.

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Specific antioxidant compounds differentially modulate cytotoxic activity of doxorubicin and cisplatin: *in vitro and in vivo study*

Panchuk RR[1], Skorokhyd NR[1], Chumak VV[1,2], Lehka LV[1], Omelyanchik SN[3], Gurinovich VA[3], Moiseenok AG[3], Heffeter P[4], Berger W[4], Stoika RS[1,2]

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Key words: antioxidants, sodium selenite, selenomethionine, D-pantethine, doxorubicin, cisplatin, cytotoxicity, tumor cells, rats.

Aim of this study was to use the antioxidant compounds (sodium selenite, selenomethionine, D-pantethine) for modulation of cytotoxic effect of doxorubicin and cisplatin towards wild type and drug-resistant mutants of several human tumor cells. Similar treatments were applied *in vivo* towards the adult male Wistar rats.

Methods: Human tumor cells of different lines (HCT-116, Jurkat and HL-60) with various mechanisms of drug-resistance were treated with doxorubicin or cisplatin, alone or in combination with sodium selenite, selenomethionine, or D-pantethine. Cell viability, induction of apoptosis and production of O_2^- radicals were measured. Activity of redox potential modulating enzymes was measured in liver and blood plasma of adult male Wistar rats subjected to similar treatments.

Results: All antioxidants used in physiologically harmless concentration inhibited by 15-30% cytotoxic action of doxorubicin towards tumor cells sensitive to chemotherapy treatment, and slightly enhanced cytotoxic effect of this medicine towards drug-resistant malignant cells. At the same time, there was no statistically significant effect of these antioxidands on cisplatin action. Such effects of sodium selenite, selenomethionine, D-pantethine were accompanied by a complete inhibition of production of superoxide radicals induced by doxorubicin. In general, the results of *in vivo* study in which adult male Wistar rats were treated with the anticancer drugs in combination with the antioxidants, are in agreement with the results obtained for *in vitro* treated human tumor cells.

Conclusion: Protective effect of specific antioxidant agents during cytotoxic action of doxorubicin was demonstrated *in vitro* in drug-sensitive human tumor cells and in adult male Wistar rats, while these antioxidants failed to protect drug-resistant sub-lines of these tumor cells from action of doxorubicin and cisplatin.

Acknowledgements: The study was supported by Cedars Sinai Medical Center's International Research and Innovation Management Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

A comparative study of neurotoxic potential of synthesized polysaccharide-coated and native ferritin-based magnetic nanoparticles

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Key words: polysaccharide-coated nanoparticles of magnetite, ferritin, glutamate uptake, the ambient level of glutamate, membrane potential, the proton gradient of synaptic vesicles, planar lipid membrane, rat brain nerve terminals.

Aims: Neurotoxic potential of synthesized nanoparticles of magnetite coated by dextran, hydroxyethyl starch, oxidized hydroxyethyl starch, chitosan, and native protein-covered magnetic nanoparticles, that is, iron storage protein complex ferritin, was analyzed.

Methods: The size of nanoparticles, their effects on the conductance of planar lipid membrane, membrane potential, acidification of synaptic vesicles, glutamate uptake, and ambient level of glutamate in isolated rat brain nerve terminals (synaptosomes) were studied using photon correlation spectroscopy, planar lipid bilayer technique, spectrofluorimetry, radiolabeled assay, respectively.

Results: No significant effect of uncoated synthesized nanoparticles of magnetite and nanoparticles coated by above polysaccharides on synaptic vesicle acidification, the initial velocity of L-[^{14}C]glutamate uptake and ambient level of L-[^{14}C]glutamate were found in synaptosomes. Also, these nanoparticles did not influence the potential of the plasma membrane of synaptosomes and conductance of planar lipid membrane. In contrast, native ferritin-based magnetic nanoparticles, while having no effect on the membrane potential, has been shown to reduce significantly L-[^{14}C]glutamate transport in synaptosomes and acidification of synaptic vesicles.

Conclusion: Polysaccharide-coated magnetite nanoparticles did not influence significantly the functional state of nerve terminals and key characteristics of glutamatergic transmission, whereas native ferritin-based magnetic nanoparticles, considerably affected the proton gradient and glutamate transport in nerve terminals. Therefore, ferritin can not be used as a prototype, analogue or model of polysaccharide-coated magnetic nanoparticles in toxicity risk assessment and manipulation of nerve terminals by external magnetic fields. Ability of ferritin to change the functional state of nerve terminals in combination with its magnetic properties provides biotechnological potential.

May 31 (Saturday), 2014

Plenary session

8:30 - 10:15

Session Chairs
S. Moein Moghimi
Srećko Gajović
Zoltan Papp

Enhanced anticancer activity and circumvention of drug resistance mechanisms by novel polymeric/phospholipidic nanocarrier

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Key words: nanoscale polymeric drug carrier, doxorubicin, KR1019, 4-tiazolidinones, cancer cell lines, drug resistance, experimental tumor models *in vivo,* systemic toxicity.

The application of most effective anticancer drugs is limited by major drawbacks such as severe acute toxicity (especially, cardio-, hepato-, nephro- and neurotoxicity) and frequent occurrence of an intrinsic and acquired drug resistance. There are multiple strategies to overcome these drawbacks, and a central approach is the development of nanoparticle formulations which should reduce the systemic side effects and increase the therapeutic effectiveness of the drug.

Here we used as drug carrier a new polymeric delivery system (poly(VEP-GMA)-graft-PEG) containing phosphatydilcholine synthesized at Lviv National Polytechnic University (Ukraine) by the team of Dr. A. Zaichenko. Its application distinctly enhanced Doxorubicin uptake by both ABC-transporter-overexpressing, as well as non-overexpressing cancer cells. They enhanced (10 times) the antineoplastic activity in tumor cell cultures, as well as anticancer activity *in vivo.* New nanocarriers were shown to transport Doxorubicin rapidly (within 10 min) and effectively into drug-resistant, as well as drug-sensitive cancer cells. Consequently, treatment with new Doxorubicin-nanocarrier complexes led to effective tumor cell cycle arrest in G2/M phase, DNA fragmentation, and other apoptosis-related changes in tumor cells. In the *in vivo* experimental models of murine NK/Ly lymphoma and L1210 leukemia, the application of Doxorubicin nano-formulation resulted in 100% cured animals at low concentration (0.1 mg/kg), while free Doxorubicin solely extended a survival time. Thus, the incorporation of phospholipid into novel PEGylated polymeric nanocarrier showed to be a promising strategy to enhance efficacy and reduce toxicity of Doxorubicin of drug-sensitive and resistant tumor models *in vitro* and *in vivo* [1].

Ruthenium anticancer drugs belong to the most promising non-platinum anticancer metal compounds in clinical practice. Although the pre-clinical results are promising regarding both activity and low adverse effects, the clinical application is currently hampered by the limited solubility and stability of such drug in aqueous solution. Application of above mentioned nanoscale polymer-based micelles loaded with the anticancer lead ruthenium compound KP1019 was characterized by an enhanced stability in aqueous solutions. Moreover, the nano-formulation facilitated cellular accumulation of KP1019 (determined by ICP-MS measurements) resulting in significantly lower IC50 values. With regard to the mode of action, an increased cell cycle arrest in G2/M phase (PI-staining), DNA damage (Comet assay), as well as enhanced levels of apoptotic cell death (caspase 7 and PARP cleavage) were found in HCT116 carcinoma cells treated with KP1019 conjugated with a new drug delivery system [2].

New derivatives of 4-tiazolidinones (3882, 3288, 3833) synthesized at Lviv National Medical University (Ukraine) by the team of prof. R. Lesyk were used in free form and after being complexed with the above mentioned polymeric nanocarrier. The activity of 7 enzymes and 3 indicators of protein metabolism were measured in blood serum of rats, and a protective effect of such complexation of the potential anticancer drugs against their systemic toxicity in the organism was demonstrated [in press].

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Preterm Birth and its consequences in rural and urban communities of India

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Key words: Preterm Birth, Rural population, Urban population, Mortality

Introduction: Preterm birth is associated with many risk factors. Behavioral and psychological factors along with nutrition, environmental exposure and medical conditions are quite divergent in rural and urban communities. Information on socioeconomic disparities in the two communities, is important to implement adequate prenatal care and reduce the risk of preterm birth.

Method: In a pilot study, a cohort of subjects was identified with at least one preterm birth (n = 347) registered in Haryana, India during 2012-2013. Rates and relative risks (RRs) of preterm birth and neonatal and maternal deaths across two rural and two similar but urban neighborhoods were compared. The data was analyzed using Chi- square and Logistic regression.

Results and Discussion: Although, the number of preterm births was not significantly different in the two communities (51.4% vs 48.5%), the rate was almost double for maternal mortality (25.0% vs 12.3%, $p < 0.001$) as well as neonatal mortality (67.7% vs 32.3%) in the rural setting. Rural cohort was recorded with 84% spontaneous preterm birth, whereas, urban population indicated only 58% of it ($p < 0.02$). There were persistent fetal sex disparities in both cohorts, male fetus being the predominant ones in rural areas (81% vs 57 %, $p < 0.05$). There was equal distribution of very early (gestational age of < 32 weeks) and early preterm birth (32-33 weeks) In rural cohort, the number of low birth weight neonates was significantly higher than the urban cohort (90% vs 60%, $p < 0.03$). The premature birth was highest for nulliparous subjects in the rural population only. Interestingly, maternal morbidity due to anemia was significantly lower in the rural population (18.7%vs 36%, $p < 0.0001$).

Conclusion: Disparities in birth outcomes in rural areas of India demonstrate an absolute need for prenatal care to improve perinatal outcome of mothers and infants

The influence of selected factors of life style on bone health – the experimental study

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Key words: ovariectomy, obesity, exercise, selenized nutritional supplement, bone

Introduction: Healthy lifestyle habits, including well-balanced diet, exercise and sufficient micro-nutrient intake are key determinants of bone mass. The aim of our study was to investigate the influence of body weight, physical activity and nutritional supplement on bone mass density (BMD) and bone mineral content (BMC) in rats with/without ovariectomy.

Methods: Sixty-four female Wistar rats (4 weeks old) were enrolled into the study. After eight weeks of feeding with standard or high fat diet (16 SD, 48 HFD), rats were subjected to either ovariectomy (8 SD-Ovx, 24 HFD-Ovx) or Sham surgery (8 SD-Sham, 24 HFD-Sham). 1/3 of every HFD subgroups ingested nutrition supplement, 1/3 underwent treadmill training and 1/3 together with both SD groups served as sedentary controls for next 10 weeks. Bone densitometry was performed before the ovariectomy and at the end of the study.
Results: HFD and Ovx significantly increased body weight, total body fat and abdominal circumference. BMD was lowered and BMC was increased in total, femur, tibia and L2-L5 vertebras. Regular physical activity as well as nutritional supplementation with selenised cookie partially suppressed these effects.

Discussion and conclusion: There are several studies that confirmed the protective effect of high BMI on BMD in postmenopausal women. Anyway, there are still more data in which overweight and "Western" diet have the negative impact on bone health. Our results are in concordance with it. We can conclude that HFD intake supports not only obesity but also the osteoporosis development. Well-balanced diet, regular physical activity and sufficient intake of essential nutrients can be benefit to healthy bone. Following determinations of bone markers, Ca and P bone content and evaluation of expression of selected genes involving in the regulation of adipocytogenesis and bone remodeling should help us to clarify the influence of body fat on bone.

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This work is part of a multi-site study "Obesity, Bone Density and Cardiovascular Diseases" supported by Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center – RECOOP Research Centers (CRRC).

SSAO enzyme activity in adjuvant arthritis and anti-inflammatory effect of a new SSAO inhibitor

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Key words: SSAO, leukocytes, chronic inflammation, arthritis

Aims: We aimed to study the Semmicarbazide Sensitive Amine Oxidase (SSAO) enzyme activity in an animal model of chronic joint inflammation and the anti-inflammatory effect of SzV-1287, a new SSAO inhibitor.

Background: SSAO has been shown to be identical to Vascular Adhesion Protein-1 (VAP-1) and its role in the accumulation of leukocytes at the site of inflammation is well established. This adhesion molecule function has been found to be dependent on the enzyme activity of the protein. SSAO inhibitors thus can decrease the leukocyte extravasation and the inflammatory process.

Methods: Chronic inflammation (adjuvant arthritis) was induced by intraplantar injection of heat killed *Mycobacterium tuberculosi*s (0.1 ml; 10 mg/ml) in rats. The size of oedema was assessed by plethysmography and measuring the diameter of the tibiodorsal joint while inflammation associated hyperalgesia determined by Randall-Selitto test. SSAO enzyme activity in the serum and the inflamed tissue has been measured by radiometric method.

Results: Unexpectedly, reduced SSAO activity has been found in the inflamed tissue samples and the sera of rets with chronic arthritis. On the other hand, the new SSAO inhibitor developed at Semmelweis University effectively ameliorated the symptoms of inflammation as well.

Conclusion: The anti-inflammatory activity of a new SSAO inhibitor has been demonstrated in animal model of chronic joint inflammation. The reduced SSAO activity in the inflammatory model suggests that the may play a role in the early phase of inflammation and might be inactivated during leukocyte extravasation process.

Transcatheter Patent Ductus Arteriosus Closure in VLBW Infants

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Key words: patent ductus arteriosus, neonatal intensive care, interventional catheterization

Introduction: Patent ductus arteriosus (PDA) increases the morbidity and mortality of premature infants worldwide. Current management of a hemodynamically significant PDA includes watchful waiting, fluid restriction, indomethacin or ibuprofen, and surgical ligation. We report the safety and efficacy of transcatheter PDA closure in very low birth weight (VLBW) infants utilizing a commercially available device.

Methods: We examined the medical records of neonates <32 weeks gestational age who received transcatheter PDA closure between 03/13 and 04/14 at Cedars-Sinai Medical Center. This study was approved by the CSMC Institutional Review Board.

Results: Seven preterm infants from 26 to 31 weeks gestational age (median, 26 weeks, range 25-31 weeks) underwent successful PDA closure with the Amplatzer Vascular Plug II (AVP II). Median weight was 1155g (900-2240g) and age 21 days (16-80). Echocardiography and fluoroscopy guided intervention without contrast was achieved in all cases, with median procedural and fluoroscopy times of 40 min (33-87min) and 7.2 min (0 – 19.5 min), respectively. No complications related to the procedure were noted. No instances of aortic or pulmonary artery obstruction were observed post-procedure.

Discussion: Patent ductus arteriosus in VLBW neonates can be safely closed via a novel, transvenous, transcatheter approach utilizing existing devices. In addition to watchful waiting, active management with indomethacin or ibuprofen, and surgical ligation, transcatheter PDA closure should now be evaluated for safety, efficacy, and healthcare value in larger clinical trials.

Acknowledgements: This study received support from the Fashion Industries Guild, the Ruth and Harry Roman Chair in Neonatology in honor of Larry Baum, and the Vera and Paul Guerin Congenital Heart Program.

N-Glycan mapping by capillary electrophoresis

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Key words: N-glylcans, biotherapeutics, separation

Introduction: Comprehensive analysis of protein glycosylation represents one of the most challenging bioanalytical tasks as glycans are very complex groups of molecules with their chemical natures ranging from neutral, linear polymer structures to branched, highly polar or even charged ones. This vast structural diversity of glycans makes carbohydrate analysis extremely difficult.

Methods: The lack of chromophore / fluorophore moieties and, in many instances, easily ionizable groups usually require derivatization of carbohydrates before their analysis by high performance bioanalytical techniques, such as liquid chromatography, electrophoresis and mass spectrometry. Full structural elucidation of glycans also utilizes consecutive enzymatic digestion by exoglycosidase arrays, followed by analysis of the digests. Hyphenation with offline weak anion exchange chromatography (WAX) fractionation is an additional powerful tool, especially to decipher highly sialylated complex carbohydrate structures.

Results and Discussion: This presentation covers the state of the art of liquid phase separation methods for structural elucidation of protein glycosylation mostly focusing on capillary electrophoresis (CZE and CGE) but also including the main approaches of liquid chromatography and microcfluidics methods, as well as their combination with mass spectrometry. Particular attention will be paid to identification of glycosylation changes in serum glycoproteins, and help to reveal alterations in their glycosylation pattern in different disease states. In pathological conditions, aberrant glycosylation patterns are reportedly observed, e.g. degalactosylation of immunoglobulins in rheumatoid arthritis, elevated level of antennary fucosylation on α1-acid glycoprotein in chronic inflammation, desialylation of transferrin in alcoholism and increased fucosylation of haptoglobin in cancer. The N-glycosylation on human immunoglobulins, especially on IgGs, plays a critical role in the bioactivity of this group of important proteins, e.g. in RA patients a decrease in the terminal galactose content of the N-linked glycans at the conserved Fc region (Asn 297) glycosylation site of IgG occurs. Changes in terminal galactose, sialic acid and N-acetil-glucoseamine content have also been reported in different conditions such as pregnancy or ageing.

Conclusion: N-glycosylation analysis is an important and dynamically growing field, especially due to the emerging field of biologics. Full characterization of the glycosylation of biotherapeutics is crucial in the biotechnology and biopharmaceutical industry, especially in clone selection, process development and lot release.

Sources of funding: MTA Momentum Program #97101

Acknowledgements: The author acknowledges the support of the MTA-PE Translational Glycomics grant.

May 31 (Saturday), 2014

Poster Session

10:30 – 11:30

Session Chairs
Iuliana Ceausu
Jana Tulinska
Charles F. Simmons

Research Achievements (2012-2014) of the Department of Regulation of Cell Proliferation and Apoptosis at the Institute of Cell Biology (NAS of Ukraine) Due to Collaboration Network Within the RECOOP-HST Association and Collaboration with Other None-RECOOP Institutions

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Key words: novel nanomaterials, treatment, diagnostics, novel anticancer drugs, tumor cells, apoptosis, drug resistance, glycocalyx, urogenital infection, catalytically active antibodies, autoimmune diseases, auto-antigens, auto-antibodies, recurrent pregnancy loss.

1) **NanoBioTech** Projects: 1) Lectin-functionalized fluorescent labeled nanoparticles for bio-imaging and isolation of apoptotic cells **[3,4,15,21].** 2) Biomedical applications of novel magnetic and polymeric particles of nano- and micro-scale with a reactive shell suitable for bio-functionalization **[11,16,23,25].** 3) Enhanced anticancer activity and circumvention of drug resistance mechanisms by novel polymeric/phospholipidic nanocarriers **[9,10].** 4) Novel oligoelectrolyte based non-viral gene delivery systems for genetic transformation of the eukaryotic (yeast, plant, mammalian) cells **[12,14,24].** 5) Evaluation of eco- and bio-safety of metal-containing nanomaterials **[2,17].** RECOOP partners: Institute of Macromolecular Chemistry (Prague, Czech Republic), Wroclaw Technical University (Wroclaw, Poland), Institute of Biochemistry (Kiev, Ukraine), and potential partner: Slovak Medical University (Bratislava, Slovak Republic). None-RECOOP partners: Lviv National Polytechnic University (Lviv, Ukraine), Taras Shevchenko Kiev National University (Kiev, Ukraine), Ternopil National Pedagogical University (Ternopil, Ukraine).

2) **Cancer**. Project: Pre-clinical study (*in vitro* and *in vivo*) of biological action of novel anticancer drugs of natural (Landomycins) and synthetic (4-thiazolidone derivatives) origin **[6,22].** RECOOP partner: Lviv National Medical University (Lviv, Ukraine). None-RECOOP partners: Institute for Cancer Research at Vienna Medical University (Austria), Institute of Food Biotechnology and Genomics, NASU (Kiev, Ukraine), Institute of Biophysics and Biomedical Engineering (Bulgarian Academy of Sciences, Sofia, Bulgaria), Institute of BioOrganic Chemistry, NAS of Belarus (Grodno, Belarus).

3) **Infection of Reproductive System.** Project: Targeting glycocalyx for detection and isolation of specific (apoptotic) cells at the uropathogenic *E. coli* infection in women and Crohn's disease patients **[8]**. Potential RECOOP partner: University of J.J. Strossmayer in Osijek (Croatia). None-RECOOP partner: University of Lille (France).

4) Immunity and Autoimmunity. Project: Specific catalytically active antibodies as a diagnostic instrument at autoimmune diseases [**1,5,7,13,18,19**] and other related issues [**26**]. RECOOP partners: Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, (Croatia), Debrecen University (Hungary). None-RECOOP partner: Medical University in Erlangen (Erlangen, Germany).

5) **Preterm Birth** Project: Novel auto-antigens and auto-antibodies in pathogenesis of the recurrent pregnancy loss (RPL) **[20]**. RECOOP partner: Faculty of Military Health Sciences, University of Defense (Hradec Kralove, Czech Republic), and potential partner: Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, (Croatia). None-RECOOP Partner: Institute of the Hereditary Pathology AMSU (Lviv, Ukraine).

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Experimental esophagitis research: new insight on animal models and translational aspects

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Key words: hydrogen sulfide, oesophagitis, animal model, IL-10; IL-17

Background: Gastroesophageal reflux disease (GERD) is the common chronic group of diseases worldwide. However, its diagnostic and therapy approaches haven't been clear completely because there are several limitations associated with the investigation oesophagus in humans. Hydrogen sulphide (H_2S) has been suggested to contribute as critical fact to gut cytoprotection. No previous data of H_2S role in oesophago protection. The present study was designed to set up a model of related to nonerosive oesophagitis (NEO) in rodents to understand mechanisms of oesophageal pre-ulcerogenic injury under such conditions as modification biosynthesis of H_2S with postprandial hyperglycemia (PHG) and water immobilization stress (WIS).

Methods: All experiments were on rats with altering H_2S production via cystathionine γ-lyase (CSE) inhibitor, DL-propargylglycine (PAG, 25 mg/kg, intraperitoneally, i.p.), cystathionine-β-synthetasa (CBS) inhibitor, β-cyano-alanine (BCA, 50 mg/kg i.p.), or NaHS (100 mlmol/kg) with PHG; and WIS by Takagi, 1964. L-Tryptophan (L-Try) and NEO was determined by Esohisto Consensus score index; IL-10, IL-17 via ELISA.

Results: Over-expression of IL-17 was achieved using PAG and BCA *vs* the control. WIS-associated oesophageal injury with inhibition of H_2S via CSE, CBS developed submucosal oesophageal edema and neutrophilic infiltration, including muscular lamina lesions and basal cell hyperplasia of OEM. Moreover, increased IL-17 and decreased IL-10 were recorded. Treatment with L-Tryp reduced NEO.

Discussion: These findings suggest that endothelial metabolism is deeply involved in pathogenesis of NEOL. These models may be useful for detecting a new therapeutic strategy NEOL, testing anti-ulcer and reparatory drugs, oesophageal destruction and epithelial inflammation.

Conclusion: Modification of activity of bioregulators of H_2S in rats providing a novel experimental model that can potentially be useful in preclinical testing of NOE-related therapeutics and helping in identifying of the physiologic impact of H_2S, as a molecular mediator associated with mucosal defense in oesophagus.

Acknowledgements: We thank Prof. John L Wallace (McMaster University, Antibe Therapeutic Inc Canada) for providing chemicals.

Cervical fluid IL-6 and IL-8 levels in pregnancies complicated by preterm prelabor rupture of membranes

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Aim: To determine the cervical fluid interleukin (IL)-6 and IL-8 levels in pregnancies complicated by preterm prelabor rupture of membranes (PPROM) and the association of these interleukins with microbial invasion of the amniotic cavity (MIAC) and histological chorioamnionitis (HCA).

Methods: Sixty women with singleton pregnancies were included in this study. Cervical fluid was sampled at the time of admission using Dacron polyester swabs, which were placed into the endocervical canal for 20 seconds. IL-6 and IL-8 levels were determined by ELISA. The management of PPROM was active management (except for in pregnancies <28 weeks of gestation) and occurs no later than 72 hours after the rupture of membranes.

Result: The women with MIAC had higher IL-6 and IL-8 levels than did the women without MIAC (IL-6: p=0.01; IL-8: p=0.003). There was no difference in IL-6 levels between women with and without HCA (p=0.37). The women with HCA had higher IL-8 levels only in the crude analysis (p=0.01) but not after adjustment for gestational age (p=0.06). The women with both MIAC and HCA had higher levels of IL-6 and IL-8 than did the other women (IL-6: p=0.003; IL-8: p=0.001). IL-8 level of 2653 pg/mL was found to be the best cutoff point in the identification of PPROM pregnancies complicated by both MIAC and HCA with a likelihood ratio of 24.

Conclusion: The presence of MIAC is the most important factor impacting the local cervical inflammatory response, which is determined by IL-6 and IL-8 levels in the cervical fluid. IL-8 levels seem to be a promising non-invasive marker for the prediction of pregnancies complicated by the presence of both MIAC and HCA.

How does maternal smoking influence the early neurobehavioral development of rat pups?

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Key words: smoking, perinatal, neurobehavioral tests

Introduction: Exposure to tobacco smoke during perinatal life is known to have various deleterious effects. Among others they worsen cognitive functioning, decrease locomotor behavior and present a risk factor for future pshychiatric disorders. The aim of our study was to investigate the influence of maternal smoking during pregnancy on the early physical and neurobehavioral development of newborn rats.

Methods: Wistar rats were exposed to whole-body smoke exposure for 2x40 minutes daily from the mating until delivery. For the treatment, TE2 manual closed-chamber smoking system and 4 research cigarettes per occasion were used. The neurobehavioral development of the pups was monitored by a battery of tests until postnatal day 21. Weight was measured daily until postnatal day 21. On the 4rd week of life motor coordination tests were carried out.

Results: Some parameters appeared earlier, like eyelid and eartwitch reflexes, forelimb and hindlimb placing, forelimb grasp. On the other hand we observed a delay in the appearance of forlimb and hindlimb reflexes.

Conclusion: These results suggest that maternal smoking during pregnancy has unfavourable effects on the early neurological development of the rat pups.

Acknowledgements: This work was supported by PTE-MTA “Lendület” program, the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 ‘National Excellence Program, Arimura Foundation, Bolyai Scholarship.

The renin release in ischemia/reperfusion kidney injury; gender differences

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Key words: acute renal ischemia-reperfusion injury, multiphoton microscopy, renin, collecting duct

Introduction: Ischemia/reperfusion (I/R) kidney injury is one of the most important risk factors for chronic allograft nephropathy. The course and severity of I/R kidney injury differs in the two genders. Our studies focused on the role of renin in I/R kidney injury. The JGA is the key anatomical site, where renin is synthesized and released and the cortical collecting duct (CD) is a newly described localization of renin release. In these studies we have investigated *in vivo* the effect of I/R kidney injury on renal renin system in male and female rats.

Methods: The left renal pedicles of mature male and female Wistar rats were clamped for 50 minutes followed by 2, 8, 16, 24 and 48 hours of reperfusion, sham-operated rats served as controls. We applied fluorescence-activated cell sorting (FACS) analysis for quantitative measurements and used multi-photon imaging to directly and quantitatively visualize the intact kidney, monitor the basic parameters of kidney function *in vivo* including (pro)renin content as well as release.

Results: Applying FACS analysis we have detected decrease in CD renin content in the first 8 hours of reperfusion, however, from the 16th hour of reperfusion renin content increased in the CD. These results were further supported by the intravital multi-photon microscopy and as a result of the local renin activation vasoconstriction was found in the kidney.

Discussion: Our studies revealed first that there is a sub-acute renin response to I/R injury not only in the JGA but in the CD segment as well. We could visualize the renin content and release *in vivo* even in this new localization and reveal that renin release is more explicit in males than in females

Conclusion: We assume, that the inhibition of renin might serve as a therapeutical possibility for the moderation of both sub-acute and chronic renal I/R injury.

Sources of funding: Supported by OTKA K-108688, SE-MTA Lendület LP2001-008/2011.

Microalbuminuria and cardiometabolic risk factors in general population of high school students

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Key words: microalbuminuria, adolescents, cardiometabolic risk

Introduction: In the adults, microalbuminuria (MA) is a marker of presence, or indicates an increased risk of development of cardiovascular and/or renal diseases. Roots of these diseases go back to childhood and adolescence. Data on prevalence of MA in adolescents are scarce, and the relationship between albumin/creatinine ratio (ACR) and cardiometabolic risk factors in this age group is not clear. We aimed to assess the prevalence of MA and the relationship of ACR with cardiometabolic risk factors in population of adolescents in Bratislava region.

Methods: We analyzed data from 2 685 adolescents (49% boys), aged 14-20 years. Anthropometric and blood pressure measurements were performed at high schools. Blood and urine samples were analyzed at central laboratory. MA was defined as ACR 2.5-25 mg/mmol for boys, 3.5-35 mg/mmol for girls.

Results: Prevalence of MA was 3.2% in boys, 3.3% in girls. Multivariate analysis showed that higher ACR in boys is associated with higher serum urea concentration, urine specific gravity and glomerular filtration rate, and lower BMI, waist/height ratio, waist circumference, body weight, total body fat, serum creatinine and pulse pressure. In girls, ACR was inversely associated with percentage of total body fat, serum albumin, BMI, body weight and age.

Discussion: Compared with studies on similiar population, prevalence of MA was rather low in both genders. No association between traditional (atherogenic lipid profile, elevated blood pressure, insulin resistance, obesity) or non-standard (homocysteine, hs-CRP) markers of cardiometabolic risk and ACR were revealed in either gender. Paradoxically, ACR associated inversely with markers of obesity. Several factors may contribute to higher ACR in lean subjects, such as different anatomic proportions, probably more frequent physical activity and possibly high income of proteins.

Conclusion: In apparently healthy adolescents of both genders, the prevalence of MA was rather low, with no significant association to cardiometabolic risk factors.

Sources of funding: Bratislava Self-Governing Region, APVV-0447-12 and VEGA 1/0637/13 grants.

Diabetes –induced impairments of the exocytosis process and the effect of gabapentin: the link with cholesterol level in neuronal plasma membranes

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Key words: diabetes, exocytosis, membrane fusion, cholesterol, gabapentin

Aim: To investigate the effects of diabetes-induced disturbances on the Ca^{2+}-triggered fusion process in cell-free system, to asses the role of neuronal plasmalemma cholesterol. To study the effect of gabapentin on the steps of exocytosis process.

Methods: The diabetes in rats was induced by one-time injection of streptozotocin- (60 mg/kg of body weight, i.p.). After 4 weeks the one group of diabetic rats were treated with gabapentin (50 mg/kg, i.p. 5 times per week) during one month. Fusion experiments were performed in the cell-free model system using fluorescent dye octadecylrhodamine B. [2-^{14}C]serotonin preloaded synaptosomes were used for assay of stimulated neurotransmitter release.

Results: In diabetes rats the cholesterol level in synaptosomal plasma membrane was on 12% higher than in control. Note that after gabapentin treatment of diabetic rats the cholesterol content in plasma membrane of synaptosomes was decreased on 5%.

The rate of synaptic vesicles fusion with plasma membranes in the presence of Ca^{2+} and synaptosomal cytosolic proteins was decreased from 23% in control to 14.5% in diabetes. Whereas, in group of rats with diabetes that received gabapentin the rate of fusion of synaptosomal membrane structures is rise to 18%. At diabetes the stimulated synaptosomal serotonin release was increased on 1.7-2 folds and was partially normalized after gabapentin treatment.

Conclusion: The detected elevation of cholesterol content in neuronal plasma membranes at diabetes impairs the membrane fusion process in neurons and provokes neuropathy. Diabetes-evoked defects of the exocytotic process, which have the pathophysiological significance, could be attenuated by gabapentin therapy.

The role of *musashi* in *Caenorhabditis elegans* – the gene of forgetting?

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Key words: forgetting, F-actin, musashi

Introduction: Understanding the molecular mechanisms of learning and memory is one of the biggest challenges in neurosciences. The role of F-actin in subcellular consolidation of memory is proven. Actin network is the result of dynamic polymerization and branching of actin-filaments. Branching is regulated by the Arp2/3 protein complex, which initiates growth of a new filament on the side of an existing one. Our group's results showed that the RNA-binding protein Musashi (MSI) is negative regulator of translation of the Arp2/3 element *arx-2* mRNA [1]. MSI selectively regulates forgetting of *Caenorhabditis elegans* by decreasing actin branching through the downregulation of ARX-2. Loss of MSI selectively impairs memory decay, but has no effect on learning.

Methods: Our aim was to visualize the presumed colocalization of ARX-2 (*arx-2*), F-actin (*utrCH*) and Glu-R (*glr-1*) in the AVA interneuron. For that, we marked these genes with green (*gfp*) and red (*rfp*) fluorescent markers. The following plasmids were constructed: [p_{rig-3}*::glr-1::gfp::unc-54*$^{3'UTR}$], [p_{rig-3}*::arx-2::gfp::unc-54*$^{3'UTR}$] and [p_{rig-3}*::utrCH::rfp::unc-54*$^{3'UTR}$]. Rig-3 promoter was used to gain neuron-specific expression in the AVA neuron. Fluorescent intensity was measured with confocal microscopy and analysed with ImageJ software.

Results: 61 *wild type (wt)* and 23 *msi-1(lf)* transgenic worms were imaged. We found significant ARX-2/F-actin and F-actin/Glu-R colocalization in the AVA neuron. ARX-2::GFP intensity was elevated in *msi-1(lf)* compared to *wt*, while synapse size not changed.

Discussion: We found difference between *msi-1(lf)* and *wt* in the steady state ARX-2 levels. Measuring ARX-2 dynamics after conditioning is to be studied in the future.

Conclusion: Our results show that loss of memory is actively regulated by MSI that acts as an inhibitor of actin branching in the Glu-R positive synaptic areas. ARX-2 is increased in msi-1(lf) worms. This results in an increased branching and more complex actin structure that might be the reason of the increase in memory retention.

Sources of funding: The Forschungsfonds of the University of Basel and the Swiss National Science Foundation (Sinergia grants CRSIK0_122691 and CRSI33_130080 to D.J.-F.d.Q. and A.P.) supported this work.

[1] Hadziselimovic N, Vukojevic V, Peter F, Milnik A, Fastenrath M, Fenyves BG, Hieber P, Demougin P, Vogler C, de Quervain DJ, Papassotiropoulos A, Stetak A. (2014) Cell 156: 1153-1166.

May 31 (Saturday), 2014

Plenary session

11:30 - 12:30

Session Chairs

András Guttman
Shubhada Bopegamage
William J. Britt

Value of hysteroscopy for endometrium evaluation in postmenopause

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Key words: bleeding in menopause, endometrial biopsy, menopause, hysteroscopy

Aim: The study compared the efficacy of blind endometrial biopsy by Pipelle with that of hysteroscopic biopsy with biopsy forceps under direct view.

Material and Methods: The study included 69 postmenopausal women in the gynecology department of the "Dr. I. Cantacuzino" Hospital selected for endometrial biopsy because of irregular bleedings in menopause or for endometrial thickness 4-6 mm at transvaginal ultrasound routine examination. The age rage was 45 and 62 years, at least after one year without any menstrual bleedings and without hormonal therapy.

Results: All women undergo biopsy with Pipelle and in 36 cases the results indicated endometrial hyperplasia, 27 with atrophy, 4 polyps and 2 cases with insufficient tissue sample. The hysteroscopy was performed after 4 weeks from the first biopsy, and enabled a selective biopsy of the suspected areas. The results were 42 hyperplasia, 16 polyps and 11 cases with atrophic endometrium. The rate of confirmation rate for polyps by Pipelle was 1 to 4, the rest of 12 polyps were initially considered atrophy or hyperplasia without atypia (3 cases). Also, 9 cases of initially considered atrophy were discovered with simple hyperplasia without athypia, and in 7 cases of simple hyperplasia it was discovered a complex hyperplasia without athypia. The conformity rate calculated by analysis between blind and under direct visual control biopsy for hyperplasic endometrium was 88.31%.

Conclusion: Hysteroscopic biopsy under visual control is necessary at least for the patients with any hyperplasic changes at the blind biopsy. Transvaginal ultrasound may be helpful for uterine cavity evaluation.

Gender differences in expression of estrogen receptor β and leptin receptor in adrenal gland after chronic and acute stress

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Key words: stress, adrenal gland, steroid hormones, leptin receptor

Introduction: To analyze clinically relevant biochemical markers for development of metabolic syndrome and differences in expression of estrogen receptor β and leptin receptor (Ob-R) in adrenal gland of rat males, non-ovariectomized females (NON-OVX) and ovariectomized females (OVX) under acute and chronic stress.

Methods: Study included 72 four-months-old Sprague-Dawley rats, 24 males and 48 females. Animals were divided into male, NON-OVX and OVX group. Groups were further divided into – control, acute and chronic stress (8 animals each). Weight, glucose tolerance test (GTT), plasma concentration of glucose, urates and cholesterol were measured. Adrenal glands were collected at the age of 28 weeks and immunohistochemical staining was performed using estrogen receptor beta (ERβ) and leptin receptor (Ob-R) antibodies.

Results: Concentration of analyzed biochemical parameters decreased after acute and chronic stress. Acute and chronic stress significantly changed GTT profile in males and NON-OVX, but not in OVX group. ERβ expression in zona glomerulosa was significantly higher in OVX than NON-OVX control ($p = 0.009$) and chronic stress group ($p = 0.004$). ERβ expression in medulla was significantly higher in OVX than NON-OVX group ($p = 0.03$). Ob-R expression in zona reticularis is significantly higher in NON-OVX than OVX after chronic stress ($p = 0.03$).

Conclusion: Supcceptibility of male groups to acute and chronic stress is reflected in changes of biochemical markers. NON-OVX and OVX animals respond differently to acute and chronic stress and differ in adrenal gland expression of Erβ and Ob-R.

Acknowledgements: This study is the part of the Women's Health and Cardiovascular Diseases Research Network of Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium formed by Cedars–Sinai Medical Center (CSMC), Los Angeles, CA, USA
Internal research grant from Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia.

5-Ylidene-4-thiazolidinones anticancer agents source: problem solution

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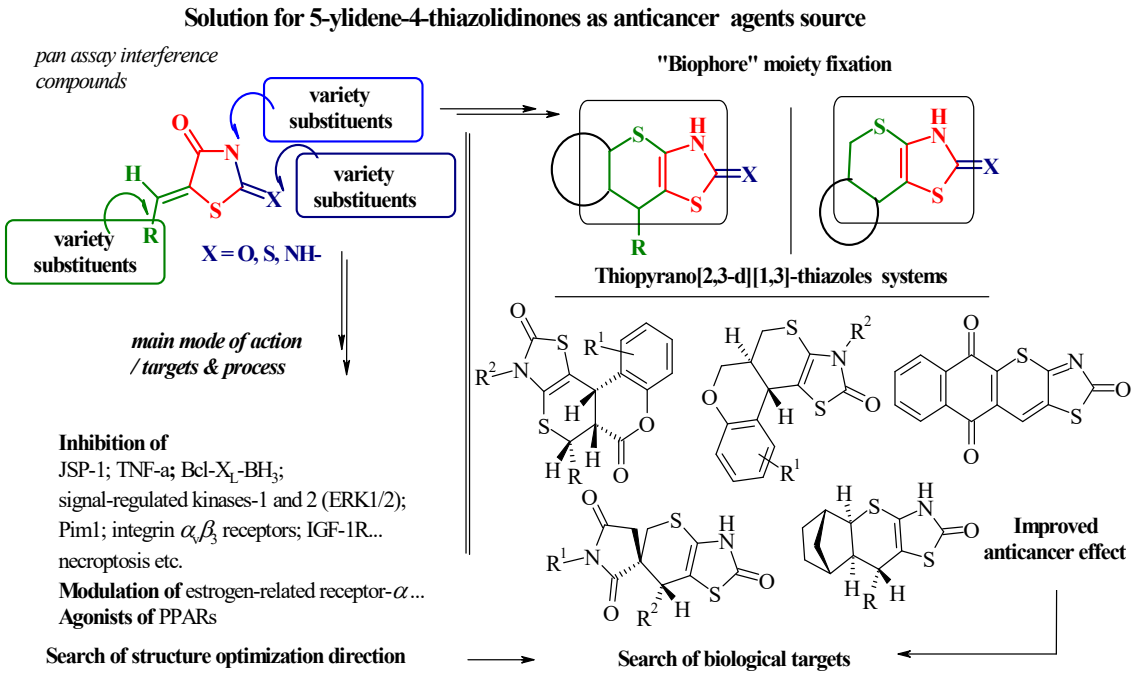
Key words: 5-ylidene-4-thiazolidinones, thiopyrano[2,3-*d*][1,3]thiazoles, anticancer agents.

The advances in the medicinal chemistry of thiazolidinones have significantly increased since sixties and reflected among others in the introduction of PPARs-agonists into the medical practice. Following the chemical diversity of 4-thiazolidinones the latter can be treated as powerful tool in the rational privileged substructure-based diversity oriented synthesis. Among variety types of thiazolidinones the 5-ylidene-derivatives are of the special interest both of chemical and pharmacological points as anticancer, antiviral, agents, etc. Nevertheless, conjugation of 5-ylidene fragment to the carbonyl group makes compounds electrophilic and potentially reactive due to possible Michael addition of the nucleophilic protein residues to the exocyclic double bond. This property characterizes 5-ylidene-4-thiazolidinones as frequent hitters (promiscuous inhibitors) or pan assay interference compounds that are useless in the drug discovery process because of their insufficient selectivity.

As an attempt to solve this confuse in the spirit of anticancer agents discovery two ways – "biological" and “chemical” have been proposed.



“Biological way” is based on the put forward and confirmed hypothesis about crucial role of the presence/nature of the C-5 substituent of 4-thiazolidinone core for biological activity realization. The argument for its favor is that the vast majority of hit- and lead-compounds belong to the 5-ylidene-4-thiazolidinones. The positive perspective may also be linked to a polypharmacological approach in drug discovery, where the affinity toward various targets is regarded as an advantage. In this context, it is worth mentioning that such Michael acceptors are effective activators of Nrf2 through the Keap1 modification that opens new perspectives in the treatment of inflammation, cancer and chemo-prevention. “Chemical way” is based on the hypothesis about highly active 5-arylidene-4-thiazolidinone fixation in fused thiopyranothiazole system that allowed conserving the activity vector and therefore opened new possibilities of molecules optimization. Thiopyrano[2,3-*d*][1,3]thiazoles could be of special interest as cyclic isosteric mimetics of 5-arylidene-4-thiazolidinone precursors. For this reason the rows of different thiopyrano[2,3-*d*][1,3]thiazoles were synthesized and their anticancer potential was confirmed.

Visualization of melanoma tumor with lectin-conjugated rare-earth nanocrystals

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Key words: melanoma, imaging, nanocrystals, lectin, glycan

Aim: The goal was to develop specific fluorescent markers for melanoma tumor visualization, which would provide high selectivity and reversible binding pattern, by the use of carbohydrate-recognizing proteins lectins, combined with ability to be imaged deep in the living tissues by utilizing red and near-IR fluorescent properties of specific rare-earth doped nanocrystals (NC).
Methods: B10F16 melanoma cells were inoculated to C57BL/6 mice for inducing experimental melanoma tumor. Tumors were removed and analyzed by lectin-histochemistry using LABA, PFA, PNA, HPA, SNA, GNA and NPL lectins and stained with hematoxylin and eosin. NPL lectin was conjugated to fluorescent $NaGdF_4:Eu^{3+}$-COOH nanoparticles (5nm) via zero length cross-linking reaction, conjugates were purified from unbound substances and then used for further visualization of histological samples. Fluorescent microscopy was used to visualize NPL- $NaGdF_4:Eu^{3+}$ with the fluorescent emission at 600-720nm range.
Results: NPL lectin selectively recognized regions of undifferentiated melanoblasts surrounding neoangiogenic foci inside melanoma tumor, while PNA lectin was bound to differentiated melanoblasts, and LCA and WGA were bound by tumor stroma. NPL-$NaGdF_4:Eu^{3+}$ conjugated NC were efficiently detecting newly formed regions of melanoma tumor, confirmed by fluorescent microscopy in visible and near-IR mode. These conjugates possessed high photostability and were compatible with convenient xylene-based mounting systems and preserved intensive fluorescent signal at samples storage for at least 6 month.
Conclusion: NPL lectin-$NaGdF_4:Eu^{3+}$ conjugated NC permitted distinct identification of contours of the melanoma tissue on histological sections using red excitation at 590-610nm and near-IR emission of 700-720 nm. These data are of potential practical significance for development of glycans-conjugated nanoparticles to be used for in vivo visualization of melanoma tumor.

Sources of funding: This work was supported by Polish National Centre for Research and Development for their financial support under the LIDER project No. 014/L-2/10, Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association), State Agency on Science, Innovations and Informatization of Ukraine, and the West-Ukrainian BioMedical Research Center.

Acknowledgements: This work was supported by Polish National Centre for Research and Development for their financial support under the LIDER project No. 014/L-2/10, Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association), State Agency on Science, Innovations and Informatization of Ukraine, and the West-Ukrainian BioMedical Research Center. The authors are grateful to Dr. V.Antonyuk for providing specific lectins and their HRP-conjugates and to Dr. Sandor G. Vari for management support.

May 31 (Saturday), 2014

Plenary session

14:00 - 15:00

RECOOP Review

SOP

FP8

Standard Operating Procedures for CRSMN at the RECOOP HST Association

Linn Defensor RN, MSHS-CRA, CCRP
Office of Research Compliance and Quality Improvement, Cedars-Sinai Medical Center, Los Angeles, CA, USA & Global Clinical Trial Leader, CRSMN, RECOOP HST Association

The Standard Operating Procedures (SOPs) for Clinical Research was created in compliance with regulations and guidance that govern the conduct of clinical research.

It is designed as a tool in the development of site specific SOPs in the Clinical Research Site Management Network (CRSMN) of RECOOP HST Association to ensure patient safety, data quality and integrity.

SOPs are defined by the International Conference on Harmonization (ICH) 1.55 as "detailed written instructions to achieve uniformity of the performance of a specific function." In section 2.13 of the ICH Good Clinical Practice (GCP) guideline it is described as "systems with procedures that assure the quality of every aspect of the trial that should be implemented." Article 1 of the EU Clinical Trial directive dated April 4, 2001 stated that "GCP becomes a legal requirement; therefore GCP inspections of investigational sites are mandated."

In compliance with these regulations, this SOP template was created for the exclusive use of CRSMN using published files from various research organizations as permitted, personal files of the author and covers various regulatory topics in the conduct of clinical research.

Bioseparation of glycans, lipids and fatty acids to understand the common mechanisms of diseases

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Extensive literature supports the role of the glycan-containing "information" in the development of autoimmune diseases and cancer [1, 2]. The degalactosylation of immunoglobulins investigated extensively in rheumatoid arthritis [3] . The elevated level of antennary fucosylation on α1-acid glycoprotein observed in chronic inflammation [4]. The desialylation of transferrin in alcoholism and increased fucosylation of haptoglobin have significant role in cancer [5, 6]. Glycosylations of IgG influences binding of rheumatoid factor [7]. Changes in terminal galactose, sialic acid and N-acetil-glucoseamine content also reported in different conditions such as pregnancy or ageing [8].

Pathogen recognition is a critical element in the immune response mechanisms to modulate the inborn immune system and control of immune cell homeostasis and inflammation. The immune activation and inflammation have defined role in neurodegenerative diseases and atherogenesis also. In Alzheimer's disease (AD) the immune activation and inflammation play remarkable role in the pathogenesis of AD and microglia the principal cellular element of brain inborn immune system likely to be involved in processes and triggers the amyloid-beta (Aβ) deposition which is the most frequent cause of dementia in the elderly [9, 10].

Atherogenesis is associated to inflammation the chronic inflammatory state initially stimulated by an aberrant accumulation of lipid molecules beyond the endothelial barrier and triggers a cascade of deleterious events mainly through immune cell stimulation with the consequent liberation of potent pro-inflammatory and tissue damaging mediators. The pronounced modifications in the atherogenetic process are the changes in endothelial cell functions and a radical change in the endothelial–leukocyte interaction [11].

Lipids

Multiple defects in lymphocyte signaling and function contribute to the pathogenesis of systemic lupus erythematosus (SLE), a autoimmune rheumatic disease. Dyslipidemia is the driving force in T cell defects contributes to the immunopathogenesis of SLE. One of the novel therapeutic strategies for the treatment of SLE targets lipid biosynthesis pathways. Glycosphingolipids (GSLs) are enriched predominantly in lipid rafts, regions in the plasma membrane that coordinate the interaction of key signaling molecules that facilitate lymphocyte activation and function. Alteration of plasma membrane GSL profile could influence the balance between positive and negative signals transduced during activation and contribute to the characteristic T cell dysfunction seen in SLE patients. In the treatment of

SLE patients the ultimate goal is restore defects in lipid (GSL and cholesterol) homeostasis and to inhibit T cell proliferation, cytokine production [12].

Considerable evidence supports the role of oxidative stress in the development and progression of chronic diseases such as heart failure (HF) and typically measured biomarkers of oxidative stress are predominantly lipid peroxidation products [13].

The major risk factor of cardiovascular disease, type 2 diabetesis obesity associated with high blood pressure and lipids. Adipose tissue is capable of expanding many-fold during adulthood. Accumulation of adipose tissue in individuals with obesity is associated with a state of persistent low-grade inflammation that seems to play a pivotal role in the pathogenesis of obesity-linked insulin resistance, diabetes, and cardiovascular diseases. The relation between inflammatory markers, adiposity and disease is extensively studied. Recent reports underlined the inverse relations between circulating immunomodulatory protein pentraxin 3 (PTX3) and increased total and visceral fat mass. The analysis of the fatty acid composition of subcutan and visceral fat could help to have a better understanding of adipose tissue accumulation and the differences in adipose tissue expansion between female and male [14].

Fatty acids

In the literature it is well documented that the symptomatic ambulatory HF patients' circulating levels of the lipid peroxidation product 4-hydroxynonenal bound to proteins (4HNE-P) are strongly associated with those of its potential precursors and with the levels of total fatty acids. In the pathophysiological events those includes all classes of fatty acids and reflect both free and bound fatty acids, to albumin or esterified to cholesterol or as triglycerides and phospholipids in lipoproteins linked to heart disease progression. Gas chromatography-mass spectrometry (GC-MS) is one of the validated methods in the measurements of lipid peroxidation products [15].

An alternative and orthogonal method is micellar electrokinetic chromatography (MEKC) to separate fatty acids could help to have a better understanding of obesity, stress and metabolic disorders.

Glycans

The N-glycosylation on human immunoglobulins, especially on IgGs, plays a critical role in the bioactivity of this group of important proteins, e.g. in RA patients a decrease in the terminal galactose content of the N-linked glycans at the conserved Fc region (Asn 297) glycosylation site of IgG occurs [16, 17]. Recent progress in bioseparation science combined with novel diagnostic methods allows identification of glycosylation changes in serum glycoproteins, and help to reveal alterations in their glycosylation pattern in different disease states [1, 7]. Most proteomics based diagnostic tests are measuring the concentration, structural and/or post-translational changes of the relevant protein markers in blood or other body fluids. It is well known that a high percentage of serum proteins are glycosylated and that this modification is vital for the parent proteins as glycosylation is responsible for the physical, chemical and biological properties of proteins [18].

The method established at the Horváth Laboratory of Bioseparation Sciences comprises affinity chromatography (Protein A) based IgG capture, PNGase F based glycan release followed by fluorescent labeling with aminopyrene trisulfonate and capillary electrophoresis

with laser induced fluorescent detection (CE-LIF) [19] using a 96 sample plate format. The glycan structures found are annotated using Glycobase database.

Using this bioseparation method is one of the most important components in the planned RECOOP research projects to understand the common mechanisms of diseases and their relevance in co-morbidities and could lead to compete for an FP8 proposals should focus on the integration of pre-clinical and clinical studies for the identification of mechanisms common of several diseases.

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Examination of the role of CREB and REST transcription factors in the brain to better understand the stress and aging induced neuronal damage and the regulation of food intake

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Recent data indicate that the neuronal networks in the brain are rather dynamic structures. Synaptic plasticity and the generation of new neurons in some brain regions play important role in the learning and memory formation as well as the regulation of mood.

Exposure to chronic stress can induce both depression and cognitive impairment [1]. Recent data indicate that both conditions are accompanied by brain atrophy, decreased number of neurons and synaptic connection. Contrary to long time belief recent results indicate neurogenesis in the adult brain. In depression a decrease in the neurogenetic process in the hippocampus was found that can be reversed by antidepressant therapy [2].

The brain derived neurotrophic factor (BDNF) plays a central role in the survival of neurons and also stimulates the synaptic plasticity and neurogenesis. Its synthesis is regulated by several transcription factors. cAMP responsive element binding protein (CREB) integrates the effect of various signaling pathways, including the neurotransmission and growth factor signals [2]. It was found that chronic stress induced depression is characterized by decreased neurogenesis in the hippocampus and reduced activity of the CREB-BDNF pathway in both sexes [3-5].

Repressor element-1 silencing factor (REST) originally described as a transcriptional repressor of neuronal genes in non-neuronal cells. It is also expressed in neurons during the developmental phase [6]. Recently its upregulation in aging brain has been found where it has neuroprotective effects and is lost in neurodegenerative disorders [7].

In the present study the role of CREB and REST mediated transcriptional activity would be examined in the stress and aging induced alterations of the brain. Activation of CREB (its phosphorylation) as well as expression of REST and BDNF on protein and/or mRNA level are to be determined in various brain regions of acutely and chronically stressed and aged animals. Investigators will study the effect of gender and ovariectomy of female animals with reduction of estrogen levels also. Hippocampus and prefrontal cortex involved in mood regulation and memory formation and thalamic, hypothalamic and brain stem regions involved in regulation of appetite, neurohormonal axes and sympathetic nervous system are to be studied.

The role of CREB and REST transcription factors in the regulation of food intake and their correlation to leptin/leptin receptor expression would also be studied. In addition to thalamic and hypothalamic regions olfactory bulb, the brain area involved in smell sensation, is to be studied. In this brain region significant nerurogenesis activity was reported in the rat [8].

Recently diet induced alteration in CREB activity was found and connected to behavioral eating [9-10]. The correlation to gender, leptin and smell sensation are to be determined.
In *in vitro* experiments the function of CREB-BDNF and REST pathways would be studied on SH-SY5Y cell line. These neuroblast cells can be differentiated to gain mature neuron phenotype. Correlation of CREB-BDNF and REST pathways to stress resistance, oxidative stress and cell viability is to be assayed.

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Pathogenesis of inflammation in CKD and atherosclerosis, role of CREB, Wnt/β-catenin signaling and SSAO activity

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Hypothesis:

Semicarbazide Sensitive Amine Oxidase/Vascular Adhesion Protein 1 (SSAO/VAP-1) is a protein with double function, it is an enzyme and an adhesion molecule. Its role in leukocyte infiltration to the site of inflammation has been proved and the dependence of the process on the enzyme activity has also been demonstrated. Adipose tissue is rich in SSAO activity that may participate in its infiltration with different classes of white blood cells and the development of proinflammatory and adipogenic cytokine environment. Serum levels of VAP-1 are associated with the severity of kidney damage or stages of kidney disease. The true mechanism which links the serum VAP-1 and CKD remains to be elucidated in further studies.

Low grade inflammation in the vasculature is a characteristic hallmark of the atherosclerotic process. The involvement of adipose tissue in the inflammatory processes in atherosclerosis can be speculated as it can produce a wide range of inflammatory mediators and obesity, especially abdominal type is well known to associate with high cardiovascular risk. In the adipose tissue the cytokines produced are only partly originated from the fat cells, the mediators partially derive from the infiltrating inflammatory cells, e. g. monocytes (1).

It is well known arginine vasopressin (AVP) is a neurohypophysial hormone regulating hydromineral homeostasis. Greenwood M et al. showed that the mRNA encoding **cAMP responsive element-binding protein-3 like-1 (CREB3L1),** a transcription factor of the CREB/activating transcription factor (ATF) family, increases in expression in parallel with AVP expression in supraoptic nuclei (SONs) and paraventicular nuclei (PVNs) of dehydrated (DH) and salt-loaded (SL) rats, compared with euhydrated (EH) controls. They identified CREB3L1 as a regulator of AVP transcription in the rat hypothalamus (1).

The role of CREB and Wntk/β-catenin signaling already reported epithelial cancers and Gastrin-mediated gastric cancer cells therefore it is feasible to investigate in CREB and Wntk/β-catenin signaling damage of endothelia in CVD and kidney diseases. (2.3)

Wnts are required for adult tissue maintenance and perturbations in Wnt signaling promote both human degenerative diseases and cancer. A critical mediator of key cell-cell signaling events during embryogenesis is the highly conserved Wnt family of secreted proteins**.** Recent biochemical and genetic analyses have greatly enriched our understanding of how Wnts signal, and the list of canonical Wnt signaling components has exploded. The literature reveals that multiple extracellular, cytoplasmic, and nuclear regulators intricately modulate Wnt signaling levels. In addition, the receptor-ligand specificity and feedback loops could help to determine Wnt signaling outputs.

The Wnt family of secreted signaling proteins plays an essential role in organogenesis, tissue homeostasis, and tumor formation (4-7). Aberrant regulation of Wnt signaling has been implicated in the pathogenesis of many human diseases in diverse types of tissues (6,7). Wnt proteins transmit their signal across the plasma membrane through interacting with serpentine receptors, the Frizzled (Fzd) family of proteins, and co-receptors, members of the LDL receptor–related protein (LRP5/6). Upon binding to their receptors, Wnt proteins induce a series of downstream signaling events involving Disheveled (Dvl), axin, adenomatosis polyposis coli, and glycogen synthase kinase 3β (GSK-3β), resulting in a protection of β-catenin from being phosphorylated by GSK-3β. This leads to the stabilization of β-catenin, rendering it to translocate into the nuclei, where it functions as a cofactor of T cell factor 4/lymphoid enhancer-binding factor (LEF) to stimulate the transcription of Wnt target genes (8 -10). In addition to this canonical pathway, Wnt proteins may also exert their activities through numerous β-catenin–independent, noncanonical intracellular signaling routes (11).

Wnt/β-catenin signaling has been shown to play a role in kidney development and diseases. Wnt4 and Wnt9b are highly expressed in the early stage during kidney development and are functionally important for nephron formation (12,13). In adult kidney, however, Wnt signaling seems to be silenced (14, 15). Dysregulation of Wnt/β-catenin signaling occurs in certain types of kidney diseases, including obstructive nephropathy (16,17). These observations clearly suggest a potential role of Wnt signaling in mammalian nephrogenesis, tissue homeostasis, and pathogenesis of kidney diseases; however, the expression of 19 Wnts and 10 Fzd receptors in adult kidney remains to be determined. Furthermore, their regulation and function in the evolution of chronic kidney diseases are poorly understood.

This study will be part of the FP8 proposal, and will support the RECOOP research activities in the understanding of the common mechanisms of diseases and their relevance in co-morbidities.

Study plan

In this study, we aim to study the role of **CREB, SSAO, Wnt/β-catenin signaling and activity**, rapid detection of glycosylation changes in sera samples by our novel enzyme-linked lectinosorbent assay (**ELISA-ELLSA**) in the inflammation of adipose **and** arterial tissues.

Intra-abdominal and subcutaneous adipose tissue samples will be taken from female and male living donor (healthy control) and chronic kidney failure patients subjected to transplantation with advanced state of atherosclerosis.

Inflammation will be determined by histology, measuring CREB, NF-κB and Wnt/β-catenin signaling activation and the level and/or expression of inflammatory cell markers and

cytokines and its correlation with SSAO activity and the clinical severity of atherosclerosis would be studied.

The difference in gender, obesity and age would also be determined.

During nephrectomies in chronic kidney failure, the following samples will be collected.

I vial (5-10 ml) blood – refrigerated

A defined segment renal artery snaps frozen (immediately frozen on dry ice).

Intra - abdominal (mesenteric) and subcutaneous (white adipose tissue) formaldehyde fixed and snap frozen (immediately frozen on dry ice).

In case living donors the following samples will be collected:

I vial (5-10 ml) blood – refrigerated

Small sample from renal artery - if during the renal artery anastomosis an adjustment of the end of artery needed - snap frozen (immediately frozen on dry ice).

Intra - abdominal (mesenteric) and subcutaneous (white adipose tissue) formaldehyde fixed and snap frozen (immediately frozen on dry ice).

Patient cohort:

Sites (2014)	Szeged		Budapest		Total
Cohorts	Male	Female	Male	Female	
Nephrectomies	6	4			10
Nephrectomies (malignant)			12	10	22
Nephrectomies (benign)			10	13	23
Living donors	2	1	22	9	33
Total	8	5	44	32	88

The following parameters planned to study:

1., cAMP responsive element-binding protein-CREB

In in vitro experiments the function of CREB-BDNF and REST pathways would be studied on cell lines.

Wnt/β-catenin signaling

To monitor Wnt pathway activation under influence of proinflammatory cytokines ***a luciferase reporter*** assay using a Bat-Luc reporter will be performed in renal cell lines, MEEC (mouse embryonic endothelial cells) and commercially available primary HUVEC (human umbilical vein endothelial cells). Cells will be treated with 10 ng/ml of TNFα, Hyper IL-6 overexpression (or Hyper IL-6 conditioned medium), 2.5 nM Wnt3a or their combinations. An adenoviral Bat-Luc reporter construct was already generated in Dr.Korchynskyi's lab to transduce HUVEC that are badly tranfectable. Most important findings will be confirmed using a chromattin immunoprecipitation assay with TCF4/LEF1-specific antibody (Cell signaling, CA, USA), essentially as before (17).

Real-Time RT-PCR amplification of Wnt ligands, Frizzled receptors, LRP4/5/6 coreceptors and direct Wnt target genes *Axin1*, *REST*, *CyclinD1* and *c-myc* mRNA prepared from renal cell lines, MEEC and HUVEC treated for 0.5, 1, 2, 4 and 6 hours with 100 ng/ml of TNFα, Hyper IL-6 conditioned medium, 2.5 nM Wnt3a or their combination in the presence or absence of the protein synthesis inhibitor cycloheximide (CHX, 5 μM) will be used to verify if *de novo* protein synthesis has a role. Culture of mentioned above cells grown in a 6-well multi-well plate (10 cm^2) is sufficient to generate 4-6 μg of total RNA, where 2-4 μg total RNA is an adequate to assess RNA quality by Real-Time RT-PCR. Total RNA will be isolated using TriZol reagent (Invitrogen) in accordance to manufacturer protocol. 3-5 μg total RNA per variant will be reverse-transcribed with SuperScript-II (Invitrogen). Real-Time RT-PCR amplification of cDNAs will be performed on StepOnePlus™ Real-Time PCR System (Applied Biosystems) Fast SYBR® Green Master Mix (Applied Biosystems, Carlsbad, CA, USA) essentially as described before (Krause at al., 2010). Serial dilutions (1:10; 1:100; 1:1000) of cDNA prepared from 2.5 nM Wnt3a-treated cells will be used to generate a calibration curve for quantification of *Axin1*, *REST*, *CyclinD1* and *c-myc* mRNA expression results. Primers for Real-Time RT-PCR amplification of mentioned above cDNAs are already designed and actively used in our work (18).

SSAO activity and cytokine content would be measured and the correlation of these parameters in the vascular and adipose tissue would be studied.

Inflammatory clinical markers: red blood cell sedimentation, serum CRP.

Inflammatory markers: tissue and serum level of the main inflammatory cytokines (e.g. TNFα, IL-1β) and adipokines (e.g. leptin) – ELISA, WB or PCR level.

SSAO activity, mRNA and protein expression.

Histology/immunohistochemistry: evaluation of the inflammation and inflammatory cell infiltration of the adipose tissue and OB receptor

Paraformaldehyde fixed and cryoprotected brain samples were cut in coronal plane (35 μm) on cryostat (Leica CM-3050-S, Leica Microsystems Nussloch GmbH, Nussloch, Germany) and free-floating immunohistochemistry was carried out to detect distribution of leptin receptor (Ob-R). Sections were pre-treated in 1% H_2O_2/1xPBS solution for 30 minutes to block endogenous peroxidase activity and then incubated in 1% BSA and 5% goat serum in 0.1 M PBS blocking solution for 2 h, followed by incubation in primary XYZ antibody (diluted 1:30, H-300, sc-8325, Santa Cruz Biotechnology, Inc., USA) overnight. Incubation in secondary antibody for 2h. After washing in 1xPBS sections were incubated in Vector Elite peroxidase kit (ABC) (Vector Laboratories, Burlingame, CA, USA) for 1h, washed several times in PBS and then incubated with peroxidase substrate kit (DAB) (Vector Laboratories, Burlingame, CA, USA). All steps were performed at +4°C without using detergents. Sections were then mounted on slides; air dried and scanned with Nikon Super CoolScan 9000 ED. After that sections were cover slipped with Vectamount (Vector Laboratories, Burlingame, CA, USA). Microscopic images were acquired using an Olympus D70 camera mounted on Zeiss Axioskop 2 MOT microscope. Multiple images were assembled in Adobe Photoshop CS5 for uniformly adjustment of contrast, intensity and brightness. Ob-R positive neurons were quantified using ImageJ software.

These experiments could help the better understanding of the:

1. Role of SSAO in tissue inflammation (adipose and artery) and atherosclerotic diseases and SSAO enzyme may serve a potential drug target to decrease the inflammatory process.

2. The regulatory role cAMP responsive element-binding protein-CREB and Wnt/β-catenin signaling in the inflammatory process of chronic disease (CKD, atherosclerosis).

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May 31 (Saturday), 2014

Breakaway sessions

15:00 - 16:00

(The sessions will be in different rooms)

Chairs:

Mother and Child Health

Chander P. Arora

Women's Health and Cardiovascular Diseases

Zoltan Papp

NanoBioTechnology

Rostyslav Stoika

May 31 (Saturday), 2014

Closing Poster session

16:00 – 18:30

Chairs
Éva Szökő
Zora Krivosikova
Tibor Ertl

Risk factors for preterm birth in Southern Croatia: a case control study at University Hospital

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Key words: preterm delivery, risk factors, maternal morbidity

Introduction: Preterm delivery (PTD) defined as delivery before completing 37 weeks of gestation, is one of the leading problems in obstetrics. Despite advances in modern care, PTD significantly contributes to perinatal morbidity and mortality worldwide. This study examined risk factors of preterm delivery associated to maternal morbidity before and during current pregnancy.

Methods: A retrospective case-control study studied a series of risk factors in all women with PTB (n=142) and equal number of controls with term delivery (TD) who delivered singleton live-born infants without malformations at Department of Gynecology and Obstetrics, University Hospital in Split during 2013. TD was defined as birth after completion 37 weeks. Data was collected from medical archives. We analyzed 34 risk factors related to mother's socioeconomic status, anthropometric and behavioral variables and other, current and previous pregnancy-related.

Results: Risk factors related to maternal morbidity before current pregnancy that were more common in PTB cases than in TD controls were: previous PTB (9.86% PTB vs. 2.11%TD), cervical surgery (27.46% PTB vs. 8.42% TD), congenital uterine anomalies (2.11% PTB vs.0.70% TD) and myoma surgery (5.63% PTB vs. 2.11% TD).
Risk factors related to maternal morbidity during current pregnancy that were more common in PTB cases than in TD controls were: genitourinary infections (34.5% PTB vs. 8.45%), vaginal bleeding (14.08% PTB vs. 3.52% TD), intrauterine growth restriction (10.56% PTD vs. 3.52% TD), HELLP syndrome (3.52% PTB vs. 1.41% TD), pre-eclampsia (11.97% PTB vs. 5.63 TD) and assisted conception techniques (11.97% PTB vs. 7.75% TD).

Discussion: Risk factors before current pregnancy that differed the most between PTB cases and controls were previous PTB and cervical surgery. The main risk factors for PTB during current pregnancy were genitourinary infections and vaginal bleeding.
Conclusion: Identification of PTB risk factors impact may provide important insights into mechanisms leading to preterm birth.

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Detection of novel auto-antigens in patients with recurrent miscarriage: Description of an approach and preliminary findings

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Key words: recurrent miscarriage, human chorion, autoantibodies, autoantigens, proteomics

Aim: To develop and test a protocol of isolation of potential auto-antigens from chorionic tissue that may be linked to recurrent miscarriage (RM) in pregnant women.

Methods: The strategy employed included; (1) isolation of IgGs tightly bound to chorionic tissue of the RM patients by Protein G chromatography, (2) construction of affinity columns using the chorionic Abs for isolation of auto-antigens (auto-Ags), (3) enrichment of auto-Ags from detergent extracted solution of chorionic proteins by affinity chromatography, and (4) separation by SDS-electrophoresis followed by identification using MALDI TOF MS.

Results: Five potential auto-Ags were detected in chorionic tissue obtained from patients with RM. They were identified as heat shock protein HSP90B1 (endoplasmin), neutral alpha-glucosidase AB, putative endoplasmin-like protein, and cytoplasmic actin 2.

Conclusion: Our results demonstrate a strategy for identification of potential auto-Ags in the chorionic tissue of women with RM. From these results we propose that the identification of auto-Ags in chorionic tissue and corresponding auto-Abs in blood from these women could be diagnostic and prognostic value in this population of pregnant women.

Acknowledgement:
The study was supported by Cedars Sinai Medical Center's International Research and Innovation Management Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) and the participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC).

Bedside assessment of amniotic fluid interleukin-6 in preterm prelabor rupture of membranes

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Objective: To test the efficiency of bedside assessments of interleukin-6 with respect to the presence and absence of microbial invasion of the amniotic cavity and/or histological chorioamnionitis in pregnancies complicated by preterm prelabor rupture of membranes.

Methods*:* One hundred twenty-four women with singleton pregnancies were included in this study. The amniotic fluid was sampled by transabdominal amniocentesis at the time of admission. Interleukin-6 levels were assessed with an immunoassay.

Result*:* The presence of microbial invasion of the amniotic cavity, histological chorioamnionitis, or both microbial invasion of the amniotic cavity and histological chorioamnionitis was associated with higher amniotic fluid interleukin-6 levels upon crude analyses and analyses adjusted for gestational age. The amniotic fluid interleukin-6 level of 1000 pg/mL was determined to be the best cutoff value for identifying pregnancies complicated by microbial invasion of the amniotic cavity (sensitivity of 50%, specificity of 95%, positive predictive value of 82%, negative predictive value of 81%, and likelihood ratio of 8.4) or both microbial invasion of the amniotic cavity and histological chorioamnionitis (sensitivity of 60%, specificity of 94%, positive predictive value of 75%, negative predictive value of 88%, and likelihood ratio of 9.4).

Discussion and Conclusion*:* The bedside assessment of amniotic fluid interleukin-6 seems to be an easy, rapid, and inexpensive method for identifying pregnancies complicated by either microbial invasion of the amniotic cavity or both microbial invasion of the amniotic cavity and histological chorioamnionitis.

Risk factors and clinical follow-up of patients with preterm births in a tertiary referral maternity unit in Bucharest, Romania

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Aims: We aimed to analyze the historical risk factors associated with preterm birth, on a representative sample of patients. The main purpose of the study was to analyze the potential benefit of correct prenatal care for preventing preterm birth.

Methods: Prospective study on 4078 women that gave birth at Dr. I. Cantacuzino Hospital, Bucharest, a tertiary level regional emergency referral maternity center for a large sector of Bucharest and for other counties in the south-eastern part of Romania.
The data collection was under the guidelines of the International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, California, USA and the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) Mother and Child Health Research Network –and supported by POSDRU/107/1.5/S/82839 project.

Results: Overall, the prevalence of preterm births was 11.7%. Women giving birth to preterm babies had significantly less prenatal visits during pregnancy compared to women giving birth at term. High body mass index (BMI) for 34-36 gestational weeks, a previous history of spontaneous abortion, a family history of preterm births and smoking were the most important risk factors for preterm birth.

Conclusion: Our study showed that appropriate clinical follow-up in pregnancy was associated with a decreased risk for preterm delivery. An incorrect follow-up in pregnancy, together with high BMI (for preterm deliveries at 34-36 gestational weeks), previous history of spontaneous abortion, family history of preterm births and smoking were the most important risk factors for preterm birth. The follow up the newborns of obese women with diabetes will help to understand the role of intrauterine growth and gestational diabetes mellitus (GDM) as a major antenatal factor for later overweight.
The risk factors can be reduced at least partially taking correct preventive measures during pregnancy to minimize the risk for preterm delivery.

Acknowledgements: The study was supported by Cedars Sinai Medical Center's International Research and Innovation Management Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association).

The Role of PACAP on Retinal Vascular Changes in the Rodent Model of Retinopathy of Prematurity

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Key words: PACAP, ROP, Rat model

Introduction: Premature birth can be associated with a series of disorders affecting future life quality. One of these conditions is a neurovascular disease of the retina, called retinopathy of prematurity (ROP). Despite striking advances in neonatology ROP still remains the leading cause of vision impairment in childhood. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors occur throughout the nervous system, including the retina. The in vivo protective effects of PACAP have been shown in several model of retinal degeneration including diabetic or ischaemic retinopathy. Therefore we aimed to study PACAP administration in the well described animal model of ROP, the oxygen induced retinopathy (OIR).

Methods: Sprague-Dawley rat pups were kept in alternating oxygen concentration for 2 weeks to provoke OIR. Pups were treated either with intravitreal (3x3ul) or intraperitoneal (7x100ul) PACAP injections. After 21 days the rats underwent functional examinations and their eyes were processed to flat mount lectin staining and immunohistochemistry.

Results: Quantification of avascular to whole retinal areas showed that PACAP administration decreases the vasoobliterated territory by almost 50 % and there was also a significant reduction in the number of neovascular tufts. These findings were further supported by functional and immunohistochemical examinations.

Discussion: The rat OIR model is a widely used model of ROP, because it mimics the human disease. Nowadays more and more efforts are taken to prevent or treat the affected prematures but until today there is no effective treatment. Several studies have proven the neuro and retinoprotective effect of PACAP, but not in ROP. We have succeded to use this polypeptide in rat pups with retinopathy and it can ameliorate the vascular impairments and can reduce the degree of ROP.
Conclusion: Our results suggest that PACAP can be a novel target in the treatment of retinopathy of prematurity.

Acknowledgements: This work was supported by PTE-MTA "Lendület" program, the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 'National Excellence Program, Arimura Foundation, Bolyai Scholarship'.

Shared decision making in life style and nutrition for intervention in women with risk factors in cardiovascular health

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Key words: cardiovascular health, risk factors, life style, nutrition, women

Introduction: Cardiovascular diseases (CDV) are the leading cause of death for women in industrialized countries. The aim of our study was to determine the impact physicians' interventions have on women's decisional conflict surrounding lifestyle changes necessary to minimize CDV risks.

Methods: This prospective, interventional pilot study included 21 women between 45 and 60 years with different reproductive status and one or more of cardiovascular risk factors: overweight or obese, high cholesterol, tobacco exposure, and high blood pressure. Prior and post the educational intervention, which included a lecture on life style and diet habits associated with CDV, as well as individually tailored decision aids accompanied with calculated 10-year CDV risk; we measured decisional conflict (DC), state hope (IHS) and participants knowledge of CDV and lifestyle changes associated risks and benefits.

Results: So far, the pilot included 21 women, aged 51.4±3.8, whose CDV risk ranged from 1.1 to 19.7 (M=5.2, SD=4.5). Their DC score significantly decreased after the intervention (M1=35, 95%CI 28-41 vs. M2=20, 95%CI 16-25; P<0.001 paired samples t-test), while their hope remained unchanged (M1=99, 95%CI 91-106 vs. M2=103, 95%CI 96-110; P=0.08, paired samples t-test). Most women (n=18, 86%) expressed willingness to change their life habits, 2 (9%) remained undecided, and 1 (5%) decided against making lifestyle changes.

Discussion: Detection, treatment and life style interventions are meant to reduce major risk factors and instances of cardiovascular diseases. Although our individually tailored intervention lowered the decisional conflict of women regarding their necessary lifestyle changes for reducing CDV risks, their hope of actually reducing them remained the same.

Conclusion: Intervention of primary care physicians is an effective first step for inducing lifestyle changes needed to decrease cardiovascular risks. With the continuing of our study we aim to find if such interventions will lead to actual behavior and risk change.

Acknowledgements: We thank Dr. Sandor G. Vari, MD, Director of the International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA and President of the RECOOP HST Association for the initiation of the project and helpful comments.
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We confirm that we have not had any sources of research support in the form of financial support or grants.

Astrocyte toxicity to motor neurons is dependent on age

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Key words: aging, astrocytes, amyotrophic lateral sclerosis, cellular senescence, glial-derived neurotrophic factor

Introduction: Astrocytes play a crucial role in supporting motor neurons in health and disease. In amytrophic latereal sclerosis (ALS), a neurodegenerative disease characterized by the death of motor neurons, astrocytes are toxic to motor neuron survival. Although age is the greatest risk factor in ALS, there has been little attempt to understand how aging may influence an astrocyte's ability to support neurons. In this study we are interested in observing how aging astrocytes contribute to neurodegeneration in ALS.

Methods: We generated primary astrocyte cultures from young and old rats from wildtype and $SOD1^{G93A}$ rats. $SOD1^{G93A}$ rats express an ALS mutation that recapitulates motor neuron loss seen in patients. We profiled gene expression of astrocytes using quantitative PCR. We next co-cultured them with wildtype motor neurons derived from rat embryos or mouse embryonic stem cells. Using stereology we counted motor neuron survival after one week in culture.

Results: There is a direct correlation between astrocyte age and its ability to support neurons. There is no difference in motor neuron survival regardless of genotype when astrocytes are derived from young rats. Adult rat astrocytes are significantly less supportive to motor neurons compared to young astrocytes. Furthermore, adult SOD1 astrocytes were extremely detrimental to motor neuron survival. We also found astrocytes show an age-dependent senescence phenotype, leading to a significant reduction in motor neuron support. In SOD1 rats, the rate of astrocytes acquiring senescence was accelerated. Astrocyte toxicity can be partially reversed by priming astrocytes with glial-derived neurotrophic factor (GDNF) before culturing with neurons.

Conclusion: Replacing aging astrocytes with young ones producing GDNF may have a significant survival promoting affect on aging motor neurons and those lost in ALS.

Acknowledgements: We would like to thank Genevieve Gowing, Jessica Latter and Maximus Chen from Cedars-Sinai Medical Center, Los Angeles, California, USA for helpful discussion and technical assistance.

Synthesis and surface functionalization of $NaGdF_4:Yb^{3+},Er^{3+}$ nanocrystals for their biomedical applications

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Key words: nanocrystals, rare earth ions, ligand exchange, silanization

Aims: The aim of our work was the synthesis of fluoride nanocrystals doped by erbium Er^{3+} and ytterbium Yb^{3+} ions and adaptation of their surface to water environment.

Introduction: Considerable interest has been focused on lanthanide doped nanocrystals LaNCs due to their possible application in biomedicine. In contrast to conventionall organic dyes (like fluorescein or cyanines) or semiconductor quantum dots, LaNCs can be effectively excited in NIR thanks to up-conversion process. This is especially beneficial for application in the living organism, because in NIR tissues posses the lowest absorbance. Additionally lower toxicity, reduced photobleaching and lack of autofluorescence pose additional advantages. Placement of Gd^{3+} ions in crystal lattice opens new attractive application paths for nanocrystals as multifunctional optical and magnetic markers.

Methods: $NaGdF_4:Yb^{3+}$, Er^{3+} nanocrystals have been synthesized by co-thermolysis method in the presence of oleic acid and 1-octadecen. The structural properties of the products were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The luminescent properties of the NCs were investigated by excitation, emission and absorption measurements. In order to transfer NCs to aqueous solvent two procedures were applied: ligand exchange and surface coating methods.

Results: The average size of obtained nanocrystals, determined from TEM measurements differs for various dopant concentrations. Both procedures of NCs surface modification have been successful what allowed for the placement of as obtained nanocrystals in different biological environments. In consequence MRI and microscopy imaging were successfully applied.

Conclusion: Using different dopant concentration and ligand/solvent volume ratio emission spectrum, size and morphology of NCs can be adjusted. What is more, successful NCs water transfer makes them applicable in biological media.

Haptoglobin N-glycome Alteration Analysis of Inflammatory and Malignant Lung Diseases by Capillary Electrophoresis

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Key words: biomarkers, capillary electrophoresis, glycan analysis, haptoglobin glycosylation, fucosylation in cancer, exoglycosidase array

Introduction: A high percentage of serum proteins are glycosylated and this modification is vital for their proper function as the glycan moiety may be responsible for some of their important physical, chemical and biological properties. In pathological conditions, aberrant glycosylation patterns are reportedly observed, e.g. degalactosylation of immunoglobulins in ovarian and stomach cancer, increased fucosylation of haptoglobin in pancreatic cancer, altered sialylation of serum N-glycome in lung cancer, just to mention a few.

Methods: Haptoglobin N-glycans were enzymatically released, fluorescently labeled and profiled by capillary electrophoresis with laser induced fluorescent detection. Disease associated changes of core and antennary fucosylation were identified by targeted exoglycosidase digestions and their levels were compared in the different patient groups.

Results and Discussion: Increased level of α1-6 fucosylated tri-antennary glycans was found in all disease groups compared to the control. Elevated amounts of core- and arm fucosylation on tetra-antennary glycans were detected in the lung cancer group compared to the COPD group. Terms of core- and arm-fucosylation degree, as well as branching-degree were introduced for easier characterization of the changes. Statistical analysis was used to examine which structures are responsible for the observed differences.

Conclusion: Our results showed that capillary electrophoresis with laser induced fluorescence (CE-LIF) detection was a high sensitivity and robust tool offering rapid analysis with high resolution and excellent reproducibility. Targeted exoglycosidase digestions in combination with CE-LIF offers a powerful method to reveal both structural and linkage information, thus monitoring highly detailed glycosylation changes.

Acknowledgements: This research was supported by the Bridge Fund of University of Debrecen and the MTA-PE Translation Glycomics Grant (#97101).

RECOOP HST Association Mother and Child Health (M&CH) Research Network

Study protocol for the project Coxsackievirus infections during pregnancy

Bopegamage S, Borsanyiova M, Kacerovsky M, Vari SG

Infections by enteroviruses are highly prevalent, but often sub-clinical or cause a mild flu-like illness. Sero-epidemiological surveys have associated enterovirus infections during pregnancy with increased risk for offspring to become diabetic, even years after birth. Neonatal infections and chronic diseases where autoimmunity and/or viral persistence may be involved such as type 1 diabetes (T1D), chronic myocarditis and idiopathic heart failure, are associated with Coxsackie B viruses (CVB), of which 6 serotypes are known (CVB1-6), belong to the *Human Enteroviruses B (HEV-B)* species

Laboratory diagnosis of enterovirus infection may be achieved by isolation of viruses from stool and throat swabs, cerebrospinal fluid (CSF), blood, or biopsy of the affected organs.

Traditionally throat swabs used in the diagnosis of enteroviruses are collected in virus transfer medium in which they are stable at ambient temperatures. Techniques applied for detection of the viruses in the collected specimens range from tissue culture isolations, antigen detection to molecular analysis. PCR has increasingly replaced other methods in the routine diagnosis due to high sensitivity and rapid results.

1. The standard throat swab collection method was modified by standardization using different control parameters to check its applicability and for the stability of enteroviruses (without the transport medium).
2. This modified standardized dry swab method was tested by the project team for PCR diagnosis by carrying out a pilot screening study to check the feasibility

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Aim:
Systematic data and sample collection for determining
Phase I
1. incidence of CV infections during pregnancy in different ethnical groups and countries in Central and Eastern Europe.
2. effect of CV infections on the course of pregnancy
3. risk factors (smoking, nutritional, social status as these factors may influence the course of pregnancy of the infected mothers).
4. transmission of the virus to fetus/neonate and resulting complications due to infections
Phase II
1. follow-up of infected mothers and children during and after pregnancy

Countries involved in sample/data collection and testing: Hungary, Czech Republic, Romania and Slovak Republic

Sample collection:
Inclusion: Singleton pregnancies
Exclusion: Maternal age below 18 years, pregnancies with structural and chromosomal abnormalities and multiple pregnancies

Group A) All pregnant women
1. Sera of all mothers - 1st, 2nd and 3rd trimesters
2. Throat swabs of mothers 1st, 2nd and 3rd trimesters and the newborn
Throat Swabs:
Step 1: collection of throat samples on swabs,
Step 2: air dry for 1 hour in laminar (preferably) with open tubes.
Step 3: close the tubes after drying and
Option A: freeze at -80C, if not it is available then at -20C. Transfer of material on ice/dry ice.
Option B: In the case the samples will be transferred immediately to Bratislava then at has to be kept on 4C.

Group B) Mothers reported fever with symptoms of infection, suspected viral infection or detected fetal anomalies during ultrasound

Step 1. Whole blood and throat swabs from mothers during acute illness
Step 2. Serum (paired sample -1st during acute illness, 2nd four weeks later) frozen in aliquots
Step 3. Blood sample and throat swabs mothers and newborns at the time of delivery in all cases either preterm or term birth.

Group C) all steps of Group B and not mandatory, optional sample collections: from mothers reported fever with symptoms of infection, suspected viral infection or detected fetal anomalies during ultrasound

1. Placenta frozen in aliquots
2. Umbilical cord frozen in aliquots

Data collection:
Participants will use FlexiForm Aps for the collection of a structured data.
(www.flexiform.eu)

Mother: history of diabetes, smoking and nonsmoking, nutritional habits, socio- economic status

Newborn: the newborns of mothers found to be infected have to be followed to report the presence of diabetes/myocarditis

Laboratory tests: PCR for enteroviruses, Serum antibodies against coxsackieviruses

Expected Results:
Systematic and conclusive data of CBV infections during pregnancy.
Identify transmission course if there is, the pathway of transmission
Complications consequences of CVB infections in newborn

Genotyping and characterization of Varicella zoster clinical isolates from Hradec Kralove, Czech Republic

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Key words: virulence assays, mass spectrometry analysis, DNA, real-time PCR

Introduction: Varicella zoster virus (VZV) belonging to the herpesvirus family is the causative agent of varicella (chickenpox) and zoster (shingles). Infections of VZV are strongly restricted to human and simian origin host cells. The primary infection (chickenpox) is followed by the latent phase and the viral reactivation causing the shingles occurs mostly in immunocompromised and elderly individuals. VZV with a genome of ~125 kbp encode at least 70 unique open reading frames (ORFs) with GC content of 46% in average.

Methods: Clinical isolates of VZV from 6 patients were propagated using NHLF cells. Cell-free virus was quantified using plaque assay and isolated DNA was subjected to real-time PCR assay. Next, the viral DNA was genotyped for single nucleotide polymorphism in ORF22 and ORF50 to distinguish new viral variants. For host membrane proteins analyses, the virus was purified using cation exchange chromatography followed by SDS-PAGE separation and mass spectrometry analysis.

Results: The clinical isolated was collected during three-year period from 2010 to 2013. We found out differences in pathogenicity and virulence of the clinical isolates. All clinical isolates were genotyped and determined into clades based on ORF22 and ORF50 analyses.

Discussion: VZV is highly infectious pathogen capable to effect the whole human population. The personal-to personal transmission reaches high rates (up to 95%). Although the mortality rate is quite low, the infection can cause very severe complication in adolescents, pregnant women and immunocompromised persons. Detection of mutations in functionally important genes and their effect on the viral phenotype can provide better understanding to the process of pathogenesis.

Conclusion: We isolated and propagated six strain of VZV collected in Hradec Kralove, Czech Republic. To elucidate the pathogenicity and genotypes of all isolates, analyses comprising genotyping, virulence assays and membrane proteins analyses were undertaken.

Entero-salivary nitrites recirculation is an endogenous modulator of leukocyte-mediated inflammation in experimental oesophagitis

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Key words: Nonerosive osophagitis, entero-salivary nitrites recirculation, NO, IL-1ß, GRO/CINC-1

Introduction: Nonerosive oesophagitis (NEO) is an inflammatory condition of oesophageal mucosa (OEM) with healing options which leads to causes of Barrett's oesophagus, oesophageal carcinoma. The entero-salivary nitrites recirculation (ESNR), a depot of potential nitric oxide (NO) bioactivity and participant of cytoprotection, corrects NO/Nitrous Oxide Systems (NOS) signaling, natural resistance. This study is aimed to detect an effect of modification ESNR via blocking cholinergic, histaminergic and nitroergic pathways on rat oral mucosa, OEM integrity, and inflammation.

Methods: All experiments were provided on rats with ESNR on NEO formation, evaluating oral mucosa and OEM, which was induced via histamine H-2-receptor antagonist ranitidine (R), antagonist of the muscarinic acetylcholine receptors, atropine (A) given separately or in combination with the non-selective blocker NO L-Nitroarginine Methyl Ester (L-NAME). The reproducible NEO was done by selecting an optimal time for ESNR in 3 time periods. NEO was determined by Esohisto Consensus score index; serum NO by bioassay, IL-1ß, Growth-regulated gene product/cytokine-induced neutrophil chemoattractant (GRO/CINC-1) via ELISA.

Results: ESNR by A had a significant increased IL-1ß (32,8%) over 2 days, while over 3 days a similar pretreatment GRO/CINC-1 increased by 13,9% vs control. The dual blocking by R with A caused significant increase of GRO/CINC-1 (137,1%) after 2 days of treatment *vs* control. The effect of R, A and L-NAME realized in increased IL-1ß (by 14,3%), GRO/CINC-1 (by 191,4%) appearing after 3 days vs control.

Discussion: NO content showed an increasing tendency with a maximum rise after 3 days of blocking of R and both R and A, as well a triple blocking R, A, L-NAME. These treatments caused obvious oral and OEM lesions: subepithelial layer edema, submucosal vascular dilation, moderate leukocyte intraepithelial infiltration, keratosis.

Conclusion: The dysfunction of ESNR leads to low-grade inflammation of the foregut inducing erosive lesions in oral and oesophageal mucosa.

May 31 (Saturday), 2014

Plenary session

Closing Remarks

18:30 – 19:30

RECOOP Research Networks

Mother and Child Health

Chander P. Arora

Women's Health and Cardiovascular Diseases

Zoltan Papp

NanoBioTechnology

Rostyslav Stoika

Overview of the RECOOP Association's General Assembly

Sandor G. Vari

June 1, 2014 (Sunday)

Departure

Goodbye

See you CSMC - RECOOP RESEARCHER at 5th TriNet Meeting in Wroclaw on October 17 -19, 2014

RECOOP Visegrad Scholarship Program

Visegrad Scholarship http://visegradfund.org/scholarships/

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences have the opportunity to apply for International Visegrad Fund (IVF) Scholarship and receive the RECOOP Young Scientists Matching Fund. The Visegrad Scholarship is the Visegrad Four European Macro-Region's Fulbright Program. Therefore it could be important to link the Visegrad Scholarship and the Fulbright Foreign Student Program.

RECOOP HST Association in 2014 won two Visegrad Scholarship:

Post-Master's Scholarship:

Ivana Koborová
Institute of Molecular Biomedicine
Medical Faculty, Comenius University, Bratislava, Slovak Republic

Research project at the Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary from September 2014 to January 2015:

"Relationship of SSAO/VAP-1 and insulin resistance in adolescents"

In-Coming Scholarship:

Alexander Karmash
Intern at the Department of Regulation of Cell Proliferation and Apoptosis
Institute of Cell Biology, NASU, Lviv, Ukraine
(Department of Biochemistry, Ivan Franko Lviv National University, Ukraine)

Research project at the Horváth Laboratory of Bioseparation Sciences at the Research Centre for Molecular Medicine, University of Debrecen, Hungary, September 2014 – January 2015:

"Role of disease-related changes in immunoglobulin IgG glycosylation"

Visegrad Scholarship Program (VSP)

The International Visegrad Fund offers Master's and Post-Master's scholarships awarded to selected scholars for periods of 1 or 2 semesters (with the exception of Master's scholarships within the Visegrad Scholarships schemes where 1– to 4-semester scholarships can be awarded).

The following scholarship schemes are available:

Intra-Visegrad Scholarships
In-Coming Scholarships
Out-Going Scholarships
Scholarship Program for Belarusian Students
Scholarship Program for Ukrainian Students
Visegrad Scholarships at OSA Archivum (separate program)

If selected each scholar receives the scholarship funding at the beginning of each five-month period (semester) upon a written confirmation from the host university/institution. Deadline for all scholarship applications is **31 January**. Results are announced by mid-May.

CSMC – RECOOP Research Centers (CRRC) the Center of Excellences of the RECOOP HST Association. They host young scientists, PhD students with CSMC – RECOOP (IVF – CSMC - RECOOP) Scholarship. The RECOOP HST Association Scientific Advisory Board selects the young scientists could compete for IVF – CSMC - RECOOP Scholarship.

The selected young scientists (preferably PhD students) will spend maximum four semesters at the host organization and receive: €2,300 / semester and the corresponding host universities/institutes receive €1,500/semester/scholar. The host CRRC will get $1,500 for laboratory expense and consumables from CSMC – RECOOP HST Association. Applicants whose current (i.e. at the time of applying) university or employer is further than 1,500 km from the selected host university/institute are eligible for a one-time travel grant.

RECOOP HST Association Members from the Visegrad Group Countries:
IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic
University of Debrecen, Hungary
University of Pecs, Hungary
University of Szeged
Slovak Medical University, Bratislava, Slovak Republic

RECOOP HST Association Member Organizations allegeable for the In-Coming scheme
Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

RECOOP HST Association's Cedars – RECOOP Research Center (CRRC) could participate
Semmelweis University, Budapest, Hungary
Comenius University in Bratislava, Slovak Republic
Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland
University Hospital in Hradec Kralove, Czech Republic

Welcome to Split

Please be advised that you need to take care of your airport transfer and local transportation – this cost will not be reimbursed

Information package

Getting to Split

Split is served by airport located in the city of Kaštela (about 25 km outside of Split), ferry and bus transport (last station of both train and long-haul buses is located in the city center, at the port). You can also comfortably arrive by car; Croatia has built an excellent network of highways in recent years.

Alternatively, there is an airport in Zadar (http://www.zadar-airport.hr/en/), which is served by Germanwings, Ryanair and Danube wings. If you decide to fly to Zadar, you can take aiport bus to get to the bus station for long-distance buses in Zadar, and take another bus from there to Split – drive from Zadar to Split is about 2 hrs long.

Getting to your hotel from the airport

By airport bus: There is official airport bus, located immediately at the front of the airport building. When you exit the airport, turn to the right. Bus schedule: http://www.plesoprijevoz.hr/split.html (departures from Split airport). You can buy ticket at the driver. One-way bus ticket costs 30 HRK (about 4 Eur). The driver does not accept Euros so you should change currency at the airport. This bus does not have any stops before Split, and it takes about 30 min to get to Split. Ask the driver to stop for you on the bus station in front of the Atrium hotel in Split. From that bus station, it is a short walk to hotel Dujam (Map 1) and hotel Art (Map 2).

By local intercity bus: The official airport bus has limited departures. If it happens that the bus is not leaving for Split around your arrival time, you can take local bus #37 that drives from Trogir to Split. The local bus stop is just 100 m below the airport, on the road. When you cross the airport parking, cross the road and the station will be right there. You can buy ticket at the driver (you should enter the front door). The bus ticket costs about 20 HRK (around 3 Eur). The driver does not accept Euros so you should change currency at the airport. This bus has numerous stops along the way, and it will take about 50-60 min to get to Split. Ask the driver, or fellow passengers on the bus, to warn you when the bus will stop in front of the Atrium hotel in Split.

By taxi: Multiple taxis are available in front of the airport. It costs at least 200 HRK (about 30 Eur). Please check the price before entering the taxi. The drive will take about 30 min to get to the meeting venue.

Getting to hotel Dujam /Best Western/ Atrium from the train and bus station in the city port

To get to the hotel Dujam from the central train/bus station in the city center: after exiting the railway/bus station, one needs to turn left, and walk for about 200 m to get to the station of the local bus #9. Take the bus #9 and exit the 6th station (you can also ask the driver to warn you about the station of the Dujam hotel). Bus ticket can be bought inside the bus from the driver, and it costs 12 kn (about 1.5 Eur). The driver does not accept Euros so you should change currency at the station. One can also walk from the railway station to the hotel Dujam – the walk is 1.8 km long and it could take 25-30 min (Map 3).

Walking from hotel Dujam /Best Western/ Atrium to the city center

The hotels are located about 20 min of slow walk from the city center. If you would like to take the local bus, you can take bus #9 near the hotels. Please refer to Maps 1 and 2 to see how to get to the nearest local bus station.

What to see and do in Split

Split is a historical city, 1700 years old. There are plenty of things to see and do.

Diocletian's palace was built by Roman emperor in the 4th century AD, and the city grew around the palace. The palace is the very center of the city, so do not expect to visit some isolated and empty palace. Many people live in the palace, and it is full of shops and restaurants.

Cathedral and Bell Tower of St. Domnious are right in the center of Diocletian's palace. You can walk all the way to the top of the bell tower and the entrance ticket is 30 kn (about 4 Eur). Not recommended for people afraid of heights and open spaces, as the bell tower is quite 'airy'.

Riva is a promenade at a seaside front of the Diocletian's palace, facing the city. Riva is the living room of Split. We go there to see and been seen. Take a stroll through Marmont street and along Riva, and then have a coffee at one of numerous cafés.

West coast is a recently renovated and expanded part of the promenade that goes from the end of Riva to the Sustipan. Very nice and relaxing walk, highly recommended. There are several cafés and one restaurant right in the middle of the West coast.

Coffee culture is very strong in Split and you will see numerous people sitting in cafes. People from abroad always ask us is anyone working at Split, when there are so many people in cafés. Considering our high unemployment rates in Croatia, the most accurate answer is – very few people actually work. The price of beverage in cafes is the same if you sit or if you drink at the bar. To give you a rough estimate of prices to expect in cafes – plain espresso coffee is around 8 kn (cca 1 Eur), coffee with milk or espresso around 10 kn (cca 1.5 Eur), soft drink around 12 kn (cca 1.7 Eur), small beer about 15 kn (cca 2 Eur).

Marjan hill is a small hill (highest point 178 m) with forest and recreational facilities. It is highly recommended to go to Vidilica – the observation point above Split. It is about 15 min walking uphill from the city center. When you get there, you will be rewarded with fantastic views of Split, sea and islands. And, of course, there is a café there.

Meštrović gallery houses works of a world renowned Croatian sculptor Ivan Meštrović. The gallery and surrounding park are truly worth a visit. Take the bus #12 from Riva to get there.

Bačvice beach is only 10 min walking away from the city center. It is a beautiful sandy beach, with a Blue Flag. This is not one of the beach resorts, and it does not have any mega hotels. A good place to relax, have an ice cream, or – a drink in one of numerous bars along the beach.

Islands Brač and Šolta are one hour away with a ferry from Split. **Island Hvar** is two hours away. Going to islands would require a day trip and staying in Split more days before or after the meeting.

Cities of Trogir and Omiš are one hour away from Split by local buses. Those are beautiful historical cities, pearls of Adriatic.

Popular tourist attractions further away from Split are **national parks Krka** and **Plitvice Lakes**, city of **Dubrovnik** and city of **Mostar in Bosnia and Herzegovina**. Visiting those places requires at least a full-day trip.

Public transport in Split

Split has bus lines numbered from 1 to 19. Day buses 1 through 18 run from 05:00 to 23:00. There is only one night bus, number 19, which runs on Fridays and Saturdays. Maps and schedules for each line can be found at their respective stops. Tickets can be purchased on the bus for 11 kn or from kiosks near each bus stop for less. The company that operate Split's buses is called Promet Split, so make sure the kiosk has that name on it before trying to buy a ticket. Split is covered by one zone, so a ticket is good for one trip anywhere in the city. Sukoišanska is the main station from which you can catch buses for Trogir, Omiš, the airport and other destinations outside of Split. Sukoišanska's ticket office operates from 06:00 to 20:00 on weekdays, 06:00 to 12:00 on Saturdays and is closed on Sunday. To contact the Sukoišanska station, dial (021) 48 06 56. For general information regarding bus services, call (021) 40 79 99.

http://www.promet-split.hr/

http://www.promet-split.hr/sadrzaj/interaktivna/interaktiv_10_preview.html

Taxis

The simplest way to call a taxi is to dial 970. The starting fee for a taxi trip is 20 kn, with a 10 kn fee added per kilometer and 2.5 – 10 kn added per each piece of luggage. There is no

additional charge for traveling at night. Taxis wait in front of most major hotels, Firule and Križine hospitals, at the ferry port, at the main bus station and near the Riva.

Practical tips

Tap water in Split is potable. Numerous public places have water fountains with potable water as well.

Currency is Croatian kuna (HRK). The exchange rate is approximately 1 Eur = 7.5 HRK. There are many currency exchange offices around Split and your hotel might also provide this service.

Split is generally a **safe city**, but exercise caution and common sense. Protect your valuables.

Official language is Croatian.

Majority of **population** is Croatian (90.4%), Catholic and conservative. Population of Croatia is 4.2 million.

Croatia joined **EU** on July 1, 2013.

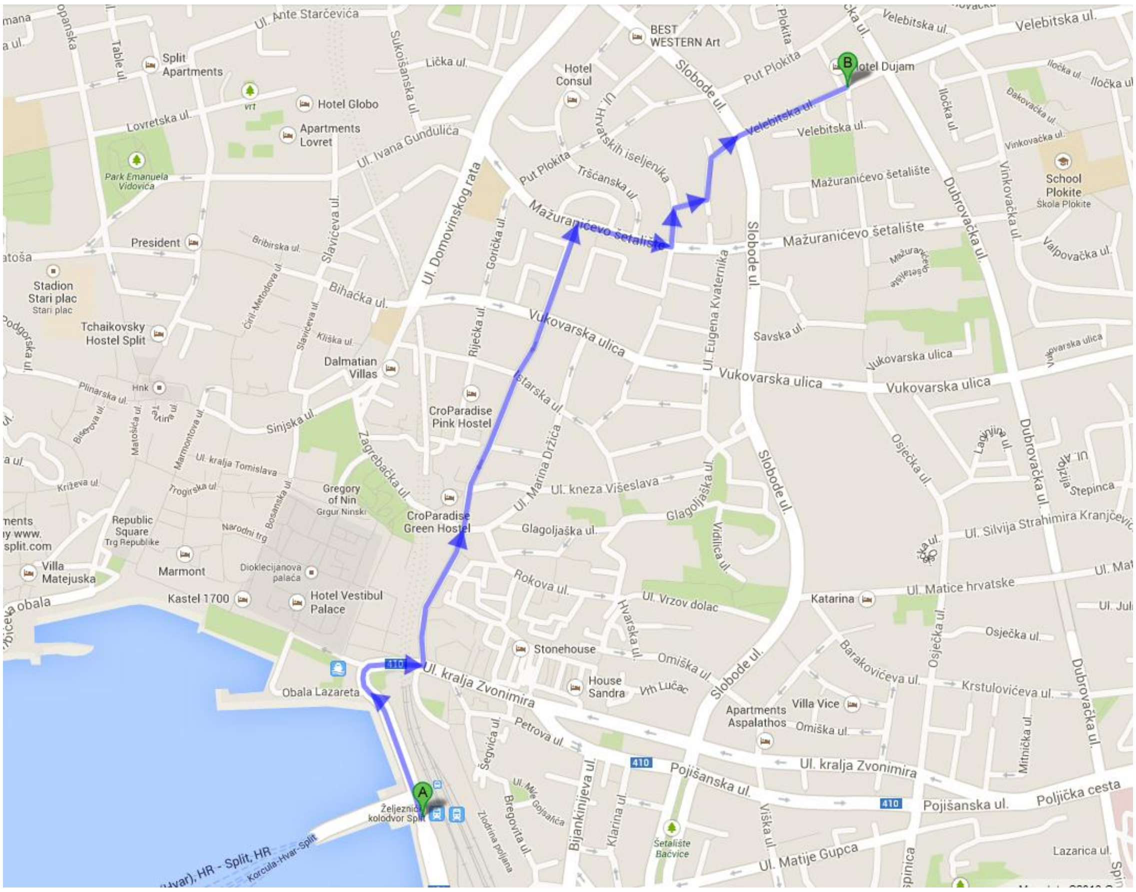
Useful links:

http://www.split.info/

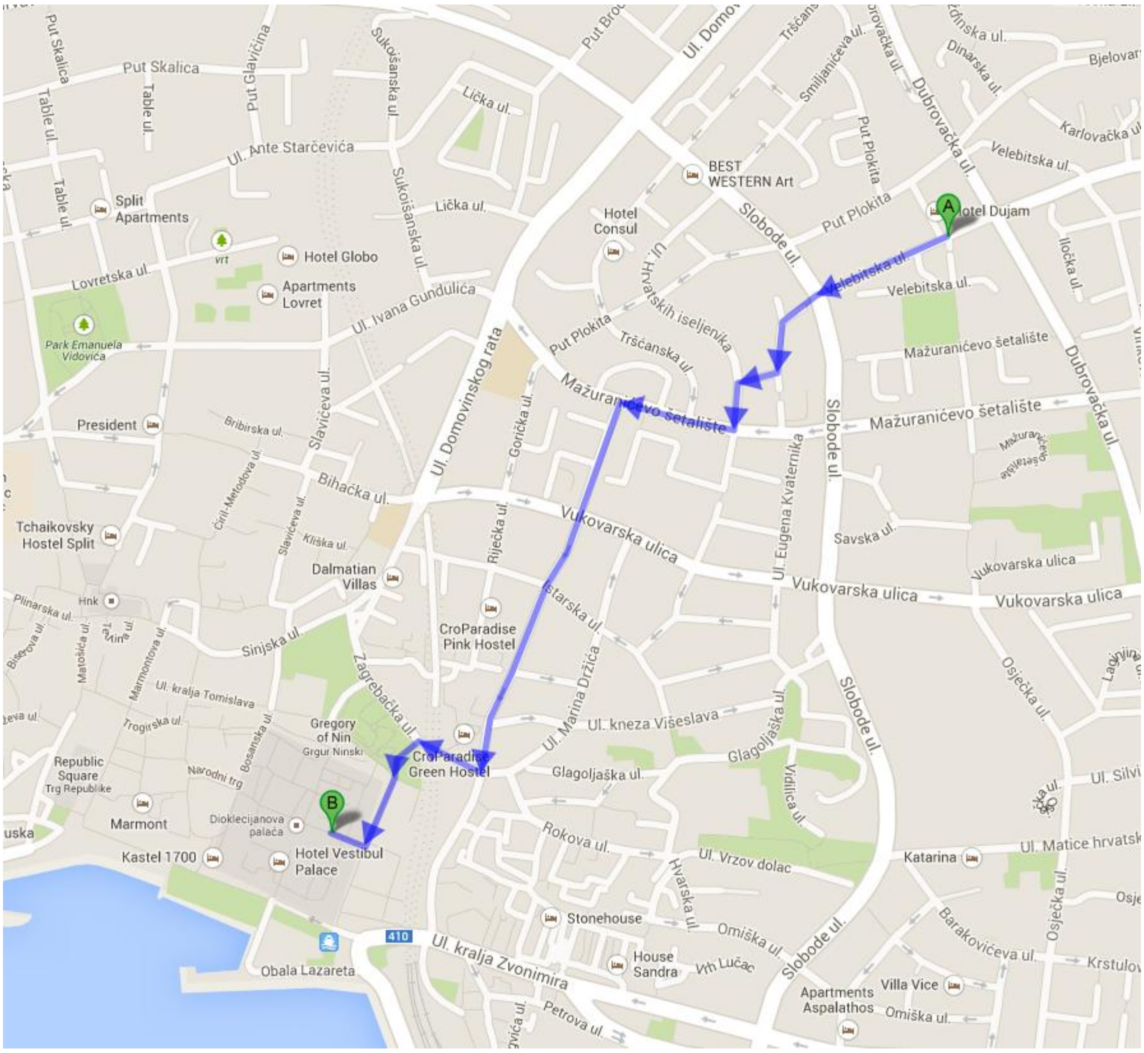
www.croatia.hr

www.visitsplit.com

Map 1. Walking from bus/train station to hotel **Dujam /Best Western/ Atrium** (1.8 km, cca 25 min)


Hotel Dujam
Velebitska ul.
Mažuranićevo šetalište
Slobode ul.
Vukovarska ulica
Ul. Domovinskog rata
Ul. kralja Zvonimira
Pojišanska ul.
Dubrovačka ul.
Željeznički kolodvor Split
Obala Lazareta
Hotel Vestibul Palace
Dioklecijanova palača
Gregory of Nin
CroParadise Green Hostel
CroParadise Pink Hostel
Dalmatian Villas
Tchaikovsky Hostel Split
Stadion Stari plac
President
Hotel Globo
Apartments Lovret
Split Apartments
Hotel Consul
BEST WESTERN Art
Park Emanuela Vidovića
Republic Square
Villa Matejuska
Marmont
Kastel 1700
Stonehouse
House Sandra
Apartments Aspalathos
Villa Vice
Katarina
School Plokite
Šetalište Bačvice
Ul. Matice hrvatske
Ul. Silvija Strahimira Kranjčevića
Poljička cesta
Ul. Matije Gupca

Map 2. Walking from hotel **Dujam /Best Western/ Atrium** to the city center (Diocletian's palace)



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