RECOOP HST CONSORTIUM

3rd Bridges in Life Sciences
Annual Scientific Review
Regional Cooperation for Health, Science and Technology

October 2008 Volume 2 Number 1
3rd Bridges in Life Sciences Annual Scientific Review

The Official Publication of the Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium

Bridges in Life Sciences Annual Scientific Review is periodical of the scientific advances in RECOOP HST Consortium.

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Mission Statement
Cedars-Sinai Medical Center (CSMC) and the RECOOP HST Consortium Partners formed a Strategic Alliance that is grounded in the high quality scientific output of the individual Partners. The Strategic Alliance is helping the Partners provide high quality research & innovation management, expand biomedical research and increase research capacity. The Strategic Alliance’s main goal is to train and motivate scientists to capture and maximize the value of the laboratories and turn the “outputs” - the research results - into “outcomes” – commercially available products: new diagnostic tools, new medical devices and new drugs - for the benefit of the public.

EDITOR
Sandor Vari, MD, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA

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The Bridges in Life Sciences Second Annual Scientific Review Meeting, Zagreb, Croatia, October 4, 2008

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Bridges in Life Sciences
Second Annual Scientific Review Meeting of the
Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium
October 4, 2008 Zagreb, Croatia

Venue:
Hotel International Zagreb
Miramarska 24
Zagreb 10000, Croatia

Program Chair:
Professor Calvin Hobel, Vice Chair, Department of Obstetrics & Gynecology, Miriam Jacobs Chair, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Program Co-Chairpersons:
Professor Filip Culo, Department of Physiology and Immunology, Zagreb University School of Medicine, Zagreb, Croatia

Professor Aleš Macela, Faculty of Military Health Sciences, Hradec Králové, Czech Republic

Professor Constantin Mircioiu, Scientific Secretary, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

Professor Oleh Pinyazhko, Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Professor Tomas Trnovec, Institute of Preventive and Clinical Medicine, Slovak Medical University, Bratislava, Slovakia

Sandor Vari, MD, Director, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA
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<tr>
<th>Time</th>
<th>Session</th>
<th>Speaker/Abstract</th>
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<tr>
<td>08:45</td>
<td>Opening Remarks</td>
<td>Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board</td>
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<td>Professor Matko Marusic, Editor in Chief, Croatian Medical Journal</td>
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<td>Zagreb University School of Medicine</td>
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<td>09:00</td>
<td>Summary of the Scientific Review</td>
<td>Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board</td>
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<td>09:15 – 11:30</td>
<td>Genomics and Proteomics (10 minutes presentation)</td>
<td>Chairman: Professor Aleš Macela, Faculty of Military Health Sciences, Hradec Králové, Czech Republic</td>
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<tr>
<td>#</td>
<td>Name</td>
<td>Title of the Abstract</td>
</tr>
<tr>
<td>1</td>
<td>Sergii Kropyvko</td>
<td>Cloning of Full-Length Transcripts Human ITSN1 Gene, and Expression Analysis in Human Tissues.</td>
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<tr>
<td>2</td>
<td>Rostyslav Bilyy</td>
<td>Identification and Way Of Appearance of Novel Membrane Glycomarkers Of Apoptotic Cells</td>
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<tr>
<td>3</td>
<td>Aleksandr G. Kondratov</td>
<td>Detection of genetic and epigenetic abnormalities in epithelial tumors by NotI-microarray technology.</td>
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<tr>
<td>4</td>
<td>Adela Straskova</td>
<td>Molecular and functional profile of Francisella tularensis FTT1103 deletion mutant.</td>
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<tr>
<td>5</td>
<td>Ganna Livshyts</td>
<td>The study of mutations in FSH receptor and INHα1 genes in women with ovarian dysfunction from Ukraine.</td>
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<tr>
<td>6</td>
<td>Jiri Dresler</td>
<td>The analysis of <em>Francisella tularensis</em> native protein complexes.</td>
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<tr>
<td>7</td>
<td>Pavlo F. Tatarskyy</td>
<td>Development of Test-System Prototype for DNA Analysis of the Detoxification System Genes Polymorphisms.</td>
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<tr>
<td>8</td>
<td>Klara Kubelkova</td>
<td>The specificity and the role of circulating antibodies produced in the course of Francisella tularensis infection in mice.</td>
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<tr>
<td>9</td>
<td>Dmytro Morderer</td>
<td>Intersectin 1 interacts with cytoskeletal protein MTAP6</td>
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<tr>
<td>10</td>
<td>Oleksandr Solovyov</td>
<td>Use of Real-Time PCR Techniques for Mutation Detection</td>
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**10:45 Questions – Panel Discussion**

**11:15 Coffee Break**

**POSTERS**

**Chairmen:**
Professor Aleš Macela, Faculty of Military Health Sciences, Hradec Králové, Czech Republic

| 11 | Adrienn J. Veres  | Cell surface pattern of HLA and ICAM 1 receptors on HLA II expressing and deficient human B cells | University of Debrecen |
| 12 | Maksim Skaldin    | Ephrin binding switches on a zipper-like mechanism of Eph receptor dimerization | Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine |
| 13 | Jurica Arapovic   | Regulation of NKG2D ligands by mouse cytomegalovirus | Medical Faculty University of Rijeka, Croatia |

**Questions – Poster Discussion**

Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board and Miriam Jacobs Chair, Cedars-Sinai Medical Center, Professor, Obstetrics/Gynecology & Pediatrics UCLA School of Medicine, Los Angeles, CA, USA

Sandor Vari, MD, Director, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA
### Pharmaceutical Research (10 minutes presentation)

**Chairmen:**
**Professor Constantin Mircioiu,** Scientific Secretary, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

**Professor Oleh Pinyazhko,** Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

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<tbody>
<tr>
<td>14</td>
<td>Dmytro Havrylyuk</td>
<td>Synthesis and anticancer activity of novel 4-(3-phenyl-5-aryl-4,5-dihydropyrazol-1-yl)-5H-thiazol-2-ones</td>
<td>Danylo Halytsky Lviv National Medical University, Lviv, Ukraine</td>
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<tr>
<td>15</td>
<td>Danylo Kaminskyy</td>
<td>Rhodanine-3-succinic acid utilization as the scaffold in design of novel anticancer agents</td>
<td>Danylo Halytsky Lviv National Medical University, Lviv, Ukraine</td>
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<tr>
<td>16</td>
<td>Tatiana Goriushkina</td>
<td>Development of Amperometric Biosensors Based on Immobilized Oxidases For Ethanol, Glycerol, Glucose and Lactate Determination</td>
<td>Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine</td>
</tr>
<tr>
<td>17</td>
<td>Yevhen Filyak</td>
<td>Doxorubicin Inhibits TGFβ-Signaling Via Blocking Translocation of Smad-Proteins into Nucleus of Tumor Cells</td>
<td>Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine</td>
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<tr>
<td>18</td>
<td>Valentin Didkivskyi</td>
<td>Study of Fullerenes’ C60 Effects on Apoptosis Induced in Normal and Transformed T-lymphocytes</td>
<td>Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine</td>
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**12:30** Questions – Panel Discussion

**13:00** Lunch Break

### POSTERS

**Chairmen:**
**Professor Constantin Mircioiu,** Scientific Secretary, Faculty of Pharmacy, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

**Professor Oleh Pinyazhko,** Department of Pharmacology, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

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<tr>
<td>19</td>
<td>Corina Arama</td>
<td>Ion pair RP HPLC in basic drug analysis. Assay of midazolam.</td>
<td>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania</td>
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<td>20</td>
<td>Bozhena Vynnytska</td>
<td>A combinational approach in anticancer therapy based on arginine deprivation</td>
<td>Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine</td>
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<td>21</td>
<td><strong>Volodymyr Chernyshenko</strong></td>
<td>Echis multisquamatis venom enzymes acting on haemostasis</td>
<td>Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine</td>
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<tr>
<td>22</td>
<td><strong>Dmytro Atamanyuk</strong></td>
<td>Leads structure optimization aiming improvement of antitumor profile using the strategy of privileged scaffold based on thiopyran[2,3-(\beta)]thiazol-2-one derivatives</td>
<td>Danylo Halytsky Lviv National Medical University, Lviv, Ukraine</td>
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**17:45 Questions – Poster Discussion**

Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board and Miriam Jacobs Chair, Cedars-Sinai Medical Center, Professor, Obstetrics/Gynecology & Pediatrics UCLA School of Medicine, Los Angeles, CA, USA

Sandor Vari, MD, Director, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA
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<tr>
<td>23</td>
<td>Katya Volkova</td>
<td>Studies of T-284 and SH-516 cyanine dyes as fluorescent probes for specific α-synuclein fibrils detection</td>
<td>Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine</td>
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<td>24</td>
<td>Tünde Rente</td>
<td>Energy transfer measurements using different dye-to-protein labeled antibodies</td>
<td>University of Debrecen, Faculty of Medicine</td>
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<td>25</td>
<td>Martina Valachovicova</td>
<td>Selected products of oxidative damage in relation to nutrition and age</td>
<td>Slovak Medical University, Bratislava, Slovakia</td>
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<tr>
<td>26</td>
<td>Andreea Letitia Arsene</td>
<td>Relationships between cerebral monoaminergic status and a targeted pharmacotherapy of depression in a murine model of behaviour</td>
<td>Carol Davila University of Medicine and Pharmacy, Bucharest, Romania</td>
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<td>27</td>
<td>Olena Kuchmenko</td>
<td>Correction of bioenergetical, antioxidant and apoptotic properties of heart tissue with the help of precursors and modulator of coenzyme Q biosynthesis under ageing</td>
<td>Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine</td>
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### Clinical Research

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<tr>
<td>27</td>
<td>Zuzana Dzupinkova</td>
<td>Metabolic syndrome and diabetes mellitus type 2 and SNPs in genes PPARD and PPARGC1A</td>
<td>Slovak Medical University, Bratislava, Slovakia</td>
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<td>28</td>
<td>Ivanna Romankevych</td>
<td>The particularities of the functional status of the cardiovascular system in children with the juvenile rheumatoid arthritis (JRA)</td>
<td>Danylo Halystky Lviv National Medical University, Lviv, Ukraine</td>
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<td>29</td>
<td>Peter Boor</td>
<td>Platelet-Derived Growth Factor – D (PDGF-D): a novel treatment target in kidney diseases</td>
<td>Slovak Medical University, Bratislava, Slovakia</td>
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<td>30</td>
<td>Nataliya Rohovyk</td>
<td>Thread of nosocomial salmonellosis in the view of antibiotic resistance.</td>
<td>Danylo Halytsky Lviv National Medical University, Lviv, Ukraine</td>
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<td><strong>15:50</strong></td>
<td><strong>Panel Discussion</strong></td>
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<td><strong>16:15</strong></td>
<td><strong>Coffee Break</strong></td>
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| **POSTERS**
Chairmen:
Professor Tomas Trnovec, Institute of Preventive and Clinical Medicine, Slovak Medical University, Bratislava, Slovakia,
Professor Filip Culo, Department of Physiology and Immunology, Zagreb University School of Medicine, Zagreb, Croatia |
<p>| 31 | Maria Takacova | Prevalence of HIV CCR5-Δ32 in the HIV-positive versus HIV-negative Slovaks | Slovak Medical University, Bratislava, Slovakia |
| 32 | Liudmyla Kapustian | Changes of molecular Chaperon HSP60 Expression in Cardiac Muscle at Dilated Cardiomyopathy Progression | Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine |
| 34 | Sola Paryzhak | Cells-based amperometric biosensors for formaldehyde monitoring in biological samples | Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine |
| 35 | Iryna Fomenko | Lipoperoxidation processes in heart tissue of rats under COX-2 blockage and dual COX/LOX inhibition | Danylo Halytsky Lviv National Medical University, Lviv, Ukraine |
| <strong>17:45</strong> | <strong>Questions – Poster Discussion</strong> | | |
| Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board and Miriam Jacobs Chair, Cedars-Sinai Medical Center, Professor, Obstetrics/Gynecology &amp; Pediatrics UCLA School of Medicine, Los Angeles, CA, USA |
| Sandor Vari, MD, Director, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA |</p>
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<tr>
<td>16:30 – 19:00</td>
<td>Human Development (10 minutes presentation)</td>
<td>Chairman: Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board and Miriam Jacobs Chair, Cedars-Sinai Medical Center, Professor, Obstetrics/Gynecology &amp; Pediatrics UCLA School of Medicine, Los Angeles, CA, USA</td>
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<td>36</td>
<td>Katarina Vukojevic</td>
<td>Is there neurogenesis after final settlement of neural crest cells in spinal ganglia?</td>
<td>School of Medicine University of Split</td>
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<td>37</td>
<td>Jana Babjakova</td>
<td>Allergic diseases in children of preschool age from 2 environmentally different Slovak regions</td>
<td>Slovak Medical University, Bratislava, Slovakia</td>
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<td>38</td>
<td>Khrystyna Slivinska</td>
<td>Sex assignment of females, suffering from the virilizing form of congenital adrenal hyperplasia with high grade of virilization</td>
<td>Danylo Halytsky Lviv National Medical University, Lviv, Ukraine</td>
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<td>39</td>
<td>Kristína Klenovicsová</td>
<td>Content of Maillard reaction products (MRPs) in breast milk and infant formulas and their impact on selected biological parameters in 5-7 months old infants</td>
<td>Slovak Medical University, Bratislava, Slovakia</td>
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<td>17:30</td>
<td>Panel Discussion</td>
<td>Posters Chairman: Professor Calvin Hobel, Chairman of the RECOOP HST Scientific Advisory Board and Miriam Jacobs Chair, Cedars-Sinai Medical Center, Professor, Obstetrics/Gynecology &amp; Pediatrics UCLA School of Medicine, Los Angeles, CA, USA Sandor Vari, MD, Director, International Research and Innovation Program Cedars-Sinai Medical Center, Los Angeles, CA, USA</td>
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<td>40</td>
<td>Olga Martsenyuk</td>
<td>Cystathionine B-Synthase and Proliferation at Hyperhomocysteinemia in Human Placenta</td>
<td>Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine</td>
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<td>41</td>
<td>Olga Fesai</td>
<td>Correlation of AR CAG tract length with impaired spermatogenesis</td>
<td>Institute of Molecular Biology and Genetics, National Academy of Science of Ukraine, Kyiv, Ukraine</td>
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<td>8:30</td>
<td>REVIEW THE SCIENTIFIC ACTIVITIES AT PARTICIPATING ORGANIZATIONS</td>
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<td>Poster Session and Visegrad Four (V4) Scholarship Marketplace</td>
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<td>17:45</td>
<td>Questions – Poster Discussion</td>
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<td>Obstetrics/Gynecology &amp; Pediatrics UCLA School of Medicine, Los Angeles,</td>
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<td>Scholarship Marketplace</td>
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<td>Marketplace for young scientists to engage with the RECOOP Czech, Hunga</td>
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<td>rian and Slovakian member organizations and apply for the Visegrad Fou</td>
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<td>r (V4) Scholarship Programme offers Master's and Post-Graduate (Post-M</td>
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<td>aster's) Scholarships for study/research projects in the length of 1 t</td>
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<td>18:30</td>
<td>Closing Remarks – Professor Calvin Hobel, Chairman of the RECOOP HST</td>
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<td>Scientific Advisory Board Department of Obstetrics &amp; Gynecology, Miri</td>
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<td>am Jacobs Chair, Cedars-Sinai Medical Center, Los Angeles, CA, USA</td>
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<td>19:30</td>
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<td>Announcement of the winner of the “RECOOP HST Best Young Scientist&quot;</td>
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<td>will be awarded a CSMC Travel Grant to visit Cedars – Sinai Medical C</td>
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<td>enter, Los Angeles, USA and spend two weeks at Burns and Allen Rease</td>
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<td>ice President for Finance and Chief Financial Officer, Cedars-Sinai M</td>
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<td>edical Center, , Los Angeles, CA, USA</td>
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<td>The “RECOOP HST Best Young Scientist” will be invited to submit a ful</td>
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<td>l paper to the Croatian Medical Journal will be published in English.</td>
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<td>Professor Matko Marusic, MD, PhD, Editor in Chief, Croatian Medical J</td>
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<td>ournal Zagreb University School of Medicine</td>
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<td>The six best presentations will be invited to attend the “Bridges in L</td>
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ABSTRACTS

GENOMICS IN BASIC AND CLINICAL RESEARCH
Cloning of Full-Length Transcripts Human ITSN1 Gene, and Expression Analysis in Human Tissues

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Alternative splicing has recently become one of major mechanisms, increasing the diversity of transcriptome and has important application in physiology, development and genesis of diseases.

Intersectin 1 (ITSN1) is an evolutionary conserved, multidomain protein involved in clathrin-associated endocytosis, signal transduction, and cytoskeleton rearrangement. ITSN1 mRNAs encode two major isoforms, a short and a long one. The short form is ubiquitously expressed and consists of two EH domains, a coiled-coil region, and five SH3 (A-E) domains. The long form is predominantly expressed in neurons and includes three additional C-terminal domains, namely, DH, PH, and C2. All the domains have their protein-partner.

Recently, we have identified eleven alternative splicing events affecting mouse and human ITSN1 transcripts. Five of them isoforms have deletions or insertion in resulting protein, which may affect binding properties and functions of ITSN1. Here we present results on cloning and characterization of fifteen full-length human ITSN1 transcripts comprising different combinations of alternatively spliced exons. Moreover, we have found additional alternative splicing event affecting exon 5 of human ITSN1 gene.

Analysis of GenBank databases showed the possibility of existence of exons 1a and 1b (EST DA507184 and DA738410), which potentially can regulate translation of ITSN1. Their expression was found non-uniform in different human and cancer tissues.

We have shown lower expression of long form transcripts, transcripts with skipping of exon 20 and exon 35 in human glial tumors in comparison to normal brain. Short form transcripts, transcripts with deletion of exons 25-26 and insertion of exon 22a did not change expression in these tumors.

Thus, tissue and development specific splicing may influence the interaction of ITSN1 with their partners and contribute to the regulation of ITSN1 protein function in endocytosis and signal transduction.
Detection of genetic and epigenetic abnormalities in epithelial tumors by NotI-microarray technology.

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Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations (amplifications, deletions) and epigenetic changes (methylation of gene promoter region). It has been shown that human chromosome 3 is frequently rearranged in epithelial tumors.

Therefore to perform the global search for genetic and epigenetic alterations of genes from chromosome 3 in epithelial tumors (kidney, cervical, ovary, breast and colon) we used a novel NotI-microarray approach, which allows simultaneous detection of genetic and epigenetic alterations. NotI-microarrays revealed significant part of genes from chromosome 3 with deletion or promoter methylation. Among identified genes with detected high level of changes were known tumor suppressor genes like RARbeta1, VHL, RBSP3 and others. Many of identified genes have known function (ZIC4, UBE2E, GNAI2) and some is unknown loci (LOC285205, Hmm144092, Hmm144092). The methylation status of investigated genes has been confirmed by bisulfite sequencing. The aim of further investigation will be characterization of selected genes as diagnostic and prognostic markers in epithelial tumors.
The study of mutations in FSH receptor and INHα1 genes in women with ovarian dysfunction from Ukraine.

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Objectives: Premature ovarian failure (POF) is a secondary gonadotrophic amenorrhoea affecting 1-3% of females. FSH (follicle stimulation hormone) and its receptor (FSHR) play a major role in the development of follicles and regulation of steriogenesis in the ovary. Mutations in the FSHR might theoretically lead to an impaired signal transduction and thereby to a diminished ovarian reserve. Genes encoding the three inhibin subunits can be proposed as candidates for POF due to its role in the negative feedback control of FSH. We investigate Asn680Ser and Thr307Ala transitions in FSHR gene and Ala257Thr transition in INHα1 gene as markers for diminished ovarian reserve in women: with clinical POF diagnosis and women with poor response (less than 4 oocytes after standard protocol of FSH stimulation) – “poor responders”.

Results: The frequency of Ala307-Ser680 (AS) allele of FSHR gene was significantly higher both in POF group and in “poor responders” group comparing to control group. The carriers of INHα1 gene Ala257Thr transition predominated in the “poor responders” group. Quantity of oocytes after stimulation ovulation in women with INHα1 gene Ala257Thr transition was significantly decreased in comparison to patients without such mutation. Our data shows the prevalence of FSHR gene AS allele in both patients groups: group of POF patients (45,7%) and “poor responders” group (52,8%), comparing to control group (35,1%).

Conclusions: Our data about FSHR and INHα1 genotype association with ovarian reserve and response to FSH represent that the best stimulation protocols can be based on the individual’s genetic profile in order to reduce side-effects and costs and improve the delineation stimulation protocols.
Development of Test-System Prototype for DNA Analysis of the Detoxification System Genes Polymorphisms.

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The etiology of recurrent pregnancy loss (RPL) remains largely unclear. Epidemiological studies have suggested that the risk of spontaneous abortions associated with exposure to endogenous or exogenous substances may be modified by the genetic variation in individual metabolic detoxification activities, thus in the phase I/phase II balance. Phase I enzymes such as P4501A1 (CYP1A1) metabolize organic compounds to reactive compounds which can damage cells and DNA. N-acetyltransferase 2 (NAT2) is involved in the initial biotransformation metabolism of aromatic amines and hydrazines and catalyses the transfer of an acetyl group from acetyl CoA to the nitrogen of the substrate. Glutathione S-transferase (GST) catalyze the binding of a large variety of electrophils to the sulphydryl group of glutathione, they are involved in the detoxification of free radicals and have a main function in binding and transport of a wide variety of harmful compounds. GST is present in large amounts in many tissues including those of the genital tract and placenta. They are expressed very early in embryonic development in reproductive tissues, the placenta and decidua in particular.

The GST is a family of enzymes that are believed to exert a critical role in cellular protection against toxic foreign chemicals and oxidative stress. The aim of this study was to develop a prototype test-system and investigate the role of CYP1A1, GSTM1, GSTT1, GSTP1 and NAT2 polymorphisms in the pathogenesis of RPL. We studied the polymorphic variants of genes encoding enzymes in 49 women (case group) with recurrent pregnancy loss and in 169 women (control group) with an uncomplicated obstetric history. The frequency of GSTM1 0/0 genotype in case group (0.77) was significantly (p=0.018) higher than in control group (0.50). The frequency of GSTT1 0/0 genotype in the case group (0.25) was higher than in control group (0.19) but this difference was not significant. The frequencies of NAT2 "slow alleles" were practically identical in both analyzed groups. It had been shown that GSTM 0/0 variant really can be involved in process recurrent pregnancy loss, which may be connected with changes in steroid hormones level.

The identification of GSTM 0/0 genotype can be used as a marker of RPL high risk. Our test-system can be used for diagnostics in clinical practice, mass and selective screening in genetic testing programs of families which are planning to conceive a child and to avoid pregnancy loss.
Use of Real-Time PCR Techniques for Mutation Detection

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Traditional methods for mutation detection such are labour-intensive and time-consuming. The use of Real-Time PCR techniques can essentially shorten the time of analysis; they do not include the electrophoresis stage and, thereby, avoid contamination that is very useful for molecular-genetic diagnostics. Our aim was to develop a closed-tube system for mutations scanning using Real-Time PCR techniques: melting analysis of amplicons and allele-specific Real-Time PCR.

We studied a deletion of 3bp in exon 10 CFTR gene dF508 using melting analysis. We designed specific oligonucleotide primers and optimized the PCR conditions for amplification product of 44 bp for the sample without deletion and 41 bp for the sample with dF508. It was shown that SYBR Green I dye is stabilizing the DNA duplex, thereby increasing the melting temperature of the complex. SYBR Green I dye is supplied with the concentration 10000x. We determined that the optimal dye concentration was 5x. The difference in the Tm between the normal sample and the sample with dF508 was 0.9-1ºC. The samples were checked by heteroduplex analysis to confirm the presence of dF508. We have also studied deletions/insertions in genes BRCA1/2 and CHEK2 – the susceptibility genes for hereditary breast cancer: BRCA1 5382insC, BRCA1 185 delAG, BRCA1 4153delA, BRCA2 6174 delT, CHEK2 1100delC using allele-specific Real-Time PCR. This method allows discriminating heterozygous carriers of mutations using two pairs of primers, one of which is homologous to normal DNA sample and the other one – to the DNA with mutation. We designed specific oligonucleotide primers and optimized the PCR conditions for amplification with SYBR Green I. We used samples with abovementioned mutations as positive controls in our study.

Thus, the developed assays for mutation detection using melting analysis and allele-specific Real-Time PCR in combination with direct sequencing can be used for molecular-genetic diagnostics and screening programmes.
ABSTRACTS

PROTEOMICS IN BASIC AND CLINICAL RESEARCH
Identification and Way of Appearance of Novel Membrane Glycomarkers of Apoptotic Cells

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Surface markers of apoptotic cells are of great interest as potential targets for non-destructive detection and study of these cells. They are also important for apoptotic cell recognition and subsequent clearance by cells of the immune system. Recently, it was found that apoptosis is accompanied by not only the loss of plasma membrane asymmetry detected by Annexin V, but also by changes in cell surface glycoconjugates. These novel markers of apoptosis are α-D-mannose and β-D-galactose-specific plasma membrane glycoproteins whose expression is substantially increased after induction of apoptosis and sialyl-rich glycoproteins whose expression is decreased during apoptosis (Bilyy 2003; Bilyy 2007). The aim of current work was to 1) isolate and identify glycoproteins with altered membrane exposure during apoptosis; and 2) to identify mechanism of glycoprotein changes during apoptosis.

By means of lectin-affinity chromatography, gradient PAGE and subsequent MALDI-TOF mass spectrometry, we have identified as AMID - apoptosis-inducing factor (AIF)-like mitochondrion-associated inducer of death (accession number AAH38129, sequence coverage 31%) the glycoprotein from the plasma membrane fraction of apoptotic murine leukemia L1210 cells, lacking in the intact cells. The obtained results suggest its possible glycosylation that was further suggested by finding N-glycosylation sequon in the signal peptide of AMID protein (in silico), and by predicting transmembrane localization of its N-terminal part. Using monoclonal antibodies to AMID, we demonstrated an increased expression of AMID in human leukemia Jurkat T-cells after apoptosis induction. Immunocytochemical study suggested its association to the plasma membrane (Bilyy 2008).

While determining mechanism of glycoprotein redistribution during apoptosis few hypotheses were considered, namely: a) de novo glycoprotein synthesis; b) modification of preexisting plasma membrane glycoproteins. Inhibitors of early stages in GP synthesis and processing (tunicamycin and 2-deoxy-D-glucose), inhibitor of GP traffic between Golgi compartments (monensin) as well as inhibitor of transcription (actinomycin-D) and translation (cycloheximide) were used with the aim to test de novo glycoprotein synthesis hypothesis.

Neuraminidase inhibitor DANA was used to verify modification hypothesis. Besides, activity of membrane-associated and soluble neuraminidases was determined and acid desialation of isolated plasma membrane GP was done. mRNA expression level of enzymes, responsible for synthesis of sialil-containing glycoproteins - hST6Gal.I (sialyltransferase 1 isoform a; CMP-N-acetylneuramininate beta-galactosamide α-2,6-sialyltransferase), hST3Gal.III (GaLa1,3/4GlcNAc α2,3-sialyltransferase), hST3Gal.IV (Galβ1,3GalNAc/Galβ1,4GlcNAc α2,3-sialyltransferase); or degradation of preexisting GPs - NEU1 (neuraminidase 1 or lysosomal sialidase), NEU2 (cytosolic sialidase; N-acetyl-alpha-neuraminidase 2), NEU3 (neuraminidase 3; N-acetyl-alpha-neuraminidase 3), NEU4 (neuraminidase 4 or lysosomal sialidase4) was checked. We also used fluorescent neuraminidase substrate (4-MU-NA) to determine neuraminidase activity on the surface of alive, early and late apoptotic cells, and tested the possibility of neuraminidase exposure from internal cellular membranes and their
possible influence on the neighbor cells. Obtained results suggest an activation of membrane-associated neuraminidase activity as primary cause of plasma membrane glycoprotein redistribution during apoptosis, specific enzymes involved in glycoprotein redistribution were also determined.

References:
Molecular and functional profile of Francisella tularensis FTT1103 deletion mutant

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Francisella tularensis, the causative agent of severe disease - tularemia, is characterized as a Gram-negative and facultative intracellular pathogen. There are four subspecies recognized, however, only highly virulent F.tularensis subsp. tularensis (type A) and the moderately virulent subsp. holarctica (type B) are associated with human disease.

Limited knowledge about main F.tularensis virulence factors and mechanisms of interaction with host cells are the main reasons why construction of efficient and safe vaccine has not been successful so far.

In our previous study we have applied the comparative proteomic approach with strains of different virulence to identify novel potential virulence factors. Based on the obtained results we have discovered several proteins with different level of expression in F.tularensis strains. One of them was classified as a hypothetical lipoprotein FTT1103 with homology to DsbA – a protein disulfide oxidoreductase A. This protein is known as important virulence factor in many other bacteria such as Pseudomonas aeruginosa, Salmonella enteritica or Shigella flexneri. For detailed characterization of its role in F.tularensis pathogenesis „in-frame“ deletion mutant in F.tularensis live vaccine (LVS) and in fully virulent clinical isolate subsp. holarctica FSC200 was constructed. Deletion of this gene have shown attenuation of virulence in mice, inability to spread from the site of infection to spleen, lungs and peritoneum, moreover inability to replicate in host cells and finally decreased cytotoxicity. Furthermore, the FTT1103 mutant has a potential to elicit protective immunity against fully virulent type B strain. To investigate the biological function of FTT1103 deletion we have performed comparative proteomic analysis of wild and mutant strains. We have identified several proteins whose levels were significantly altered in the FTT1103 deletion mutant. In all of these proteins signal peptide consensus sequences were found indicating their outer membrane or periplasmic localization or even secretion.

In conclusion, these data indicate that DsbA-like lipoprotein FTT1103 is an important virulence factor of F. tularensis with a potential to be a possible target for future vaccine development.
The analysis of *Francisella tularensis* native protein complexes

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Since many complex cellular processes e.g. metabolic, developmental and regulatory pathways are carried out by sophisticated multi protein machines, the investigation of protein interactions becomes far more important. This integrative view could lead to deeper insight into their physiological as well as pathologic function and cell behaviour.

*Francisella tularensis*, a gram-negative facultative intracellular bacterium, is a causative agent of the rare but potentially severe and fatal disease – tularemia. This bacterium is capable of survival and replication in macrophages, but the detailed strategy of its virulence is still not clear. In order to obtain deeper insight into the mechanism of its pathogenicity, we focused on protein complexes residing in the bacterial membrane, due to their crucial role in the host-pathogen interaction.

To analyse the complexes in their native form, the membrane fraction of *F. tularensis* live vaccine strain was separated by Blue Native polyacrylamide gel electrophoresis (BN PAGE). To investigate of subunit composition of these complexes, the SDS PAGE was subsequently applied in second dimension. The bands resulted from 1D-BN PAGE as well as the spots from the 2D-BN/SDS PAGE were cut of the gel and the proteins were analyzed by LC-nanoESI-MS/MS or MALDI-MS/MS.

Various membrane proteins of *F. tularensis* were detected, including the complexes involved in bacterial metabolism and physiology e.g. oxidative phosphorylation, stress response, heat shock proteins and secretion systems. Furthermore, some new interactions among the membrane proteins were proposed. BN PAGE thus appears to be a powerful tool suitable not only for protein complex research but also for investigating very lipophilic membrane proteins, usually inaccessible by traditional SDS electrophoresis.

This work was financially supported by Ministry of Defence of Czech Republic (project No. MO0FVZ0000501).
The specificity and the role of circulating antibodies produced in the course of *Francisella tularensis* infection in mice

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The host immune response to a pathogen improve specific and nonspecific humoral and cellular defense mechanisms to reach effective protection. Successful defense against microorganisms is nearly always the result of synergy and cooperation between the various branch of the immune system. The cellular and molecular basis of immune mechanisms that control *Francisella tularensis* (*F. tularensis*) infection are not fully understood, yet. The older information concerning the mechanisms of immune response against tularemia originated from natural infection of men or from immunization studies. Recently, new information are obtained from model animal studies, exploiting the fact that murine tularemia caused by LVS strain is analogical to natural human tularemia. Both specific antibodies and B cells may contribute to control of primary infection or vaccination-induced protection in some circumstances, particularly against lower virulence *Francisella* strains.

The dependency of protective response on cell-mediated immunity based on activation of T cells and subsequent enhancement of microbicidal activity of macrophages was already successfully documented. Moreover, IFN-γ is required for antibody-Ab)mediated protection, it is likely that either CD4 and/or CD8 T cells contribute to the humoral response. The efficacy of humoral immunity against *F. tularensis* is generally employed by demonstrating protection after passive transfer of specific antibody and/or correlating protection against infection with the presence of serum antibody. Recently, the systematic monitoring of the ability of polyclonal sera and hybridoma antibodies to confer passive protection against *F. tularensis* vindicates the humoral immunity, based on specific antibodies, as an equivalent partner to cell-mediated immune mechanisms in formation of protective immune response. Thus, the humoral immune responses play a significant role in the elimination of *F. tularensis* microbes and that a cooperative interaction between cell- and Ab-based mechanisms can provide prophylactic as well as post-exposure protection against this intracellular biothreat. Essentially, the understanding the role of Ab-mediated immunity against *F. tularensis* infection is important for fundamental immunology, development of immunotherapy and vaccine design.
Intersectin 1 interacts with cytoskeletal protein MTAP6

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Intersectin 1 is a multifunctional evolutionally conserved adaptor protein that is implicated in clathrin-mediated endocytosis, MAP-kinase signaling pathway and regulation of actin dynamics. Human intersectin 1 (ITSN1) was mapped to locus 21q22.1-q22.2 which is a “critical region” for Down syndrome. To date more than 15 partners are known to interact with intersectin 1.

In order to find new intersectin 1 partner, we cloned its SH3 domains into pGEX-4T-3 expression vector. Recombinant proteins were purified and used for GST pull-down assay with mouse brain lysate. After incubation and washing, bound proteins were eluted and separated by SDS-PAGE. The bands of interest were cut from the gel for identification by MALDI-TOF mass spectrometry.

For the SH3A domain of intersectin 1, a band of 125 kDa was observed. Using MALDI-TOF we identified this band as MTAP6 (STOP) protein. The possibility of such interaction was also predicted by Scansite server (www.scansite.mit.edu). Mass spectrometry results were confirmed by independent methods: firstly, GST pull-down assay revealed that MTAP6 can bind SH3A and with less affinity SH3C and SH3E domains. Additionally, intersectin 1 – MTAP6 complexes were coimmunoprecipitated from mouse brain lysate with anti-STOP and anti-ITSN1-EH2 antibodies. Finally, interaction between intersectin 1 and MTAP6 was visualized in HeLa and MCF-7 cell lines using bimolecular fluorescence complementation (BiFC) approach.

MTAP6 is a main factor that determines Ca-calmodulin-regulated microtubule (c missing) cold and drug stability. Absence of MTAP6 in mice leads to synaptic defects such as depleted synaptic vesicle pools and impaired synaptic plasticity, associated with severe behavioral disorders. Interestingly, these disorders can be modulated by long-term treatment with neuroleptics – antipsychotic agents used in schizophrenia. MTAP6 was shown to be phosphorylated by multifunctional enzyme Ca-calmodulin-dependent kinase II (CaMKII). Phosphorylated form of MTAP6 does not bind microtubules but co-localizes with actin microfilaments in neurites and clusters of synaptic proteins giving the role for MTAP6 in synaptic transmission.

Both intersectin 1 and MTAP6 are implicated in functioning of synapses. Together they can be involved in regulation of certain stages of synaptic vesicle recycling. Thus, functional investigations of their interaction are of great interest and further research efforts are needed.

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Cell surface pattern of HLA and ICAM 1 receptors on HLA II expressing and deficient human B cells

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Cell surface proteins have an important role in signal transduction processes. The distribution and association of different antigens is in the focus of many research groups [1, 2].

We have established a stable human B cell line expressing HLA-DQ6 by transfecting a HLA II-deficient BLS-1 (Bare Lymphocyte Syndrome) cell line with pCIneo expression vector using Amaza nucleofection [3]. After testing the transcription of the DQA1*0102 and DQB1*0602 genes on mRNA level with RT-PCR analysis and the cell surface expression of HLA-DQ6 with flow cytometry, we have determined the expression level of HLA I, HLA II and ICAM 1 proteins. On HLA-DQ6 transfected cells a decreased expression of HLA I was detected (~30%).

Homo- and hetero associations of HLA I, HLA II and ICAM 1 molecules were determined on EBV-transformed lymphoblast JY, HLA class II-deficient BLS-1 human B and HLA-DQ6 transfected BLS-1 cell lines by flow and image cytometric FRET (Fluorescence Resonance Energy Transfer) methods. No notable differences were found in the heteroassociation of ICAM 1 with HLA I and HLA II on JY and HLA-DQ6 transfected cells. However, compared to the JY cells, HLA-DQ6 nucleofected BLS-1 showed a significant decrease (from 25% to 15%) in energy transfer efficiency between HLA I and HLA II antigens (using HLA I as donor and HLA II as acceptor, and vice versa). Based on the above data we concluded that the absence and presence of HLA-DQ6 on other cell surface heavily influences the cell surface pattern of other receptors.

The effect of cholesterol level on the distribution of plasma membrane proteins of the three cell lines (JY, BLS-1, HLA-DQ transfected BLS-1) was also investigated by using phosphatidylcholine treatment. Both flow and image cytometric methods indicated a significant change in the expression levels and the pattern of the above receptors.

In conclusion, present data show that the expression levels of transfected molecules and modification of cholesterol level in the plasma membrane play a role in restructuring the patterns of the cell surface receptors.

References:
Ephrin binding switches on a zipper-like mechanism of Eph receptor dimerization

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Eph receptors are the largest family of receptor tyrosine kinases, and their ligands are ephrins, which are also membrane-bound molecules. Both Eph receptors and ephrins are divided into A and B subclasses.

These molecules play a significant role in regulation of development and functioning of nervous, cardiovascular, locomotorium, immune and endocrine systems, and they also involve in tumorogenesis.

The Eph receptor extracellular part (Eph-ECP) contains a highly conserved N-terminal ligand-binding domain (LBD), an immediately adjacent cysteine-rich region (CRR) and two type III fibronectin (Fn3) repeats.

All ephrins contain a conserved extracellular receptor-binding domain (RBD).

The crystal structure of EphB2-LBD – ephrin-B2-RBD complex has shown a unique mode of ligand-receptor recognition, where two receptor molecules bind two ligand molecules to form a circular heterotetramer.

However, further studies showed that ligand-receptor complex EphA3 and ephrin-A5 similar to EphB2 and ephrinA5 only form heterodimers both in the crystals and in solution. These data suggest that higher order oligomerization may require regions outside of the LBD.

The conformational changes of the EphB2-ECP induced by the ephrinB2-RBD were studied with a complex of biophysical methods in solution. Differential scanning calorimetry revealed that separated EphB2-LBD forms with the ephrin-B2-RBD very stable complex that is melting as a single co-operative unit with the temperature of melting (Tm) higher than 60°C (the Tm for the separated EphB2-LBD and ephrin-B2-RBD is about 55°C). Surprisingly, the Tm of the EphB2-LBD as a part of the whole EphB2-ECP is lower (about 52°C) than for the separated EphB2-LBD (55°C). After binding the ephrin-B2-RBD the stability of the EphB2-LBD as a part of the whole EphB2-ECP is increasing to 55°C. The construction EphA3-LBD-CRR is melting in a single peak of heat absorption (Tm is about 52°C), thus, either LBD interacts with CRR forming a single co-operative unit or, alternatively, they have similar thermal stability.

The circumstance of cardinal importance is the significant stabilization of a domain that is melting separately from the LBD and CRR in the second peak of heat absorption. It is evident that peak corresponds to one of Fn3 domains. This means that at least the thermal stability of one of Fn3 domains studied has increased as a result of the ephrin-B2-RBD binding by the EphB2-LBD.

The results obtained together with molecular modeling allow suggesting that high-affinity binding of the ligand by the Eph-LBD switches on a zipper-like mechanism of Eph receptor dimerization via Fn3 domain(s). The suggested model can explain mechanism dimerization and signaling in the case when Eph receptors only form heterodimers with ligand.

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Regulation of NKG2D ligands by mouse cytomegalovirus

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NK cells play a crucial role in the control of many viruses and are among the first cells to sense the release of proinflammatory cytokines, as well as the perturbations in the expression of MHC class I molecules and other surface molecules induced by viral infections. The recognition of virus infected cells by NK cells is regulated by the balance of signaling via inhibitory and stimulatory receptors specific for cell surface ligands which are either of host or viral origin. NKG2D is a dominant activating NK cell receptor implicated in immune response to viruses, graft rejection and in autoimmunity. It recognizes several MHC class I-like ligands like MICA, MICB, ULBP and RAET1 in humans as well as MULT-1, H60 and RAE-1 family proteins in mice. Both, human and mouse cytomegalovirus (HCMV and MCMV) have evolved strategies to disrupt innate immune response based on the NKG2D-mediated control. We and others have characterized four MCMV genes encoding immuno evasion proteins involved in down-modulation of all cellular ligands for NKG2D receptor.

The first described MCMV immuno evasionin was glycoprotein gp40, encoded by the gene m152, which is implicated in down-modulation of MHC class I molecules and, thus, in the protection from CD8-mediated T cell control. Later, it was shown that this viral protein also interferes with the expression of the RAE-1 family of proteins. However, based on our observation that in some mouse strains (that differently express RAE-1 proteins) NKG2D-dependent MCMV control is preserved in spite of the expression of viral inhibitors, we have postulated that there must be some of these ligands that are resistant to the virus mediated down-modulation. Indeed, we have found that RAE-1 proteins differ in their susceptibility to the MCMV immuno evasionins. In contrast to RAE-1□ representing the sensitive isoform, RAE-1□ remains present on the surface of MCMV infected cells. Our data demonstrate that the difference in susceptibility of RAE-1 isoforms to down-regulation by MCMV is rather due to the increased stability of RAE-1□ on the cell surface, than to the different sensitivity to the viral gp40 protein. We have found that the stability of RAE-1□ can be attributed to the absence of PLWY motif, which is otherwise present in RAE-1□.
Synthesis and anticancer activity of novel 4-(3-phenyl-5-aryl-4,5-dihydropyrazol-1-yl)-5H-thiazol-2-ones

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Perspectives of introduction in anticancer treatment of thiazolidine derivatives are associated with their affinity to anticancer biotargets, such as JSP-1, tumor necrosis factor TNFα, anti-apoptotic biocomplex Bcl-X₁-BH₃, etc. It must be emphasized, that combination of thiazolidine template with other heterocycles is a well-known approach for drug-like molecules build-up, which allows to achieve new pharmacological profile, action strengthening or toxicity lowering (Lesyk 2004). The aim of our research was synthesis of non-condensed thiazolidones with pyrazoline moiety and studying of their anticancer activity.

For structure modelling of new substances were used 4-(5-aryl-3-phenyl-4,5-dihydropyrazol-1-yl)-5H-thiazol-2-ones. Original heterocyclic system is synthesized by the reaction of 5-(2-hydroxyaryl)-3-aryl-4,5-dihydro-1H-pyrazoles with 4-thioxo-2-thiazolidinone in refluxing ethanol. The Knoevenagel condensation of 1 with aromatic aldehydes yielded the group of 5-arylidenedederivatives 2. Following utilization of compounds 1 in nitrosation and diazotization reactions the group of nitroderivatives 3 and diazo compounds 4 were obtained. 4-(3-Phenyl-5-aryl-4,5-dihydropyrazol-1-yl)-5-ethoxymethylene-5H-thiazol-2-one 5 was synthesized by the reaction of compounds 1 with triethylorthoformate and used as intermediate for derivatives 6 yielding in reaction with aromatic amines.

The structures of compounds were determined by ¹H, ¹³C NMR and X-ray analysis.

Twenty six of synthesized compounds were tested and most of them displayed antitumor activity on leukaemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. The most efficient antitumor agent – Les-3120 was found to be active with average lgG1₅₀ and lgTGI values: -5.00 and -4.42 respectively and exhibited all cancer cell lines in the NCI60 human tumor cell line anticancer drug screen (Shoemaker 2006).

Rhodanine-3-succinic acid utilization as the scaffold in design of novel anticancer agents

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4-Azolidone cycle belongs to the group of privileged structures, the latter make it’s derivatives attractive objects in “drug-design”. Anticancer potential discovery of 4-azolidones combined with novel molecular bio-targets identification, to which the affinity of mentioned class of heterocyclics has been possessed, is the platform for the rational design of novel drug-like molecules as innovation drugs prototypes with anticancer activity. Among 4-azolidone derivatives the group of 4-azolidone-3-carboxylic acids derivatives is one of the most perspective groups of biologically active substances.

The aim of our work was synthesis of the new rhodanine-3-succinic acids derivatives row and evaluation their anticancer activity.

The rhodanine-3-succinic acid was used as a matrix for chemical modification, what was substantiated by our previously investigations. The modification of mentioned scaffold 1 was realized in two directions: in position 5 of 4-thiazolidone cycle and carboxylic groups of succinic acid moiety. This approach was motivated by influence of the substituents in these positions on the realization of biological activity. Chemical transformation was carried out via reactions of condensation, cyclization as showed in the scheme.

The series of 5-ylidene-rhodanine-3-succinic acids 2, their diamides 3, cyclic imides 4, 5 and more complex derivatives with peptide fragment in molecules 6 were obtained in the result of chemical part of our study. The structure and purity of synthesized compounds were confirmed by TLC and spectral data (1H NMR, mass).
Anticancer assays of the compounds were performed according to the US NCI protocol (National Cancer Institute (Bethesda, USA) within Developmental Therapeutic Program. The tested compounds showed different strength of anticancer activity depends on nature of introduced moiety; some of them possess the significant specific influence on some cancer cell line. The derivatives 4, 5 & 6 possess the stronger anticancer effect as compared with initial acids 2 and diamides 3. Anticancer activity studies identified the several “lead-compounds” among groups of testing compounds.

The flexible docking of active compounds was performed to several biological target (Bcl-XL-BH3 protein complex (1BXL), PPARs-γ (1FM6 & 1NYX), tubulin (1SA1) as possible pathways of anticancer mechanism for 4-thiazolidones derivatives. The QSAR analysis was performed based on obtained data and several multivariate linear models: \( \text{anticancer activity} = \sum x_i a_i + b_i \) (\( x_i \) molecular descriptor) were obtained. These equations will be used for prediction of anticancer activity of 4-azolidone derivatives and structure optimization of lead-compounds in the raw of rodhanine-3-succinic acids derivatives will be performed.
DEVELOPMENT OF AMPEROMETRIC BIOSENSORS BASED ON IMMOBILIZED OXIDASES FOR ETHANOL, GLYCEROL, GLUCOSE AND LACTATE DETERMINATION

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Nowadays, quantitative determination of biochemical compounds of human body fluids has great practical significance to assist in diagnosis and to assess disease. Glucose is the major metabolite measured today, mainly for diabetes care. The detection and quantification of ethanol is very important in clinical and forensic analysis due to the specific influence of this alcohol to human organism. Glycerol analysis is also important in clinical diagnostics for control of the triacylglycerides level in blood since the monitoring of these components has prognostic value to drop the risks of diseases of the cardiovascular system. And the lactate concentrations measurement is of essential importance in sports medicine for fitness monitoring and to investigation of tissue damage after training.

So, the demand for fast, reliable and continuous measurements of biochemical species in medicine and other fields has evolved the need for small, easy to handle and inexpensive analytic devices, such as biosensors. These analytical systems, containing enzymes of microbial origin, combine the main advantages of bioanalytical, electronic and information technologies and are characterized by high selectivity and sensitivity, fast results, facility of data processing and low analysis prime cost. That’s why biosensors can be effectively used for clinical monitoring of numerous compounds of human body fluids.

This work was aimed at development of enzymatic amperometric biosensors with immobilized oxidases for quantitative analysis of ethanol, glycerol, glucose and lactic acid and investigation of immobilized enzymes for optimization of its working characteristics. Laboratory prototypes of amperometric biosensors on the basis of platinum printed electrodes SensLab and alcohol oxidase, glycerol oxidase, glucose oxidase and lactate oxidase electrochemically immobilized in the polymer poly(3,4-ethylen dioxythiophene) (PEDT) were designed. The developed biosensors showed a linear response to ethanol, glycerol, glucose and lactate within the concentration range 0.16 – 20.5, 0.05 – 25.6, 0.04 – 50.0 and 0.008 – 0.256 mM, respectively.

Optimum pH of the developed amperometric biosensors based on alcohol, glycerol, glucose and lactate oxidases immobilized in PEDT was determined to be 7.2. The values of buffer volume and background electrolyte concentration in buffer were shown to have no effect on the work of created biosensors. Selectivity, operational and storage stability of created biosensors were investigated. Ethanol, glycerol, glucose and lactate concentration in model solutions was successfully measured using developed amperometric biosensors based on immobilized oxidases.
Doxorubicin Inhibits TGFβ-Signaling Via Blocking Translocation of SMAD-Proteins into Nucleus of Tumor Cells

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Introduction: Doxorubicin (Dx, adriamycin) is a DNA-damaging anticancer drug, possessing both cytotoxic and anti-metastasis activity. However, the anti-metastasis activity of Dx cannot be explained by earlier described mechanisms of its action. TGFβ1 is a cytokine that is often up-regulated in human tumors and can decrease cytotoxic action of anti-cancer drugs and induce metastasis-formation.

Methods: Western-Blotting, RT-PCT, Luc-reporter assay, immunohistochemistry and nuclei-isolation, etc. were used for investigation of gene-expression, protein-phosphorylation and protein-translocation. Radioactive 35S and 3H were used for DNA and protein labeling. Transfection of A549 cells was performed using our new developed, highly efficient transfection reagent.

Results: Developed by us transfection agent was checked for transfection efficiency, toxicity and specificity.

We showed that antitumor drug Doxorubicin can inhibit TGFβ-signaling in human lung adenocarcinoma A549 cells, transfected with our new developed, highly effective transfection reagent. Namely, doxorubicin (Dx) blocked TGFβ1–induced activation of Smad3–responsive CAGA12–Luc reporter, as well as five other Smad-dependent reporters. However, it didn’t affect functioning of TGFβ-independent c–Myc–Luc reporter. That was observed as early as after 1-3hrs of treating these cells with Dx, while such DNA-damaging drugs (cisplatin or methotrexate) didn’t alter activation of CAGA12–Luc reporter under the same conditions. Besides, after 1hr action, Dx abrogated TGFβ–induced translocation of Smad3–protein from cytoplasm to the nucleus. Down–regulation of expression of Smad2-, Smad3-, Smad4–proteins, and TGFβ-receptor type-II mRNA, and up-regulation of inhibitory Smad7–protein upon Dx treatment, were found after 12-24hrs of Dx treatment. Phosphorylation of Smad2- and Smad3–proteins was also affected by Dx.

Conclusions: Our study is the first successful, showing the potential molecular explanation of the antineoplastic action of drug Doxorubicin towards tumor cells. We showed that tumor cell treatment with Dx is resulted in inhibition of TGFβ-signaling at both early (1hr) and later (12hrs) stages of the drug action. Such inhibition can be a potential molecular explanation of the antineoplastic action of Dx towards tumor cells. New developed by us transfection reagent is more efficient and less toxic than commercially available chemical and liposomal transfection reagents.

Publications. Some results of this study were partly published in the European Journal of Pharmacology, Cell Biology International journal, etc.
Study of Fullerenes’ C₆₀ Effects on Apoptosis Induced in Normal and Transformed T-lymphocytes

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Acute lymphoblastic leukemia is a commonly encountered oncological disease, especially widespread among young patients (13-20 years old). Such traditional methods of therapy as use of cytostatic agents and antibiotics as well as high-dose chemotherapy are extremely harmful for patients’ health. Thereby, the improvement of existing treatment modes and exploration of new ones is an actual and perspective research task.

Fullerenes C₆₀ belong to a new class of carbon nanoparticles which have a spherical form. Though fullerenes are chemically inactive substances, they reveal biological effects even at low concentrations (10⁻⁵- 10⁻⁷M). Moreover, fullerenes are nontoxic for most cell types. These data suggest a possibility of fullerenes use as new pharmacological agents. The aim of our study was to explore protective effects of fullerenes on normal and transformed T-lymphocytes upon apoptosis induction. Cytosine arabinoside (Ara C) was used as apoptosis inducer of transformed (Jurcat T-lymphoma cells) and normal (rat primary thymocytes) T-lymphocytes. MTT-test was used for estimation of cell survival.

Both normal and transformed T cells were pretreated with fullerenes C₆₀ for 1 hour before addition of AraC. No protective effect of fullerenes in Jurcat cells was observed. By contrast, fullerenes protected normal thymocytes from AraC-induced cell death decreasing the number of apoptotic cells from 50% to 20% as compared to untreated control. The observed selectivity of action of fullerenes might be helpful for the development of complex approaches to therapy of human lymphomas.

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Midazolam is a relatively new benzodiazepine derivative with specific chemical and pharmacological properties (i.e. increased stability to hydrolytic degradation and a rapid, metabolic inactivation, mainly through oxidation)\(^3\).

Identification and assay methods used in the analysis of midazolam are mostly chromatographic (liquid chromatographic methods are using mainly conventional RP chromatographic systems)\(^1\).

Ion-pair RP HPLC (IP-RP HPLC) is a convenient efficient less explored alternative method for the separation and quantification of midazolam and its impurities (due to its marked basic character). The optimum experimental conditions for the separation and assay (a C18 stationary phase-Inertsil 5C18 column 250x4.6 mm I.D., 5 µm particles and a mixture of MeCN and aqueous phosphate buffer as mobile phase) were established. The counter ion used was 1-heptane-sulfonate\(^2\).

We studied all critical parameters and thoroughly validated the newly developed method, our final purpose being its suitability for the analysis of pharmaceutical dosage forms containing midazolam.

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A combinational approach in anticancer therapy based on arginine deprivation

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It was previously established that some human tumors are more sensitive to arginine deprivation in comparison with normal tissues *in vitro* and *in vivo*. Such elevated requirement for normally semi-essential arginine in many tumor cells provides a rational basis for the development of novel selective anticancer therapies, including combinational approaches. It was also shown that arginine analogue of plant origin canavanine exhibits antitumor effect *in vitro* and *in vivo*, but unfortunately has high overall cytotoxicity. Prior this work, no reports regarding the use of arginine starvation in combination with canavanine administration for cancer treatment were available. Also, the molecular basis of enhanced sensitivity of tumor cells to arginine deprivation has not been established.

In our work human epithelial cancerous (keratinocytic carcinoma - A431, lung adenocarcinoma - A549, cervical carcinoma – HeLa, hepatocellular carcinoma – HepG2, breast adenocarcinoma - MCF7, melanoma - WM1158) and pseudonormal (embryonic kidney - HEK293) cell lines were utilized as models.

We demonstrated that arginine starvation has strong growth inhibitory effect on all cell lines tested but does not significantly affect cell survival. Examination of canavanine effect on cell proliferation and viability revealed that for pseudonormal cell line canavanine growth inhibitory and cytotoxic concentrations in arginine-free medium are essentially the same as in arginine-rich medium. By contrast, for all cancerous cell lines canavanine growth inhibitory and cytotoxic concentrations in arginine-free medium were 10-1000 times lower than those obtained in arginine-supplemented medium (Table 1). These data suggest that under arginine deprived conditions canavanine is more toxic for cancerous cell lines relative to the pseudonormal cell line.

**Table1. Canavanine growth inhibitory (GI50) and cytotoxic (IC50) concentrations for cancerous and pseudonormal cell lines**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Canavanine, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GI50</td>
</tr>
<tr>
<td></td>
<td>CM</td>
</tr>
<tr>
<td>A431</td>
<td>1</td>
</tr>
<tr>
<td>A549</td>
<td>2</td>
</tr>
<tr>
<td>HeLaS3</td>
<td>0.07</td>
</tr>
<tr>
<td>HepG2</td>
<td>5</td>
</tr>
<tr>
<td>MCF7</td>
<td>0.5</td>
</tr>
<tr>
<td>WM1158</td>
<td>2</td>
</tr>
<tr>
<td>HEK293</td>
<td>7</td>
</tr>
</tbody>
</table>

To understand the mechanism underlying canavanine toxicity we investigated its effect on DNA fragmentation, caspase activation and PARP cleavage. We showed that arginine starvation induces pronounced time-dependent apoptosis in only four (A431, HeLa, HepG2, WM1158) of six tested cancerous cell line. Combination of arginine starvation with low dose (0.1 mM) canavanine treatment caused apoptosis induction in all cancerous cell lines. We showed that both in case of arginine deprivation alone and in combination with
canavanine treatment apoptosis in cells of different origin is triggered through intrinsic mitochondria-mediated pathway (Figure 1).

Figure 1. Caspase activation and PARP cleavage in A431 cells upon combination of arginine starvation and low dose canavanine treatment

Importantly, arginine administration even after 72 h of incubation with low dose of canavanine in arginine-free medium could efficiently rescue growth of pseudonormal cell line. By contrast, all tested cancerous cell lines were not able to restore their growth upon arginine re-supplementation even after 24 h of incubation in arginine-free medium with canavanine. We suggest that combination of arginine deprivation with canavanine therapy can be a promising approach for selective sensitization of tumors that are otherwise not sensitive to arginine limitations alone, to improve the corresponding therapy. We continue studies to elucidate other efficient combinational approaches for anticancer therapy based on arginine deprivation.
Snake venoms contain complex mixtures of biologically active proteins and polypeptides. The large diversity of snake venom proteins affecting hemostasis contrasts with exact specificity of each individual component. The high selectivity of snake venom proteins for individual blood coagulation factors renders these components potentially useful tools to study the mechanisms of action, regulation and structure-functional relationships of coagulation factors. Furthermore, this specificity can be used for development of laboratory tests (Braud 2000).

We studied functionally active compounds of the *Echis multisquamatus* venom. Whole venom was separated using ion-exchange chromatography on Q-sepharose. Analyzing obtained fractions we’ve found fibrinogenolytic and prothrombin-activating activity in two of them.

The active Q-sepharose fraction was further purified by gel-filtration on sephadex G-75. The composition of active fraction was determined by SDS-PAGE according to the method of Laemmli. Obtained enzyme was called ecamulin. It is a metalloproteinase that efficiently activates prothrombin and produces meizothrombin. Ecamulin activation of prothrombin is Ca\(^{2+}\)-dependent. The enzyme was shown to activate functionally inactive forms of prothrombin (prethrombin and PIVKA-prothrombin). These properties of ecamulin can be used for diagnostics, research of prothrombin activation process and prothrombin derivates (Solovjev D.A., 1996).

Another studied compound is a fibrinogenase. It was purified using a two-step chromatographic protocol (ion-exchange chromatography followed by affinity chromatography on heparin-sepharose). Purified enzyme was called FLIT. Since its amidase activity was completely inhibited by benzamidine but not PCMB or EDTA, it is a serine proteinase.

Since FLIT has shown ability to cleave-off the α-chain of a fibrinogen molecule, it is an α-fibrinogenase. This fact seems to be very interesting, because most of snake venom α-fibrinogenases are metalloproteinases (Swenson 2005). Thrombin-like amidase activity of FLIT was shown, but not the thrombin-like effects on fibrinogen. Such properties suggest FLIT’s usability for fibrinogen structure and interactions studies.

Leads structure optimization aiming improvement of antitumor profile using the strategy of privileged scaffold based on thiopyrano[2,3-d]thiazol-2-one derivatives

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Rapid development of novel rational approaches in medicinal chemistry to drug discovery prompts the usage of modern strategies to discovery and optimization of new lead-compounds, as potential drug candidates. Recent progresses in molecular biology of cancer-related biotargets, as well as antitumor activity studies of thiazolidinones derivatives forced our research group to make several discoveries in the area of synthesis and biological activity screening among condensed derivatives based on 4-thiazolidinones (Lesyk 2006, Atamanyuk 2008, Matiychuk 2008). This allowed us to identify a number of lead-compounds, thiopyrano [2,3-d] thiazol-2-one derivatives, with high level and certain specificity of antitumor action. The latter yielded into new project development on studies around lead-compounds’ structures optimization.

Lipinski rules of “drug-likeness” return the search of potential drug candidates within the arrays with the molecular weight up to 500. That is we aimed to work out synthetic pathways for obtaining of low-molecular weight thiopyrano [2,3-d] thiazol-2-one derivatives.

It was previously investigated, that [4+2]-adducts of the hetero-Diels-Alder reaction between 5-ethoxymethylidene-4-thioxo-2-thiazolidinone (1) with dienophiles in the medium of acetic acid undergo ethanol molecule elimination and forms endocyclic double bond. Our investigations allowed observing new special options for the synthetic utilization of mentioned heterodiene. Reaction of 1 with acetylenedicarboxylic acid and its ester, aroylacrylic acids and 1,4-naphthoquinone are carried out with spontaneous transformations of [4+2]-adduct, which includes not only ethanol elimination, but also decarboxylation and coupled double bonds system transformation, depending on nature of dienophile, which allowed us to synthesize the row of novel building blocks 2-6, which will be used for lead-compounds structure optimization with anticancer profile, containing privileged thiopyrano [2,3-d] thiazol-2-one scaffold. Reactions flow is interpreted by 1H-, 13C-NMR and mass-spectroscopy. Decarboxylation processes at obtaining of 2, 4, 5 could be explained by protonation of intermediate sulfur atom, which initiates charge transfer and CO2 molecule elimination. Obtained functionalized “building blocks” allow us derivatization of highly active substances in few different directions and use combinatorial chemistry workflow for them.

Virtual screening methodology, including docking and QSAR studies are used for most probably important routes identification and selection of lead-compounds derivatives for synthesis. Obtaining of modified lead-compounds derivatives, based on building blocks 2-6 and their anticancer activity screening are in progress.
Acknowledgements

We thank Dr. V.L. Narayanan from Drug Synthesis and Chemistry Branch, National Cancer Institute, Bethesda, MD, USA, for in vitro evaluation of anticancer activity.


ABSTRACTS

TRANSLATIONAL RESEARCH
Studies of T-284 and SH-516 cyanine dyes as fluorescent probes for specific α-synuclein fibrils detection

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One of the cardinal pathologic features of Parkinson's disease is the accumulation of amyloid fibrils made of α-synuclein (ASN) inside brain cells. Recently we firstly proposed fluorescent mono- (T-284) and trimethine (SH-516) cyanine dyes for specific detection of ASN fibrils (Volkova 2008). These dyes are suitable for quantitative detection of ~1.5-25 µg/ml of fibrillar ASN, which is comparable to the detection limits of commercially available dyes.

As continuation of previous studies the ability of T-284 and SH-516 dyes to selectively detect fibrillar proteins of various amino acid compositions and to monitor the kinetics of proteins fibrillogenesis process was studied. Also T-284 dye/ASN fibril complexes were characterized by means fluorescence lifetime analysis.

Studies of the dyes selectivity were carried out on various fibrillar proteins (insulin, lysozyme, wild-type ASN and the Parkinson disease-related ASN mutants A30P and A53T). Both dyes exhibited fluorescence response in the presence of fibrillar proteins species, while addition of monomeric proteins hardly affected the emission intensities of the dyes. T-284 demonstrated particular sensitivity to wild-type and A53T ASN, for trimethine SH-516, the least protein-to-protein variability was admitted.

Both dyes appeared to have ability to follow the step-by-step transition of monomeric wild-type, A30P and A53T ASN proteins into fibrils, demonstrating very good results reproducibility that enables development of reliable fluorometric assays for monitoring ASN amyloid fibril formation. Such an assay may be adapted for high throughput screening of potential inhibitors of ASN aggregation. The morphology of protein fibrils assembled was studied with high-resolution AFM.

The fluorescence lifetime studies of the T-284 in free state and in presence of fibrillar ASN demonstrated, that dye binding to protein yields three-exponential fluorescence decay, while for the free dye the two-exponential decay was observed. Appearance of additional long-time component of the dye in fibrils presence may be related to protein-embedded dye molecules.
We consider that obtained results could help to get inside into mechanism of cyanine dye/amyloid fibril complex formation and are actual for exploration of possible applications of amyloid-sensitive dyes.


This work was supported by a FEBS fellowship.
Energy transfer measurements using different dye-to-protein labeled antibodies

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Different kinds of cell surface receptor clusters have recently been discovered using flow cytometric fluorescence resonance energy transfer (FCET) measurements [1]. This method is capable for identifying molecular interactions; however the exact distances remain obscure, because the classical Förster efficiency-distance relationship is valid only in the case of one donor one acceptor systems. This condition can not be fulfilled when cell surface molecules are labeled with monoclonal antibodies carrying the fluorescent donor and acceptor molecules [2].

Our aim was to carry out FCET measurements on such cell surface receptors, where the distances are constant, and the only changing parameter is the donor-acceptor ratio of the used labels. In our study, we demonstrated the dependence of resonance energy transfer efficiency on the dye-to-protein labeling ratio of the antibodies.

NCI-N87 gastric carcinoma cell line was used, since it expresses high amounts of ErbB2 on the cell surface. We used two anti-ErbB2 monoclonal antibodies: Trastuzumab (Herceptin) and Pertuzumab (Omnitarg). Aliquots of antibodies were conjugated with succinimide-derivates of Alexa Fluor 546 and Alexa Fluor 647. The dye-to-protein ratio (D/p) was determined by spectrophotometry and its values were in the range of 0.8 to 6. FCET data were collected on a cell-by-cell basis using a FACS Array flow cytometer. Energy transfer efficiencies were calculated by ReFlex software developed in our laboratory [3].

We have applied different D/p (between 1-5) labeled antibodies on a scale which is mostly common in fluorescent measurements. Using the combination of these donor-acceptor pairs we have analyzed the influence of different D/p and Donor/Acceptor ratios on the obtained FRET efficiency on biological systems (Figure). The possible changes of the correction factors were also examined resulting 20-30% change in S2 (a correction factor in FCET). According to our results, larger donor number results in higher transfer efficiency values and the transfer efficiency of a given donor increases by increasing the D/p of the acceptor. The measured transfer efficiency with a given acceptor increases only slightly with the increasing D/p of the donor.
donor. This approach will give us the possibility to relate FRET efficiencies to real distances of cell surface receptors.

References:


Selected products of oxidative damage in relation to nutrition and age

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Background: Evidence that diet is a key environmental factor affecting the incidence of many chronic diseases is overwhelming. Antioxidant substances in such a diet enhance the DNA, protein and lipid protection by increasing the scavenging of radical oxidative species that occur during metabolic reactions. The lack of balance between the amount of „unhealthy“ and „healthy“ food leads to the accumulation of unrepaird damage, initiating DNA instability and inducing cancer development. It is known that oxidative damage to tissue macromolecules increases during aging.

Subjects and methods: The main goal of this study was to assess the products of oxidative damage to DNA, lipids and proteins in relation to nutrition and age. Here were compare two nutritional regimens: a vegetarian diet with predominance of plant food with consumption of dairy products, eggs as well as ≤ 2 times monthly consumption of white meat vs. a non-vegetarian traditional mixed diet. The other comparison was young vs. older subjects at condition of a vegetarian nutrition and a traditional non-vegetarian nutrition. The study population consisted of 161 healthy non-smoking adult women aged 20-30 years and 60-70 years. Plasma concentrations of vitamin C, E, A, β-carotene were detected by HPLC. The level of lymphatic DNA damage in the peripheral blood was evaluated by the Comet assay. The plasma concentrations of conjugated dienes of fatty acids and protein carbonyls were measured by spectrophotometric methods. The intake of vitamins, mineral and trace elements only in natural form was considered (no supplementation).

Results: In groups of young women, no differences in values of oxidative damage to DNA, lipids and proteins were observed between vegetarians and non-vegetarians. The plasma values of antioxidative vitamins also were similar in both nutritional groups. In older vegetarian group the significantly reduced values of DNA breaks with oxidised purines, DNA breaks with oxidised pyrimidines as well as lipid peroxidation product were found if these values were compared with those in older non-vegetarians. Older vegetarians vs. older non-vegetarians have the significantly increased plasma values of vitamin C and β-carotene. Significant age dependences of measured parameters (increase in all oxidative damage products and decrease in plasma vitamin concentrations in older women) were noted only in non-vegetarians. Vegetarian values of older women vs. young women were similar or non-significantly changed.

Conclusion: The results of presented study suggest that increase of oxidative damage in aging may be prevented by vegetarian nutrition.

Acknowledgments: This work was supported by Research and Development Support Agency under the contract No. APVT-21-017704.
Correction of Bioenergetical, Antioxidant and Apoptotic Properties of Heart Tissue with the Help of Precursors and Modulator of Coenzyme Q Biosynthesis under Ageing

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Coenzyme Q (CoQ) is the key component of cellular bioenergetics, an important antioxidant, and a regulator of mitochondrial permeability transition pore (MPTP). CoQ biosynthesis is disrupted under pathological conditions and in ageing. This may cause an imbalance in myocardial energy supply, which in turn may lead to a number of cardiovascular systems’ disorders. Administration of CoQ is used to treat such cases, but this may lead to inhibition of its endogenous synthesis.

The aim of present work was to study the effect of precursors and modulators of CoQ biosynthesis on bioenergetics, intensity of free-radical oxidation and sensitivity of MPTP to inductors of its opening in old rats’ heart tissue.

Complex of vitamin E, 4-hydroxybenzoic acid, and methionine was administered per os daily for 10 days to male rats of 24 month age.

The results of our research demonstrate that CoQ and vitamin E content in rat heart mitochondria increase 1.5 and 1.8 times accordingly in comparison to control under administration of biologically active substances. Treatment with precursors and modulator of CoQ biosynthesis normalizes functional activity of enzyme complexes of electron-transport chain, namely NADH-CoQ oxidoreductase, succinate-CoQ oxidoreductase and cytochrome c oxidase. This may be interpreted as an improvement in respiratory processes in heart tissue. According to works by other authors, ageing is accompanied by intensification of free radical oxidation of macromolecules. Indeed, in our experiment we have shown an increase in content of products of lipid peroxidation, such as conjugated dienes and TBA-reactive products, and of protein peroxidation in old rats. Animals treated with complex of biologically active substances had substantially decreased content of mentioned products of lipid and protein free-radical oxidation. Administration of said complex led to a reliable decrease in sensitivity of MPTP to inductors of its opening, Ca²⁺ and phenylarsine oxide in concentrations 10⁻⁴–10⁻⁷ M in comparison to control animals. MPTP opening was fully prevented in presence of its inhibitor cyclosporine A (10⁻⁵ M). This ascertains the fact that MPTP opening played a role in observed mitochondrial swelling.

The results obtained may be the basis for further development of the medicals of metabolic type to be used successfully in prophylaxis and treatment of different cardiac pathologies, and in ageing.
Cells-based amperometric biosensors for formaldehyde monitoring in biological samples

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Formaldehyde (FA) is a ubiquitous environmental contaminant of our planet. It is found in paints, clothes, medicinal and industrial products, and is a component of diesel and gasoline exhaust, as well as being endogenously produced in all living organisms as a result of metabolism (methionine, histamine, methanol, and methylamine), spontaneous dissociation of 5,10-methylene tetrahydrofolate, or oxidative demethylation of DNA and RNA. It is present in fruits, vegetables, meat, and fish. Concerns about safety have focused on FA in part because high concentrations of FA can damage DNA and cause cancerous changes in cells in the laboratory. That is why it is necessary to control the level of this extremely toxic agent in consumer goods and biological samples. This requires the development of simple, cheap, sensitive and selective methods for FA monitoring.

The existing enzymatic methods of FA assay are laborious, not enough selective and specific, and are still unavailable at the world market. To solve this problem, a number of attempts to develop biosensors for the detection of FA were reported including amperometric sensors, potentiometric detection schemes and optical sensors but they did not succeed in the practical applications due to numerous drawbacks.

Two types of FA-selective amperometric biosensors (intact and permeabilised cells-based) were developed. Intact and permeabilised cells of the gene-engineered thermotolerant methylotrophic yeast *Hansenula polymorpha*, with a high content of NAD⁺- and glutathione-dependent FdDH were used as the biorecognition elements for amperometric assay of FA. The yeast cells were immobilized on the graphite working electrode by physical fixation of the cell suspension by means of dialysis membrane (phenazine methosulfate was used as a free-diffusing redox mediator). The biosensor based on intact recombinant yeast cells exhibited expanded linear range toward FA as compared to similar sensors based on the permeabilized cells of *H. polymorpha* and detection limit for it was found to be 0.1 mM. The developed biosensors are selective, inexpensive and stable over several days, as well as simple to manufacture and operate. The best analytical results for biosensors-based methods were obtained using standard multi-addition protocol assay. The constructed microbial biosensors were successfully applied for FA determination in real samples of commercial chemical product (formalin), pharmaceutical (Formidron), disinfectant (Descoton forte) and rabbit vaccine against viral hemorrhage. A good correlation was observed between the biosensors’ approaches and chemical methods. The use of cell-based amperometric biosensors looks very promising due to several advantages over conventional enzyme electrodes: simplicity, cheapness, stability and versatility. Also cells-based sensors are less sensitive in comparison with NAD⁺- and glutathione-dependent formaldehyde dehydrogenase-based sensors, which is convenient for FA determination in samples with high content of this agent. Assay procedure does not require additional dilution of tested specimens and save time for analysis.

**Acknowledgments** This work was supported by the NATO (linkage grant PDD (CP)-(CPP.NUKR.CLC, 982955), NAS of Ukraine in the framework of the Program “Sensors’ Systems for Medico-Ecological, Industrial and Technological Purposes” and WUBMRC.
Lipoperoxidation processes in heart tissue of rats under COX-2 blockage and dual COX/LOX inhibition

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Cyclooxygenase-2 (COX-2) inhibitors (coxibs) are used for treatment of inflammatory conditions, but their use is associated with the increase of cardiovascular risk [1]. Lipooxygenase (LOX) pathway also plays an important role in inflammation. Compounds that combine COX and LOX inhibition present multiple advantages because they possess a wide range of anti-inflammatory activity [2]. The aim of the research was to compare changes of NO content, lipoperoxidation processes and activity of the antioxidant protection system in heart tissue of rats under application of inhibitor COX-2 celecoxib and agents possessing dual COX/LOX inhibition {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-acetic acid and 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-benzene-sulfonamide.

Methods: The research was performed on 27 white rats. Celecoxib (10 mg/kg), {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-acetic acid (10 mg/kg) and 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-benzene-sulfonamide(10mg/kg) were introduced per os for 14 days. Lipoperoxidation processes were evaluated by malonic dialdehyde (MDA) content, activity of enzymes of the antioxidant protection system was evaluated on basis of determination of SOD, catalase, glutathionperoxidase (GP) and glutathionreductase (GR) activity, Griess reagent was used to measure the content of NO, L-Arg concentration was measured in blood plasma.

Results: COX-2 inhibition by celecoxib caused the increase of MDA content by 37 %, indicating activation of lipoperoxidation. After inhibition both COX and LOX by {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-acetic acid and 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-benzene-sulfonamide MDA content was also increased in heart tissue, but less than after celecoxib action (by 28% and 30% subsequently).

NO concentration was 21% higher than normal in heart tissue after COX-2 blockage, whereas 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-benzene-sulfonamide caused increase of NO concentration only by 7%. These changes in NO concentration were accompanied by the increase in L-Arg concentration in blood plasma.

Inhibition of COX-2 as well as COX/LOX dual inhibition led to the increase activity of the antioxidant protection system enzymes (catalase, SOD, GP, GR). Celecoxib application caused increase catalase activity 28%, {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-acetic acid increased catalase activity by 24% and 4-{2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrlidin-1-yl}-benzene-sulfonamide enhanced it by 20%.
Conclusions: COX-2 inhibition by celecoxib led to intensification of lipoperoxidation processes in heart tissue, it can be the result of its thrombotic action. Activity of enzymes of the antioxidant protection system was increased under these conditions. Celecoxib increased NO content in heart, that can indicate on enhance of NOS activity. Changes appeared after prolonged dual COX/LOX inhibition, were less marked in both tissues, comparing with action of celecoxib. Obtained results can be used as the background for the further preclinical investigations.

References:
Changes of molecular Chaperon Hsp60 Expression in Cardiac Muscle at Dilated Cardiomyopathy Progression

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Introduction: Cardiovascular disease is a leading cause of death worldwide. Dilated cardiomyopathy (DCM), characterized by chamber dilatation and myocardial systolic and diastolic dysfunction, is one of the most common heart diseases. Loss of function or death of cardiomyocytes is a major contributing factor to this disease. Understanding the mechanisms involved in cardiomyocyte cell death is a topic of great interest for treatment of cardiac disease. Previous studies have shown that molecular chaperons can regulate apoptotic processes in cardiomyocytes. Hsp60 is molecular chaperon, 70-80% of which is in mitochondria, and 20-30% - in cytoplasm of cardiomyocytes. It was revealed that cytoplasmic Hsp60 binds proapoptotic molecules Bax and Bak and decrease of Hsp60 launches apoptosis. The goal of our study was to determine the possible changes of Hsp60 expression and localisation in the hearts affected DCM at the end stage of heart failure (sectional pathomorphological samples of human myocardium) and in dynamics (on the mouse model of myosin-induced autoimmune myocardial damage like human DCM).

Methods and results: The increased level of Hsp60 has been observed by Western-blot analysis in cardiomyocytes from DCM affected hearts in comparison with normal myocardium. Besides, we revealed decrease of Hsp60 in cytoplasmic fraction and significant increase in mitochondrial fraction of DCM-affected hearts by Western blotting. The similar results were obtained in mice myocardium with experimental DCM-like pathology at different stages of disease development. The data of immunohistochemical analysis have shown increase of Hsp60 expression in myocardium of model animals at different stages of model disease progression.

Conclusion: Our results allow us to suppose that decrease of cytosolic fraction of Hsp60 in cardiomyocytes may play an important role in apoptotic events in cardiomyocytes at DCM.
ABSTRACTS

CLINICAL RESEARCH
Metabolic syndrome and diabetes mellitus type 2 and SNPs in genes PPARD and PPARGC1A

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Introduction: Obesity is an important predictor and cause of type 2 diabetes and cardiovascular disease. Both, life style and genetics factors play important roles in the development of obesity. PPARGC1A, a transcriptional coactivator of PPAR genes that regulate insulin sensitivity is considered to be a master regulator of skeletal muscle metabolism (McCarty MF, 2005). The nuclear hormone receptor PPAR-δ (peroxisome proliferator-activated receptor delta) is an important regulator of lipid and energy metabolism in adipose tissue and skeletal muscle (Barish GD et al., 2006). Therefore these genes represent interesting candidate mediators of metabolic disease and a promising target for its prevention and/or therapy.

Objective: To establish whether SNPs in PPARCC1A and PPARD can play any role in the risk of metabolic syndrome and type 2 diabetes or can predict the conversion from impaired glucose tolerance to type 2 diabetes.

Methods: Blood samples were collected from 74 people with diabetes mellitus (45 – 82 years old), 85 people with metabolic syndrome (20 – 81 years old) and 74 healthy controls (19 – 47 years old); together 228 probands. Personal data were obtained from life style questionnaire. Genotyping was performed by using the TaqMan Allelic Discrimination Assays We analyzed one polymorphism in gene PPARGC1A (rs8192678) and three polymorphisms in gene PPARD (rs6902123, rs2267668 and rs2076167).

Results: There were significant differences in weight and BMI (both p=0.000) between “healthy control” group and the diabetes mellitus and metabolic syndrome group. Allele frequencies were in Hardy Weinberg equilibrium. The minor allele frequencies were 0.07 C allele for rs69021123, 0.15 G for rs2267668, 0.19 C for rs2076167 PPARD gene and 0.30 for the Ser482-encoding allele for PPARGC1A. There were no genotype differences between tested groups for gene PPARGC1A and PPARD (rs2267668, and rs2076167). Only in case of PPARD rs6902123, there were a significant differences in genotype frequencies, the number of heterozygotes were lower in “healthy control” group than in the diabetes mellitus and metabolic syndrome group (p=0.038).

Conclusions: In our preliminary study only the polymorphism rs6902123 for PPARD shows relation to type 2 diabetes and metabolic syndrome. Other investigated polymorphisms haven’t show statistically significant differences between the examined groups.


The particularities of the functional status of the cardiovascular system in children with the juvenile rheumatoid arthritis (JRA)

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Actuality: one of the organs that are mainly damaged in the patients with JRA is the heart (55-75%). A lot of papers are devoted to this question, however most of them concern only some of the questions, with the use of the limited number of the methods of the research.

The aim: the complex evaluation of the results of the electrocardiographic, cardiointervalographic, echocardiographic examination and the results of the Holter monitoring of the children with the JRA.

Materials and methods: 100 ambulatory charts of the children with JRA, who underwent the treatment in cardiological department of the Kyiv Children’s Hospital N1 in 2007-2008, of the age of 2,5-18 years with the duration of the disease from several month up to 12 years, with I, II, III activity stage, in the stage of the clinic-laboratory remission were evaluated.

In order to evaluate the functional status of the cardiovascular system electrocardiographic, cardiointervalographic, echocardiographic examination and the results of the Holter monitoring were used.

Results: 66.2% of the children suffered from the articular form, 22% of the children suffered from articular-vascular form of JRA, 7.35% had Still’s disease and 4.44% of the children suffered from Wissler-Fanconi’s syndrome (subsepsis).

To conclude: cardiovascular pathology in patients with JRA is frequent and mainly has functional character. Pathological changes on the electrocardiogram were diagnosed in 85.3% of the children. After analysing cardiointervalograms defects of the vegetative tonus were administered. Only one child with JRA did not appear to have any pathological changes of the cardio-vascular system.
Platelet-Derived Growth Factor – D (PDGF-D): a novel treatment target in kidney diseases

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World-wide epidemic of chronic kidney disease (CKD) represents a huge medical, social and economic problem. Renal fibrosis is the common end-point of CKD leading to deterioration of renal function and finally to kidney failure. Renal fibrosis represents an excellent treatment target: a large variety of pathophysiologically distinct diseases leading to CKD, including glomerulonephritis, finally lead to a single process of uniform renal fibrosis. At present, we lack effective anti-fibrotic treatment options (Boor, Sebekova 2007). The newly discovered PDGF isoform, PDGF-DD, is crucially involved in mesangioproliferative glomerulonephritis. Its role in renal fibrosis was unknown.

The rat model of progressive mesangioproliferative glomerulonephritis is in its early phase characterized by glomerular damage, resulting mainly from mesangial-cells proliferation and activation. In the late phase, progressive development of renal, in particular tubulointerstitial, fibrosis occurs, with subsequent renal failure. We showed that inhibition of PDGF-DD by a neutralizing antibody in the early phase not only reduced the early glomerular damage but also partially prevented the subsequent fibrogenesis (Ostendorf 2006). PDGF-DD inhibition in the late phase of the same model, i.e. in a phase of already established tubulointerstitial fibrosis, potently ameliorated fibrosis and disease progression (Boor, Konieczny 2007). Transgenic mice with constitutively over-expressed PDGF-DD specifically in the glomeruli under the podocyte specific podocin-promotor developed mesangioproliferative disease, glomerulosclerosis and crescentic glomerulonephritis, finally leading to tubulointerstitial fibrosis and kidney failure (in revision).

In the rats with mesangioproliferative glomerulonephritis, serum PDGF-DD concentrations increased more than 1000-fold. We hypothesized, that PDGF-DD might be of diagnostic value in patients with glomerular diseases, in particular in those with excessive mesangial proliferation. Albeit serum PDGF-DD was specifically increased in patients with mesangioproliferative IgA nephropathy (but within normal range), PDGF-DD seems not to be a reliable marker of ongoing renal injury in these patients. We found no local glomerular up-regulation of PDGF-DD mRNA in renal biopsies of patients with glomerular diseases, including those with IgA nephropathy.

In conclusion, we provided the first experimental evidence that inhibition of PDGF-DD is an effective approach for treatment of renal fibrosis, and that PDGF-DD as a single factor is sufficient for development of glomerulonephritis in mice. Serum PDGF-DD does not specifically reflect the course of glomerular diseases in patients. PDGF-DD inhibition could represent a novel therapy effective in blunting both, the underlying glomerular diseases characterized by mesangial proliferation, and the renal fibrosis.

Thread of nosocomial salmonellosis in the view of antibiotic resistance.

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Introduction: Salmonella infections have been the focus of particular concern in Ukraine, because of the associated morbidity and mortality, as well as their ability to cause serious illness in infants. This reflects ongoing problems with food safety and person-to-person transmission. Efficacy of antibiotic therapy of salmonellosis depends on rational choice of initial empirical antibacterials. In this regard, information of local antibiotic resistance patterns is extremely valuable for practitioners.

Material and methods: Medical records of 52 children with salmonellosis, aged 1 to 10, and 3 bacterio carriers (adult), were studied retrospectively for clinical manifestation of disease in 2006. All patients have developed clinical feature of diarrhea within 3 to 10 days of their placement to hospitals for common cold, pneumonia or other diseases. The sensitivity of 55 salmonella isolates, collected from those patients treated in the Infectious Diseases Hospital, Lviv, Ukraine, have been determined to following antibacterials: ampicillin, rifampin, cefotaxime, lincomycin, ofloxacin, ciprofloxacin. Sensitivity of bacteria was tested by standard method of agar diffusion using Ukrainian discs impregnated with antibiotics.

Results and discussion: All of 52 children with salmonellosis experienced gastrointestinal forms with high fever, severe dehydration, abdomen pain, loss of weight, and anemia. Among them six patients died of severe disease, four of them were infants. Their clinical picture manifested with multi-organ failure due to bacterial metastasis (meningitis, muscles abscess, lymphadenitis, otitis, osteomyelitis, and endocarditis). Young children with underlying diseases (rachitis, anemia, and hypertrophy) experienced convulsions, and mental confusion. Just 3 serotypes were revealed: S.thyphymurium in 36 patients (65.5%), S. enteritidis in 18 (32.7%), S.tennesse in one (1.8%). All of strains were multi-drug resistant. Of salmonella isolates, 5.5% were resistant to at list 3 kinds of antibiotic. It was established that 27.3%, 29.1%, 23.6% and 9.1% of isolates were resistant to 4, 5, 6 and 7 kinds of antibiotic, respectively. Only 3.6% of strains showed complete resistance to all of antibacterials tested. Almost all of Salmonella isolates demonstrated high sensitivity to quinolones (ciprofloxacin – 92.6%, and ofloxacin - 87.0%).

Conclusion: Thus, salmonella strains isolated in Lviv, Ukraine, were multi-drug resistant and caused severe life threatening diseases. This fact as well as preceded long-term being in hospital environment can be an evidence of nosocomial origin. In the setting of increasing antibiotic resistance in salmonella, preventive strategies are critical to reducing disease incidence and trace of multidrug-resistant strains spreading throughout hospital setting using PFGE and molecular typing are of urgent issues.
Prevalence of HIV CCR5-Δ32 in the HIV-positive versus HIV-negative Slovaks

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**Background:** The chemokine receptor CCR5 plays critical role in transmission and pathogenesis of human immunodeficiency virus (HIV)-type 1 infection. Individuals homozygous for 32-bp deletion allele of CCR5 (CCR5-Δ32) are resistant to HIV-1 infection while those heterozygous for this allele, who are HIV-positive, have a delayed onset to AIDS of 2-3 years. The purpose of this study was to describe prevalence of 32 bp deletion of this gene in the Slovak population.

**Methods:** Δ32 allele frequencies from HIV-negative and HIV-positive DNA samples were collected from our data. They represent 200 (100 men and 100 women) HIV noninfected unrelated individuals of Slovak nationality and 151 (127 men and 24 women) HIV infected Slovaks. Cell DNA were prepared from venous blood by commercial kit NucleoSpin® Blood (Machery Nagel). PCR amplification was performed initially by the use of the kit EliGene CCR5 polymorphism Δ (Elisabeth Pharmacon) and than by using the „in house“ designed primer pairs. The amplification products were analyzed according to their molecular weights in agarose gel electrophoresis. Variation in the nucleotide sequence of the CCR5 gene were analyzed and confirmed by sequencing of the PCR products: 199 bp fragment for the wild type allele and a 168 bp fragment for the mutant allele.

**Results:** CCR5-Δ32 was found in 1% HIV-negative individuals homozygous and in 17% HIV-negative individuals heterozygous for this mutation. The frequency of the Δ32 deletion allele in Slovak population was estimated at 9.5%, while the allele frequency in men was similar to that in women /9% vs. 10%/ In the group of HIV-positive persons no homozygosity of CCR5-Δ32 was found. Heterozygosity of CCR5-Δ32 in HIV-negative individuals was similar than in HIV-positive patients /17% vs. 16.6%/.

**Conclusions:** Study of CCR5-Δ32 prevalence in the Slovak population revealed similar results compared to those described in other EU states. Significant difference between HIV-negative and HIV-positive group was not found. Results of the study do not indicate that relatively low prevalence of HIV-infection in SR could be due to the prevalence of CCR5-Δ32.
ABSTRACTS

HUMAN DEVELOPMENT
Is there neurogenesis after final settlement of neural crest cells in spinal ganglia?

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Background: Generally ruling opinion was that neurogenesis (mitosis of neurons) is supposed to be finished after final settlement of neural crest cells (NCC) in the area of future spinal ganglia. On the other hand there is opposite information about neurogenesis in adult rat spinal ganglia (Devor 1985). For spinal ganglia in the lumbar region final settlement of NCC occurs between 10 and 14 days of mice development (Lawson 1979) which correspond to about 5th weeks of human development (Vukojevic et al. 2008).

Aims: To study the distribution and possible co-localization of the proliferation factor Ki-67 and pan-neuronal marker PGP 9.5 in tissues of human conceptuses, 5-9 weeks old, in order to elucidate the pattern of neurogenesis during early development of the human spinal ganglia.

Methods: Tissues of 10 human embryos and fetuses between 5 and 9 gestational weeks were analysed immunohistochemically and morphologically using paraffin sections (7μm thick). Mann-Whitney test was used for statistical analysis.

Results: All mitotic cells positive to Ki-67 displayed brown-stained nuclei. Ki-67 proliferation marker had the strongest expression in the 5th developmental week (58% of positive cells). Dorsal parts of spinal ganglia in comparison to ventral parts had a significantly higher proliferation rate in 5th and in 8th week of gestation (Mann-Whitney, p=0.003 and p=0.043 respectively). During the 6th developmental week, a significant drop in the number of mitotic cell was seen in both parts of the ganglion, as well as at the end of 7th week, and at the beginning of the 9th week.
All PGP 9.5 positive cells displayed brown-stained nuclei and cytoplasm. The number of PGP 9.5 positive cells remained constant during the investigated developmental period (between 42-53%), without any differences between dorsal and ventral parts of the spinal ganglia.
Double immunoflourescence staining with Ki-67 and PGP 9.5 show no co-localization in the cells of spinal ganglia (Fig 1).
Fig 1. Distribution of Ki-67 (green) and PGP 9.5 (red) positive cells in spinal ganglia. Transversal section through the ventral part of spinal ganglion of a 5-week human embryo: there was no co-localisation of Ki-67 and PGP9.5 factors. Immunostaining to Ki-67 and PGP 9.5, ×40. Scale bar, 25μm.

Conclusions: Proliferating Ki-67 and pan-neuronal marker PGP 9.5 appear in spinal ganglia simultaneously, but there is no co-localization of these factors in the same cells, implying that there is no neurogenesis in spinal ganglia during investigated period of human development.

References:


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Allergic diseases in children of preschool age from 2 environmentally different Slovak regions

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Objectives: The prevalence of allergic diseases has been increasing in recent decades, and that is particularly true in paediatric population.

Aim: To compare the prevalence of allergic diseases (AD) in children of preschool age in 2 Slovak regions with different environmental characteristics - Bratislava (BA- urban area) and Stara Lubovna (SL- rural area).

Subjects and methods: A cohort of mother-infant pairs were recruited between 2001 and 2003 in BA and SL. Infants were followed annually between 3-5 years of age (3- years old: N=181, 4-years old: N=170 and 5-years old: N=157) for allergic disease development, in cooperation with regional paediatric allergists. Mothers completed questionnaires and children were examined by an allergist.

Fig. 1: Prevalence of allergic diseases in 3-5-year–old children

Results: Total prevalence of allergic diseases (AD), as diagnosed by a physician, differed between regions; every year the prevalence of diagnosed allergies was higher in Stara Lubovna, (Fig.1). In contrast, more allergic symptoms were reported by mothers from Bratislava based on questionnaire data, compared to diagnoses made by physicians (3–years old: 37.9% vs. 25.0 %, 4-years old: 33.7% vs. 29.5%, 5-years old: 28.0 % vs. 20.9%).

The most common atopic diagnosis in our cohort at 3 and 4 years of age was atopic dermatitis (18.3% and 15.8% respectively). In 5-year old children hay fever was the most frequent allergy reported. Prevalence of allergic diseases by region is shown in Table 1.
Table 1: Prevalence of allergies in 3-5-year-old children according to clinical diagnosis, by region.

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<th>Atopic dermatitis [%]</th>
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Hay fever was more often diagnosed in children of atopic mothers (p<0.05). Families, who reported households with mold, were more likely to be diagnosed with atopic dermatitis.

**Conclusions:** Differences in AD prevalence among regions may result from different environmental exposures. Greater proportion of mothers reporting symptoms of children’s allergies in BA as compared to SL may be the result of two factors - better public health awareness of allergies in the urban region of Bratislava and a higher level of maternal education in this region.

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Sex assignment of females, suffering from the virilizing form of congenital adrenal hyperplasia with high grade of virilization

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Congenital adrenal hyperplasia (CAH) is a group of diseases inherited in autosomal recessive manner. They are caused by the mutation of the genes, responsible for the activity of enzymes, required for normal adrenal steroid biosynthesis. As a result, reduced cortisol production, excess of adrenal androgens, gonadotropin and often diminished aldosterone biosynthesis. More than 90% of cases of CAH are caused by 21-hydroxylase deficiency.[1] From the clinical point of view three types of 21-hydroxylase deficiency are known: salt-wasting (SW), simple virilizing (SV) and nonclassical.[2] There is a problem concerning this disease worth of discussion: sex assignment and social gender of females with the high grade of virilization. In general, the recommended sex assignment is that of the genetic/gonadal sex. This is especially true for females with 21-hydroxylase deficiency who have normal internal genital structures and potential for child-bearing. Some authors estimate that an exception to this rule might be the genetically female patient with completely male appearing genitalia, especially if the child has been raised as a male for more than a few months. [3] But still there are questions: will the parents of the child be honest in making that decision despite the thought of society? and what is better a female, who due to the development of medicine can give birth or a male, who will never have normal sexual life and own children?

This year our department of pediatrics and neonatology started a program, the aim of which is to find out the information about newborns with CAH, who were treated in the hospitals of Lviv during the last 15 years. This report describes the destiny of one of that newborns.

A 10-year-old patient was treated in Lviv hospital with the diagnosis: CAH (21-hydroxylase deficiency), combined SW and SV form (Prader’s grade V) in 1998. The diagnosis was confirmed by the results of laboratory tests (steroid profiling, elevated 17-hydroxyprogesterone level), genetic test (karyotype 46XX). The patient received hydrocortisone 15-20 mg/kg/d, cortinef 0,0015 g three times a day. Since that time each year 2 times a year he comes to the endocrinologist to measure his weight, height and costal age, make changes in the therapy. On the 5th of December 2005 a surgery was performed, during which normal uterus and ovaries were taken away. The parents decided to raise the child as a male. Nowadays the height of the patient is 130 sm, weight 34 kg, costal age is 1 year “bigger” than his real age. The patient receives cortef 5-5-7,5 mg and cortinef ¼ of the pill 2 times a day, considers himself as a male.

References
Content of Maillard reaction products (MRPs) in breast milk and infant formulas and their impact on selected biological parameters in 5-7 months old infants

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Thermal processing of foods results in formation of Maillard reaction products (MRPs). Excessive intake of MRPs from thermally processed foods exerts biological effects in healthy adults, e.g. increase of inflammatory markers and those of oxidative stress, nephrotoxic and diabetogenic effects, and weight gain (1). Industrial processing of artificial infant formulas (IF) requires heat-sterilization. Thus, content of MRPs in IF is much higher than in the human mother milk (2). In the present study we compared the amounts of MRPs (furosine, carboxymethyllysine-CML) in infant formulas (n=16) versus breast milk (n=56). Plasma and urine levels of MRPs (3) and selected biochemical parameters (markers of glucose metabolism, renal function and oxidative stress) were determined in healthy 5-7 months old infants exclusively breast fed from birth (n=46) or fed by IFs (n=43).

In infant formulas the mean furosine content was 320 times higher, and the mean CML content 70-fold higher than in breast milk. The hydrolyzed formulas (n=7) tended to have 3 times higher content of furosine and CML if compared with non-hydrolyzed ones (n=9). Formula feeding in 5-7 months old infants represented 25-2800 times higher daily exposure to CML and 19-3100 times higher exposure to furosine than breast feeding.

Formula-fed babies had approximately 37% higher plasma CML levels, and 40-fold higher urinary excretion of CML compared to breast-fed infants. Intake of hydrolyzed formulas was reflected by 49% higher urinary CML excretion than that of non-hydrolyzed ones, but this difference was not significant.

Breast- and formula-fed infants differed in their oxidative status. Formula fed infants showed significantly higher plasma nitrotyrosine concentration (p=0.01) (marker of oxidative protein damage) and urinary excretion of 8-OH-deoxyguanosine (marker of oxidative DNA damage) (p=0.03), but lower malondialdehyde levels (marker of lipid peroxidation) (p=0.01), and advanced oxidation protein products concentration (AOPPs) (p=0.002). Plasma concentrations of fat soluble vitamins (tocopherols, carotenoids, retinols) were significantly higher in the formula fed group.

Although within the normal range, breast fed infants were more insulin sensitive (p=0.007), and displayed lower urinary protein excretion rate (p=0.03).

At present, we are not able to conclude whether the observed effects of IF-feeding could be unequivocally attributed to their MRPs content. However, they correspond to the in vitro effects of MRPs/AGEs, and those observed in experimental animals or humans on experimental high-MRP diets.

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Cystathionine β-Synthase and Proliferation at Hyperhomocysteinemia in Human Placenta

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Background: Deficiency of folates and high levels of homocysteine (Hcy) during pregnancy lead to different malformations of the fetus, particularly spina bifida, and to spontaneous abortion, but the mechanisms of these pathologies and the genuine possibility of human placenta to withstand them are not so clear. Hcy is metabolized in the cycle of activated methyl group and by transsulfuration with cystathionine-β-synthase (CBS) as an important player. Now there are no data about CBS expression and biological processes that are severely injured by hyperhomocysteinemia in placenta. Here we aimed to investigate the effect of hyperhomocysteinemia on the proliferation and CBS expression in the villous explants of human placenta.

Methods: Placental explants (10-25 mg of villous tissues) from normal term placentas (38-40 weeks of gestation) and from abortive material (6-11 weeks of gestation) were cultivated in DMEM/F12 in the presence of 20, 40, 80 µmol/L Hcy with or without 20 nM/L folic acid for 48h at 37°C and 5% CO2, 20% oxygen. After cultivation the explants were immunohistochemically stained for proliferative (Ki-67) and apoptotic (cytokeratin 18-neo-epitope,M30) indices. Ki-67 index was calculated as the number of Ki-67 positive nuclei in cytotrophoblast cells per 100 µm of villous circumference. The CBS expression was examined by Western blot and immunohistochemical analyses with corresponding antibodies.

Results: Ki67 staining index in the first trimester explants is higher than in term explants (3 vs.0.6). The cultivation with increasing concentrations of Hcy is followed by gradual decrease of Ki67 index (0.53, 0.48 and 0.4 in term explants and 3.3, 2.7 and 2.0 in the first trimester explants). Higher concentrations of Hcy provoke apoptotic processes reflected in higher M30 staining. The addition of folic acid at the background of 40 and 80µmol/L Hcy slightly increases proliferation index up to 2.96 and 2.20 in the first trimester explants and to 0.50 at 40 µmol/L Hcy in term placenta explants. The CBS is detected in trophoblast of first trimester and term placental explants, its content significantly increases at 20µmol/L Hcy with subsequent decrease below initial level at 40 and 80µmol/L Hcy

Conclusions: Human placenta may partly withstand to hyperhomocysteinemia by CBS involved transsulfuration. The increased level of Hcy decreases the proliferation index, induces the tissue destruction, apoptosis and suppresses CBS expression.

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Correlation of AR CAG tract length with impaired spermatogenesis

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Androgens are required for male sex determination, development and spermatogenesis. Androgen activity is mediated by the androgen receptor. The androgen receptor gene (AR) located on chromosome Xq11-12. The AR has a repetitive DNA sequence in exon 1 that encodes a polyglutamine tract. Within the normal polymorphic range this (CAG)n tract length is inversely related to the transcriptional activity of the androgen receptor.

The present study investigated whether the CAG repeats number in exon 1 of AR gene is associated with impaired spermatogenesis.

DNA isolated from blood samples of 158 infertile men (with azoospermia and oligozoospermia) was amplified by polymerase chain reaction targeting the AR (CAG)n tract. DNA isolated from blood samples of 124 fertile men served as the control. For CAG repeat numbers computation fragment analysis of Cy5-labeled PCR products on an automated DNA analyzer “A.L.F.-express” were used. To determine exact number CAG repeats three different alleles were sequenced using ABI 3130 Genetic Analyzer. The nomenclature of alleles in our study corresponds to the number of CAG repeats.

The CAG repeats number of AR gene in the infertile men group was more widely distributed than in control group. There was a significant difference in CAG alleles in the infertile men versus controls (p=0.023). Short alleles contained 7 CAG repeats were detected only in the men with azoospermia. Long alleles contained 32 and 33 CAG repeats were detected only in the men with oligozoospermia. These alleles were not found in control group. Severely oligozoospermic men had longer CAG repeat length than azoospermic men. The results of our study allow as supposing that short CAG repeats can lead to disease in androgen-dependent tissues. On the other hand, men with longer alleles of AR gene within the normal range of CAG repeats may have decreased AR function that results in reduced spermatogenesis.

So, molecular-genetic analysis of the CAG repeats number of AR gene as well as genetic counseling are very important for patients with male infertility, especially if they are included in an assisted reproductive technologies program.
ABSTRACTS

REVIEW THE SCIENTIFIC ACTIVITIES AT THE PARTICIPATING ORGANIZATIONS
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Value of transvaginal hydrolaparoscopy in infertile women

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Background: Transvaginal hydrolaparoscopy (THL) is a new minimal invasive technique of culdoscopy for exploration of the pelvic cavity. The procedure involves introducing an endoscope through a small needle placed beneath the cervix and using water instead of carbon dioxide as a distention medium. This allows a clearer view of the structures within the reproductive tract, particularly the fallopian tubes. Transvaginal hydrolaparoscopy allowed the visualization of the tubes and ovaries in their normal physiologic relationship and eliminates the need to manipulate the adnexa.

Aim: Is Transvaginal hydrolaparoscopy (THL) a good, reproducible, and safe method to investigate the pelvis and its structures?

Material and Method: A hysteroscopy and a THL were performed in 14 cases of women with infertility of unknown cases, with no previous pathology. The liquid was represented by saline infusion 0.9% or glucose 5%. The patients received intravenous anesthesia, and underwent the transvaginal hydrolaparoscopy. It was used a hysteroscop modified 2.7 mm/3mm, 30 °, oval, with working channel (Bettocchi type). The main outcome measures were the rate of successful access to the pouch of Douglas, the duration of the procedure, and the rate of complications.

Results: The successful rate of access to the pouch of Douglas was 85.7%. In two cases the pouch failed and it was preformed a classic laparoscopy. There were no complications. The mean duration of the transvaginal hydrolaparoscopy procedure was 11 min in the cases with a successful access. In 3 cases there were identified mild asymptomatic adhesions, and in 1 case the tubo-ovarian adhesions were removed with the scissors. In 8 cases the results were normal, with both tubes permeable after blue dye instillations, and in 1 case was diagnosed ovarian and peritoneal endometriosis (by biopsy) with one tube impermeable. After six months, the rate of fertility was 9/14.

Conclusion: Transvaginal hydrolaparoscopy can serve as an initial screening procedure for infertile women to help with diagnosis and guide decisions about induction of ovulation and the choice of specific medications in selected cases. The diagnostic accuracy of the transvaginal hydrolaparoscopy suggests that some of standard laparoscopies for unexplained infertility could be avoided.
Minimally Invasive Surgery for Paraganglioma

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Background: Paragangliomas are a type of neuroendocrine tumor, and are closely related to pheochromocytomas. Although all paragangliomas contain neurosecretory granules, only about 1-3% have clinical evidence of oversecretion. Paragangliomas are found predominantly in the abdomen (85%) and the thorax (12%), and only 3% are found in the head and neck region. Most occur as single tumors. The main treatment modalities are surgery, embolization and radiotherapy.

Aim: Is minimally invasive surgery feasible for the removal of these tumors?

Material and Method: Two cases of paragangliomas were identified for the last two years and treated by laparoscopic surgery in the Bucharest Emergency Hospital Department of Surgery. A retrospective review of these two cases is presented.

Results: First case: A 55 years old female was diagnosed with a 5/4.7/4.3 cm retroperitoneal tumor just next to the left adrenal gland. Intraoperative aspect revealed no lymph involvement or local invasive tumor so that, an anterior transperitoneal laparoscopic surgical removal was performed with very good postoperative results. Second case: A 46 years male with a 5/4.5/2.3 cm interhepatogastric tumor (inside the gastrohepatic ligament) was operated by laparoscopic approach and the tumor was successfully removed, with no postoperative complications. Pathology for the both tumors: paraganglioma.

Conclusion: Laparoscopic approach for paraganglioma can be used for tumors with both intra- and retroperitoneal localization but with reasonable dimensions (no larger than 5 cm).
COTRASIF: Genomics Tool for Systems Biology

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Summary: A new tool has been developed (COTRASIF, conservation-aided transcription factor binding site finder) for the genome-wide identification with increased specificity of the putative transcription factor binding sites (TFBS) in eukaryotic gene promoters.

Motivation and aim: Promoter analysis and TFBS identification are essential for the understanding of gene regulatory networks. Increasing specificity of the TFBS prediction in eukaryotic gene promoters is a challenging task for modern bioinformatics.

Based on our previous research, we observed better specificity of the TFBS search when comparing promoters of orthologous genes of the evolutionary close species (e.g. rat and mouse) for the presence of the target TFBS.

Our aim was to develop an easy-to-use web-tool for genome-wide identification of putative TFBS with enhanced results quality.

Methods and algorithms: COTRASIF is built upon the semi-automatic importer of promoters from the Ensembl genome annotation database. Currently COTRASIF has 11 genomes available.

Promoters are defined as 800bp upstream from transcription start site, plus the 5' UTR coding sequence. For TFBS search, both position-weight matrix (PWM) approach and the recently developed HMM-based (hidden Markov models) approach can be used. For PWM method, frequency matrices are needed as input; for HMM – a list of at least 3 known sequences of the TFBS, plus an optional position frequency matrix.

Initial search results can be further analyzed using the built-in gene orthology filter. Orthology information is automatically obtained from the Ensembl Compara genome alignments database. If the putative TFBS is present in the promoters of the genes of both orthologous genes being analyzed, then it has higher probability of being functional.

Results: We developed a web-accessible tool (conservation-aided transcription factor binding site finder, COTRASIF) for the genome-wide conservation-aided TFBS search.

Further development includes: addition of new genomes; integration of the Gene Ontology category enrichment functional analysis (hyper geometric and Bayesian); more results output formats; specialized web-API (application programming interface) for enabling easy use of COTRASIF by other tools.
Conclusions: We identified 323 genes in rat genome which contain ISRE (interferon-stimulated response element) in their promoters, and are the potential targets of transcriptional regulation by type I interferons. Functional analysis of these genes, conducted using Gene Ontology Tree Machine, had shown the relative enrichment of 24 GO categories; of which 12 represent known IFN effects. Further investigation of interferon-induced gene regulatory networks, based on the identified primary response genes, will help to understand the mechanisms of both therapeutic and side-effects of type I interferon treatment.

Availability: COTRASIF is freely available at http://biomed.org.ua/COTRASIF/
Peculiarities of Spontaneous Karyotypic Evolution of Mouse Embryonic Germ Cells *In Vitro*

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We have isolated original cell lines (G1, G4, G6, G7) derived from gonadal ridges of 12, 5-dpc BALB/c mouse embryo. Obtained cells are expected as alternative cell resources for different kind of biotechnologies. It has been performed a complex analysis of morphology and growth properties, and studied molecular-genetic peculiarities of the karyotypic evolution of investigated cell lines during long-term *in vitro* cultivation. It has been shown that adaptation of the investigated cell lines to *in vitro* conditions is accompanied by aneuploidy, in particular hyperploidy, as well as numerous robertsonian translocations, chromosomal fragments and microchromosomes.

The expression of *Trp53* gene has been revealed at low levels in cells of G1 cell line and its sublines G1-OA and G1-T at different passages of cultivation. It has been shown by immunoprecipitation that G1 cells contain wild type and mutant p53 protein. The general trend to increase the amount of Mgmt protein was revealed during long-term cultivation of the cell line G1 and its sublines G1-OA and G1-T, as well as a correlation between reduced amount of Mgmt protein and the increased frequencies of aberrant mitoses and chromosomal aberrations. It was suggested a possible role of DNA repair enzyme Mgmt at the maintenance of balanced karyotype in the establishment of studied mouse cell lines *in vitro*. It has been shown that chromosomal instability of the G1 cell line may be associated with the failure of the mitotic checkpoint, with the appearance of the mutant tumour suppressor p53 protein and with changes in expression of one of the key DNA repair enzyme – Mgmt.
Development of Enzyme Conductometric Biosensors for Determination of Glucose, Sucrose and Lactose

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There is a constant demand for determination different carbohydrates such as glucose, sucrose and lactose for quality control of a product in food and pharmaceutical industries. Conventional methods of carbohydrate determination either seem to be very laborious and time consuming (biochemical analysis), or needed of well-trained technicians (liquid chromatography), even though the analysis are accurate. Polarimetry and density or refractive index measurements are fast and precise, but may be less accurate. In some cases conductometric biosensors have advantages in rapidity and sensitivity over the traditional techniques. Furthermore they may be sufficiently simple, cheap, highly specific, convenient, accurate, and allow solving important scientific and industrial problems.

Conductometric biosensors for determination of sucrose, lactose and glucose have been developed. It sensitive element contains enzymes (invertase, mutarotase and glucose oxidase for determination of sucrose, β-galactosidase, mutarotase and glucose oxidase for lactose and glucose oxidase for glucose) immobilized onto a conductometric transducer consisting of two pairs of planar electrodes. The biologically active membranes were formed by glutaraldehyde cross-linking of definite enzymes with BSA on the surface of one pair of electrodes, while another pair was covered by only BSA membrane. The time of carbohydrates analysis in the solution was 1-2 min. Dependence of the sensor response on characteristic of solution such as pH, ionic strength, buffer capacity has been studied. The developed conductometric biosensor is featured in high operational and storage stability and results reproducibility.
**Study on the structural basis of the bacterial-type prolyl-tRNA synthetase from *Enterococcus faecalis* editing activity by the methods of site directed mutagenesis**

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The maintenance of the amino acid specificity by aminoacyl-tRNA synthetases in some cases requires hydrolysis of missynthesized products that is known as amino acid editing. Unlike the most of eukaryotic prolyl-tRNA synthetases (ProRS), structures of the most of bacterial ones include special editing domain that exhibits post-transfer editing activity. This domain can serve as a promising target for new drugs against pathogenic bacteria. The mechanism of tRNA-dependent editing by ProRS has to be defined.

Aim of the present work is to study the structure of the active site of enterobacteria *Enterococcus faecalis* ProRS (ProRSEF) editing domain. On the base of the putative structure of the editing domain active center, based on the ProRSEF crystal structure and computer modeling data, following amino acids positions were have been chosen for the site directed mutagenesis (alanine scanning): E218, T257, K279, G331, S332, G334, H366. In the ProRSEF gene, cloned previously, mutations were obtained by QuickChange® method (Stratagene). Purification procedure of the mutant proteins included graded salting-out, chromatography on DEAE-sepharose and chromatography on Toyopearl HW-60. For editing activity checking of the ProRSEF mutant forms by alanyl-tRNA hydrolysis a chimeric tRNA, recognized by both prolyl- and alanyl-tRNA synthetases, was constructed. Mutant forms editing activity determining allowed to reveal next amino acid residues, most important for editing process: K279, G331, H366. Comparison of these obtained data with structural data enabled us to suggest the hypothesis following which K279 is involved in substrate positioning in the editing active center, G331 takes a part in catalytic water molecule binding and activating, and H366 maintains G331 in the right conformation. Existence of a binding water molecule in the editing active center was predicted by computer modeling.

In summary, in this work seven mutant forms of the ProRSEF, with mutations in the editing domain have been obtained and their editing activity was compared with wild type ProRSEF. Three amino acid residues, important for editing activity, K279, G331 and H366, were revealed. The hypothesis was suggested, that water molecule, hydrolyzing alanyl-tRNA<sub>pro</sub>, is positioned in ProRSEF editing domain active center.
Identification of proteins that bind to Bcr PH domain using proteomics technique.

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Philadelphia (Ph) chromosome is a result of reciprocal translocation t (9;22) and known to be a marker of chronic myeloid leukemia (CML). There are three forms of hybrid Bcr-Abl protein depending on breaks in Bcr moiety whereas Abl part remains the same size in all chimeric Bcr-Abl proteins. It’s suggested that transforming potential of Bcr-Abl is determined by Abl tyrosine kinase, which is deregulated. However, the Bcr component contribution to the hybrid protein functions needs to be clarified.

In the focus of our research is PH domain that is absent in the Bcr-Abl shortest variant p190 but is found in two other types –p210 and p230. p190 Bcr-Abl corresponds to acute lymphoblastic leukemia and p210 is found in chronic myelogenous leukemia cases. PH domain is known to bind to lipids but its protein-protein interactions are not investigated well. To determine Bcr PH domain binding partner’s recombinant histagged PH protein was used. K562 cells were labeled with $^{35}$S-methionine and cell lysates were loaded on PH-bound column. Column with his-tag protein was used as a control. After incubation with K562 lysates columns were washed several times. Bound proteins were eluted and resolved by two-dimensional gel electrophoresis. Spots corresponded to K562 proteins bound to Bcr PH domain were selected. Interacting proteins were identified by peptide mass fingerprinting by matrix-assisted laser desorption-dimensional time-of-flight mass spectrometry (MALDI TOF MS). We identified 20 proteins that formed complexes with PH domain. To verify some of the interacting proteins immunoprecipitation assay and pull down experiments were performed. Binding to SMC1 (structure maintenance of chromosome protein) and β-tubulin was observed in vitro in pull down assay. Interaction with PLCε and zizimin1 was confirmed in vivo.

Taken together, the study reveals important functions of Bcr PH domains that could affect Bcr-Abl signal pathways. It’s established that the cellular compartment in which Bcr-Abl is localized is important in determining whether the outcome of its deregulated kinase activity is pro- or antiapoptotic. PH domain is a possible regulator of Bcr-Abl localization since it’s able to bind lipids of cellular membranes or form complexes with various proteins. Our results display PH domain as a protein binding partner that could associate to cellular proteins and bring Bcr or p210 Bcr-Abl to functional protein complexes. Moreover, detecting the roles and relative importance of Bcr-Abl domains in leukemogenesis in vivo should help to understand the molecular mechanisms underlying the phenotypes of leukemia and thus to identify targets for developing therapeutic interventions.
Different Members of ITSN Family Share Common Partners Except for Adaptor Molecule CIN85/RUK

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Human intersectins (ITSN1 and ITSN2) are members of a conserved family of adaptor proteins encoded by two paralogous genes. ITSN1 is known to participate in multiple cellular processes including endocytosis, mitogenic signaling, actin cytoskeleton rearrangements and apoptosis while the function of ITSN2 is to be elucidated. In this work we intended to examine interaction of ITSN2 SH3 domains with protein partners that could be common to ITSN1 or different.

Using GST pull-down assays we showed an interaction of ITSN2 SH3 domains with dynamin 1, molecule thought to drive endocytosis late events. Dynamin 1 strongly bound to SH3A, SH3C and with highest affinity to SH3E domain but not to SH3B and SH3D. No significant difference was observed comparing ITSN1 and ITSN2 SH3 domains interaction with dynamin 1.

The interaction of ITSN1 SH3A domain with Ras exchange factor SOS1 was reported. It is known also that expression of ITSN1 is detected in both proliferating and differentiating neurons, while ITSN2 is mainly expressed in latter. Given that we analyzed ITSN2 participation in signal transduction mechanisms through SOS1 binding. We showed that ITSN2 SH3A, SH3C and SH3E domains interact with SOS1 but the affinity is less when compared to ITSN1 SH3A domain. Obtained results indicate the role for ITSN2 in linking endocytosis and signal transduction pathways.

Considerable differences were observed while examining ITSN2 interactions with ubiquitin ligase c-CBL and adaptor protein CIN85/Ruk. Comparing to ITSN1 only two of ITSN2 SH3 domains (SH3C and SH3E) were involved in binding to c-CBL in vitro. It is worthwhile to mention that SH3A domain is the most divergent one when intersectin 1 and 2 are compared which might impose differences in its ability of binding partners. The results of pull-down assays showed that only SH3A domain of ITSN2 binds to CIN85/Ruk with low affinity. This allowed us to suggest that interaction of ITSN2 with CIN85/Ruk does not occur in vivo since there is an intramolecular interaction of CIN85/Ruk own SH3A domain with its own proline-rich region.

We identified new intersectin interacting partner semaphorin 6A (Sema6A) implicated in retrograde signaling and cytoskeletal rearrangements during neurogenesis and organogenesis. The interaction of Sema6A with ITSN is mediated by SH3A domain of ITSN2 and SH3A, SH3C and SH3E domains of ITSN1.

Our results suggest that ITSN1 and ITSN2 have similar functions but differences in intracellular localization and binding properties with aforesaid partners are the evidence of functional diversification of intersectins. Further research is needed to study the precise role of ITSN family members in cellular processes.

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Establishing of cellular models for the analysis of sodium-dependent phosphate transporter Napi2b, a potential marker for ovarian cancer

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Ovarian cancer is the most common gynaecologic cancer that is usually far advanced before it is diagnosed and thus patients have a poor prognosis and survival rate. Identification and characterization of novel ovarian cancer markers is important for understanding the molecular mechanisms of malignant transformation and for the development of novel diagnostic and immunotherapeutic approaches in gynaecologic oncology.

Recently, we have identified the sodium-dependent phosphate transport protein 2b (NaPi2b) as new ovarian cancer antigen based on immunoscreening of ovarian cancer cell line (OVCAR3) cDNA library with MX35 monoclonal antibodies obtained at Ludwig Institute for Cancer Research by mice immunization with ovarian carcinoma cells.

The human protein, sodium-dependent phosphate transport protein 2b encoded by SLC34A2 gene is involved in the homeostasis of inorganic phosphate. Napi2b is a membrane protein with the NH2- and COOH-termini located on the cytoplasmic side of the membrane, 8 transmembrane domains and a large extracellular loop (188-361aa). Previously, we have generated monoclonal antibodies (L2/20) against the large extracellular loop (188-361aa) of Napi2b and determined the region of transporter molecule that includes the epitope for these antibodies (311-340aa) which happened to be the same as for MX35.

The aim of present study was to create the model for functional activity investigation of a new marker of ovarian cancer - sodium-dependent phosphate transporter Napi2b in malignant cells. For this purpose we have created stable cell lines expressing wild type and mutant forms of Napi2b by stable transfection of HEK293 cells. We have chosen mutations of Napi2b that according to the data base search could be potentially associated with ovarian cancer. There are deletion in 6aa in C-terminus and point mutation T330V in extracellular loop of Napi2b. The expression of Napi2b by stable cell lines was confirmed by Western-blot analysis using tag-specific and anti-Napi2b antibodies.

In addition we have shown that amino acid substitution T330V resulted in loss of Napi2b recognition by both antibodies in Western-blot analysis that could be explained by epitope destruction for anti-Napi2b antibody. The experimental system described here will be used for the further investigation of the Napi2b function in normal and pathological states.
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“Ahh-32” – a novel plasminogen activator isolated from *Agkistrodon halys halys* snake venom.

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**Introduction:** The venom of most snakes belonging to the Crotalidae and Viperidae families contain many pharmacologically active biopolymers which act upon the different stages of blood coagulation. At present time one of the most essential for clinical matter is, proteins isolated from snake venoms belonging to the Crotalidae and Viperidae families are plasminogen activators. The aim of this investigation was to characterize a new plasminogen activator obtained from *Agkistrodon halys halys* venom.

**Methods:** A novel plasminogen-activating proteinase (named “Ahh-32”) has been isolated and homogeneity purified from crude *Agkistrodon halys halys* venom using affinity and anion-exchange chromatography on *Blue Sepharose FF* and *DEAE Sepharose FF* respectively. The characteristics of obtained protein were estimated by 2D-PAGE and analytical size exclusion chromatography. Interaction of “Ahh-32” with main components of haemostatic systems was investigated using chromogenic substrates and enzyme PAGE.

**Results:** The concluded research show that purified enzyme consists of the single peptide-chain with molecular weight of 32 kDa and can convert free plasminogen into plasmin via an enzymatic reaction. The purity of protein was about 99% and yield nearly 4.2% of total venom proteins. The “Ahh-32”, catalyzing the hydrolysis of several p-nitroanilide substrates, does not activate nor degrade prothrombin, factor X or protein C and does not clot fibrinogen nor fibrino(geno)lytic activity in the absence of plasminogen. The activity of “Ahh-32” was inhibited by DFF and benzamidine. Besides, the enzyme influences significantly the activation of plasminogen by streptokinase without effect on analogical process in case of usage of tissue plasminogen activator.

**Conclusions:** Obtained results make possible to draw a conclusion that serine proteinase isolated from *Agkistrodon halys halys* venom is a novel plasminogen activator which can be used as an instrument under investigation of protein-protein interactions in haemostasis system and for the development of new cardiovascular therapeutic agents.
Hypothesis of the Epigenetic Nature of Ageing

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We hypothesize that a specific set of microRNAs expressing in embryonic stem cells supports pluripotent immortal status of these cells by active renewing of proper profile of the epigenetic markers through microRNA-directed DNA methylation even in case of demethylation of both DNA chains. In this way minimal possible level of the mobile genomic elements activity is achieved. However, cell differentiation, starting with the most early stages, must be accompanied with repression of genes of some microRNAs from the primary set, otherwise these microRNAs would prevent expression of genes participating in the differentiation processes. Therefore cells can lose slowly the repressive chromatin markers with time, and this will excite sooner or later the derepression of some silent transposons and other mobile elements. Derepression of latent mobile genomic elements should be facilitated due to disruption of links between nuclear lamina and chromatin, particularly in case of the Hutchinson-Gilford progeria syndrome.

In our opinion, the activity of derepressed transposases as well as other recombinases should cause recombinations not only over the DNA length, but first of all in telomere capping structures. In that case the T-loops converse into rings and, accordingly, telomeres are shortened for the length of the lost circled DNA. We suppose that this process can cause quick exhaustion of one or more cell telomeres, which is not prevented by normal or, especially, decreased telomerase activity in spite of greater accessibility of telomeres, also partially liberated from epigenetic markers, to the telomerase. Normal cells, in which DNA recombination must take place at the certain development stage, protect their own telomeres from the shortening during this time by means of the telomerase activity increasing. Therefore, telomere exhaustion must cause senescence, cell cycle arrest and apoptosis of cells, in which the illegitimate activation of recombination process becomes apparent, and which can be transformed consequently. This aspect points that telomeres are guardians for genomic stability. Unfortunately, some transformed cells can escape this impediment either through telomerase hyper expression or through complete loss of epigenetical labels linking telomeres and nuclear lamina together and thus preventing the alternative lengthening of telomeres.

We hypothesize that it is with age when large quantity of organism cells reach the threshold of illegitimate activation of silent mobile genomic elements; following apoptosis of most of these cells causes the ageing as it is. Note that the higher stability of genome is characteristic, the slower telomere exhaustion can take place and the shorter telomeres can be. This assumption explains the much long lifespan of Homo sapiens in comparison with majority of other animal species, despite the telomeres of human chromosomes are rather short.
New Approach for Correction of Ischemic Lesions of Heart

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The disorders of bioenergetical processes are the main cause of development of heart ischemia. Ubiquinone (coenzyme Q, CoQ) is the key component of cellular bioenergetics as a transporter of electrons in mitochondrial electron-transport chain and is an important lipid-soluble endogenously synthesized antioxidant. Endogenous CoQ biosynthesis is a multi-step process with subtle regulation mechanisms that are often disrupted under different pathological conditions and in healthy organism under irrational feeding, deficit of vitamins, negative environmental impact, and in ageing. Additional administration of CoQ is often used in the form of CoQ medicals to provide an organism with sufficient amount of this coenzyme, but this may lead to inhibition of its endogenous synthesis.

The aim of the present work was to study the effect of complexes of precursors and modulators of coenzyme Q biosynthesis (α-tocopherol acetate, 4-hydroxybenzoic acid, methionine without or with dimethylsulfoxide - C1 and C2 respectively) on bioenergetics and pro- to antioxidant balance in rats’ heart and liver under adrenalin-induced ischemia in vivo and on hearts’ contractility under ischemia-reperfusion on isolated hearts.

Adrenalin-induced ischemia was modeled on male rats by intramuscular infusion of adrenaline. The complexes were administrated per os both as preventive measure and for treatment after administration of adrenaline.

Under adrenalin-induced ischemia Q content increases in liver homogenates and decreases in heart and liver mitochondria. C1 and C2 administration leads to increase in Q content in mitochondria. Activity of NADH-Q-oxidoreductase and cytochrome-c-oxidase decreases in liver and heart mitochondria under AC. C1 and C2 administration normalizes their activities in liver and heart mitochondria. Activity of succinate-Q-oxidoreductase in liver and heart mitochondria does not change under adrenalin-induced ischemia as well as C1 and C2 correction.

C1 and C2 administration leads to normalization of free radical lipid peroxidation (indicated by content of conjugated dienes and TBA-reactive products) and protein peroxidation and activity of antioxidant enzyme systems (catalase and superoxide dismutase) in heart and liver which are changed under AC.

The following physiological parameters were analyzed: myocardial contractility (left ventricular pressure, diastolic pressure), coronary flow, and heart rate under ischemia-reperfusion on Langendorf perfused hearts. The results obtained demonstrate the protective qualities of C1 and C2 complexes, administration of which contributes to decrease in level of reperfusion lesions in ischemic heart.

The results obtained provide ground for application of said complexes for reducing the ischemic disorders of heart.
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Oral Lichen Planus (OLP) as Extrahepatic Manifestation of HCV

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Introduction: It is known, that HCV is associated with wide series of extra hepatic manifestations. Various studies conducted in different parts of the world have proved or disproved a causative role of HCV in OLP [1, 2]. Indian scientists, for example, have not found any association between lichen planus (oral and non-oral) and HCV infection [1]. Otherwise, French authors suggest a strong relationship between erosive OLP and HCV-related chronic hepatitis [2].

The aim of our work was to determine a correlation between HCV-infection and OLP on the basis of complex examination of patients with viral-related hepatic disorders by different specialists (hepatologists, dermatologists and dentists).

Subjects and Methods: At the DHLNMU Department of Hepatology, 54 patients (48-males, 6-females; mean age – 42.5 years) with HCV-infection and 33 patients (26-males, 7-females; mean age – 45.5 years) with HBV-infection were tested for the existence of oral lichen planus. Serum HBV-DNA and HCV-RNA were detected by polymerase chain reaction. The diagnosis of OLP was established on the basis of clinical and histological investigations.

Results and Conclusions: The diagnosis of OLP was clinically and histologically proven in 3 patients with HCV-infection (5.5%) but in none (0.0%) with HBV-infection. The performed work suggests that HCV could be involved in the pathogenesis of OLP. We conclude that patients with OLP should be systematically evaluated for HCV infection. Changes of oral mucosa, especially OLP, must be taken into consideration by internists, hepatologists as extra hepatic manifestation of HCV infection.

References:
The Connection between Crystaluria and the Somatic Pathology

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At the present time the matter of crystalluria is being robustly discussed both in Ukraine and abroad. We decided to explore the problem of crystalluria more thoroughly, as almost 1/6 of the children in Ukraine appear to have it.

**The aim:** of our research is to discover which pathologies are usually accompanied by crystalluria, whether they carry any important information and what their diagnostic meaning is in the practical medicine.

**Materials and methods:** 400 patient histories of the children with the diagnosis of crystalluria, who were treated in the department of nephrology in the Lviv Children’s Hospital in 2003-2006.

Given that the reasons of crystalluria are: increased income with food, increased absorption in the stomach and increased creation in the glomeruli, the basis of our research was the pathology of the renal and digestive systems.

According to our calculations in 72% of the cases crystalluria is accompanied by the renal pathology, in 58% by the pathology of the digestive system, in 43% of the cases they combine, and only in 10.4% of the cases crystalluria is isolated. Oxalate crystalluria is the most frequent one, according to our calculations 172 children out of 400 have it.

In order to receive the most detailed and accurate analysis, children were divided into 4 age groups. In each of them the amount of girls and boys, the frequency of digestive and renal pathology were calculated, and the diseases that are most frequently accompanied by crystalluria were outlined.

**Conclusions:**
1. Crystalluria is without doubt a marker of the renal and digestive system pathology;
2. The gained results show that oxaluria is the most frequent type of crystalluria;
3. Girls suffer from crystalluria more than boys;
4. The frequency of the crystalluria raises together with the age, reaching its peak at the age of 10-15 years;
5. Crystalluria most frequently accompanies chronic pyelonephritis out of renal diseases and biliary diskinesia out of the digestive system pathologies;
6. As a rule, crystalluria accompanies chronic diseases of the renal and digestive systems;
7. In the age of 1-6 years, children with crystalluria mainly suffer from such renal pathology as chronic pyelonephritis and glomerulonephritis, renal abnormalities, cystitis, etc.;
8. In the age of 6-9 years renal, as well as digestive pathology is observed;
9. In the age of 10-15 years such diseases of digestive system: as biliary dyskinesia, chronic gastritis, reactive pancreatitis and dysbacteriosis are dominating;

Gained results show the extreme necessity of profound examination of the children with crystalluria by gastroenterologist and nephrologist in order to prevent diseases that might develop in the future.
Excessive Antibiotics Use in Children with Acute Gastroenteritis

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Introduction: Diarrhea, especially acute diarrhea, remains a major public health problem in the world. In developing countries estimated 12 or even more diarrheal episodes per child per year which occur with first 5 years of life. Virus is the most frequent causative agents of acute gastroenteritis in infants and young children. Clinical exam and basic lab investigation can not help in accurate distinguishing between viral and bacterial etiology. Viral pathogen tests are still not routinely used in most clinics for prevention of rotaviral infection by vaccination in Ukraine. Because of the several days delay in getting the final bacterial tests results, most doctors prescribe antibacterial drugs every child with acute gastroenteritis. As a result, the patients are placed on the “strong” antibiotics (ceftriaxon, tienam, ciprofloxacin) or even antimicrobial drugs combination (for severe disease) with no evidence of their indication.

Methods: During a winter & spring periods (2006-2007) 179 children less than 3 years old admitted at Lviv Infection Diseases Hospital were under observation. The diseases severity was estimated using Vesikari Scores for Clinical Severity. Two stool specimens were collected for laboratory testing. One was tested for Salmonella, Klebsiella, Campilobacter, Staphylococcus aureus using standard bacteriological methods; the second was frozen until virology tests for rotavirus, astrovirus, adenovirus serotypes 40 or 41, norovirus were made. In some cases specimens for bacterial investigating were obtained after antibacterial treatment had started. We used to IFA tests R-Biopharm AQ RIDASCREEN Rotavirus, RIDASCREEN Norovirus, RIDASCREEN Adenovirus, RIDASCREEN Astrovirus, and “MULTISKAN ASCENT” reader.

Results: All children had gastroenteritis symptoms during their hospitalizations. 152 (84.9%) children were treated by PO or IV antibiotics. Monobacterial infections were detected in 38 (21.2%) patients (Salmonella enterica strains spp. in 18, Klebsiella in 11, Campylobacter jejuni in 6, Staphylococcus aureus in 3). Rotavirus antigen was detected in 35.7% of patients. Rate of norovirus was 33.5%, rate of adenovirus (serotypes 40 or 41) was 11.7%, and astrovirus was 10.0%. Out of these 79% of norovirus infections and 90% rotavirus infections were syndromic. The virus-virus coinfection was found in 25% case, such as norovirus-rotavirus coinfection was observed more often. With regard to clinical severity, rotavirus and norovirus resulted in longer hospital stay, higher rate of vomiting, leukocytosis, lower rate in stool pus cells.

Conclusions: The high incidence of rotavirus and norovirus infections (monopathogen or coinfection) as a cause of acute gastroenteritis in infants and young children were established. The antibiotics prescription in 80% of cases were not theoretical grounds, moreover it could lead to bacterial antibiotic persistency and increased rate of nosocomial infection. We recommend easy rapid tests for common use in detection of viral pathogen and give baby antibiotics only in case of negative viral test results. In the future investigations we are going to take part at some projects for nosocomial infections and antibiotic-resistance monitoring, for this reason we participate in INICC-Multinational Multicenter Study on Health-Associated Infections.

References:
Platelet-Rich Fibrin as a Substitutive Material in Maxillofacial Surgery; First Experience

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Introduction: Stimulation of osteogenesis in the remaining postoperative cavital defects of the jaws is one of the actual problems of modern maxillofacial surgery. An evident lacks of existent bone-substitutive materials of auto-, allo- or xenogenic origin, as for example, additional patient’s traumatization, high risk of transmission of diseases (AIDS, hepatitis and others), possibility of development of allergic reactions as a result of immune and gene incompatibility, promoted a introduction into medical practice of technology of preparation and employment of autologous platelet concentrates, in particular, platelet-rich fibrin (PRF) which contains the specific biological growth factors, possesses an expressed haemostatic and osteoconductive properties, serving as a matrix for migration of non-differentiated cells, that positively influences on osteogenic processes [1,2,3].

The aim of our work was the assessment of efficiency of PRF employment in substitution of postoperative defects of jaws of different etiology.

Materials and methods: A clinical and roentgenological examination of 14 patients with postoperative jaw defects (6 – after open (surgical) extraction of lower wisdom-teeth, 6 – after removal of radicular cysts, 2 – after removal of benign tumors of lower jaw) was carried out before and 1,3,6 months after surgical intervention with simultaneous substitution of bone defects by PRF. A platelet concentrate was prepared by standard procedure of single centrifugation of venous blood of patients during 12 minutes on ЕВА-20 (“Hettich”, France) appliance.

Results and conclusions: It was found a positive influence of PRF on soft-tissue coverage of the bone wound was clinically manifested by diminishing of postoperative edema, more rapid healing etc. Renewal of bone structure in the area of jaw defects in the terms of 3-6 months after surgery depending on the size of defect was confirmed by dynamic roentgenology. A technological simplicity and financial viability of the above-mentioned procedure was emphasized.

References:


In Vitro Investigation of Osteointegration Properties of Polyethylene Composition

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Introduction: A lot of polymeric materials, in particular polyethylene (PE), due to their inherent physical and mechanical properties during many years are used in reconstruction surgery and traumatology of maxillofacial area, though one of their lacks is unsatisfactory integration with surrounding tissues.

As it is known from fundamental researches of biocompatibility of J. Osborn et al. [2], polymers belongs to biotolerant materials, implantation of which is accompanied by phenomenon of distant osteogenesis - a formation on implants surface a connective tissue layer of different thickness that is the display of so-called inflammatory „foreign body” reaction. Taking into account the above-mentioned fact, S. Goodman et al. [1] experimentally studied an integration of PE particles in the presence of interleukin-10, which acts leading part in depression of immune answer for implantation of foreign materials. Authors established the considerable increase (to 48%) of activity of bone tissue ingrowths to PE micro pores in the conditions of local infusion of interleukin-10 in comparison with the use of PE alone. P. Sabini et al. [3] found out considerably more active invasion of connective tissue elements into PE micro pores in the presence of platelet -rich plasma.

The aim of our work was the assessment of integration properties of PE, impregnated by hydroxyapatite (HA) granules.

Materials and methods: In 30 experimental animals (rats) under general anesthesia a subperiosteal fixation of perforated PE disks, impregnated by HA granules, in the area of mandibular angle was carried out. Animals were killed by the overdose of ether anesthesia on 30, 90 and 180 days and fragments of tissues with implantable material were removed for histological investigation.

Results and conclusions: On the basis of performed investigation it was established, that through 1 month from the beginning of experiment the implantable material was confined by a layer of connective tissue (a capsule) of different thickness. In most preparations which were got three months later in more thick connective tissue capsules the accumulations of polymorphic shallow osteons, which were concentrated around the beams of extraneous material (HA), which directly contacted with surrounding bone tissue, were visualized. On 6 month of experiment the brightly expressed areas of osteogenesis inside a capsule, as an evidence of more deep integration of polymeric composition with surrounding tissue, were seen.

Thus, the performed work testifies the increasing in integration properties of PE-HA compositions, what is a perspective direction on a way to creation of synthetic substitutes for the purposes of reconstruction surgery of maxillofacial area.

References:
Analysis of rheumatoid arthritis basic pharmacotherapy using software product “Monitoring the process of hospital pharmacotherapy provision with drugs by the example of rheumatological patients”.

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An electronic management in a hospital would require to very precise and must result into cost cutting and efficient management.

The aim of our study is to work up and use the software product “Monitoring the process of hospital pharmacotherapy provision with drugs by the example of rheumatological patients” (author's certificate № 22547 of November 05, 2007).

The computer program was done using free widespread software (computer language PHP and Database MySQL) thus there is no problems with licensing. Program data are multiplatform and can work under OS Windows and Unix-similar operating system. This is a great advantage as this product is a system program and allows many users from different hospital subdivisions (laboratory, EKG and X-ray rooms, etc.) to work in it at the same time. Main menu consists of 6 catalogs such as “Medical card”, “Medical tests”, “Pharmacotherapy”, “Medical discharge”, “Data analysis” and “Administration”. The first four are used for inputting and revising of data and the last two are needed for analyzing information and correcting program database (Herbolka, 2008).

With a help of the software product 603 case histories of patients with rheumatoid arthritis (RA) that have been treated in rheumatological department of Lviv municipal clinical hospital were analysed. Non-steroidal anti-inflammatory drug (NSAIDs), disease-modifying antirheumatic drugs and corticosteroids are the main groups of basic pharmacotherapy drugs used for RA treatment in the determined hospital. The group of NSAIDs possesses the biggest quantity of different drugs. In 2003 this group was presented by 7 active substances and till 2007 this quantity increased to 11. From 2003 to 2007 nonselective inhibitors of cyclo-oxygenase-2 are the most often prescribed drugs but their quantity decreased in 1.5 times and quantity of prescribed selective inhibitors of cyclo-oxygenase-2 increased in 3 times. Drugs with ibuprofen and piroxicam stopped to be prescribed in 2006 and 2005 correspondingly. From 2004 drugs with ketoprofen and celecoxib, from 2006 drugs with parecoxib and from 2007 drugs with lornoxicam and dextropropofen began to be used in the hospital. Drugs with diclofenac (60.3%), especially in parenteral way of use, and drugs with meloxicam (18.1%) are the most often prescribe drugs in the hospital. On the base of gathered information drugs usage for 100 patients in 2003-2007 and prognosis of usage for 2008 was calculated.

Proposed program can be used for gathering and analyzing information in different hospital departments. It is simple in use and free of charge.

Estimation Method of Drugs Competitiveness by the Example of NSAIDs

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Non-steroidal anti-inflammatory drugs (NSAIDs) are the most widely prescribed drugs for the treatment of various diseases. Thus comparison of NSAIDs based on confrontation of their different characteristics is of great importance.

In our study we devised a method of drugs complex estimation (author’s certificate № 24556 of May 26, 2008) and used it for analyzing NSAIDs from group M01A presented at the pharmaceutical market of Ukraine.

The method is based on an expert appraisal and graphic-mathematical method of drugs competitive level calculation. As a result of the expert appraisal each drug receives weighted average values of following characteristics as level of effectiveness for treatment of rheumatoid arthritis, availability level, frequency of different adverse drug reactions and awareness about the drug. Daily dose cost of each drug also has to be determined. With the aim of including of all upper mentioned drug characteristics, graphic-mathematical method of calculation of drugs competitive level was used. Polygon built in co-ordinates is the best graphic interpretation of the model. Integrated values of the drugs characteristics have to be put on radius-vector, length of which conditionally accepted as unity. Importance of the drugs characteristics is different according to carried out regression and correlation analysis. Area of polygon represents the drugs competitive level (Herbolka, 2008) (Figure 1).

Competitive coefficients have been calculated for 44 trade names of different NSAIDs and their numerical values were between 0.64 and 1.1. Ranking of the drugs competitiveness coefficients according to active substances was done using Kruskal-Wallis ANOVA test of program STATISTICA 6.0. 51.7% of ranks sum belongs to drugs with diclofenac. 17.5% of ranks sum belongs to drugs with meloxicam. The drugs with indometacin, piroxicam and ibuprofen receive the smallest quantity, less than 1%. Among different medical forms and dosages of drugs with diclofenac 50 and 75mg drugs for peroral and parenteral way of use show the highest level of competitiveness. Among drugs with meloxicam peroral and parenteral way of use possesses higher level of competitiveness than perrectal forms. In hospital drugs with meloxicam in dose 15 mg and in polyclinic drugs in dose 7.5 mg receive higher competitive level.

Method of drugs complex estimation can be used for analysing any group of drugs and different drugs characteristics can be included for the analysis. The method is cheap and needs not so much time for execution. It helps to determine the situation within drugs in separate region.

Search for Thiazolidon Derivates Means with Immunomodulating Activity in Experimental Immunodeficiency

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Thiazolidones and its’ derivatives have been a great success in the field of chemistry and pharmacology over the last decades. The row of the thiazolidones act on the different stages of the clinical research as anticancer, antithyroid, anti-inflammatory, cardiovascular, antiviral drugs.

Objective: to identify among thyazolidon derivates means with immunomodulating activities on the base of pharmacological screening and on the model of cyclophosphan immunodeficiency to identify leading compound as potential mean of immune status correction.

Materials and Methods: pharmacological, toxicological, immunological, morphological and statistical methods were used. The experiment was held on white rats with the weight of 70-80g. Five groups of animals underwent the experiment: control group, animals with placebo, animals with immunodeficiency (cyclophosphan model), animals with immunodeficiency, which were treated by polyoxidonium in a dose of 10 mg/kg 10 days, animals with immunodeficiency, treated by experimental substances in the doses of 1/10 LD50 during 10 days.

Results: On the base of pharmacological studies, grounded by virtual screening a group of lead-compounds which are characterized by high immunomodulating activity and non-toxic was chosen. Structure-activity relationship was established. Experimental immunodeficiency modeling was induced by subcutaneous introduction of cyclophosphan in a dose of 10 mg/kg of weight during 10 days. The growth delay, delay in body weight increasing, reduction of thymus and spleen mass index in animals has been observed. The laboratory tests showed that neutrophil leucocytosis with lymphopeny in animals with immunodeficiency occurs. The research of immune system functional status showed significant reduction in such immunocompetent cells as CD3, CD4, CD16, CD22, increased level of CD8 cells, decreased immunoregulative index and increased level of circulating immune complexes. Also, reduction in IgA, IgM and IgG quantity was observed. Studied thiazolidon derivates possessed high activity in treating of cyclophosphan immunodeficiency comparing to the standard therapy (using polyoxidonium). It was confirmed, that its’ using lead to increasing of CD3, CD4, CD16, CD22 cells levels in animals with immunodeficiency. Also, increasing of IgA, IgM and IgG quantity was observed.

Conclusion: Highly active lead-compound among thiazolidones derivatives based on immunotropic activity was identified. The data obtained let us to enhance present spectrum of means – potential drugs with immunomodulating properties.
Synthesis of 2,3-disubstituted-1,3-thiazolidin-4-ones and their 5-arylidene derivatives with different biological activity profile

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The structural and therapeutic diversity coupled with 4-thiazolidones derivatives has fascinated organic and medicinal chemists. In recent years broad spectrum of 4-thiazolidones pharmacological activity and the variable chemical modification possibilities make the 4-thiazolidone cycle very favorable «building-block» for the modeling of novel heterocyclic biologically active compounds.

2, 3-Disubstituted 4-thiazolidones possess great interest for medicinal chemistry as potential antitumor, antivirus and antimicrobial agents. Thus the aim of our work was the synthesis of novel disubstitued 4-thiazolidones and their 5-arylidene derivatives for pharmacological screening. One-pot synthetic approach was used; this allowed obtaining the target heterocyclic systems, avoiding the intermediates separation. The synthesis of 2, 3-disubstituted-1, 3-thiazolidin-4-ones was carried out by two methods in different reaction conditions. In first case the condensation of corresponding amine, aldehyde and mercaptoacetic acid has been performed in presence of DCC in medium of anhydrous tetrahydrofuran. MAOS was approved for obtaining of some 4-thiazolidones derivatives that couldn’t be synthesized by the above mentioned methods. Microwave syntheses were performed in ethanol in presence of molecular sieves A4. Times of reactions were shorter in compare to other two methods.

Our previous studies showed that the presence of substituent in position 5 of thiazolidone cycle is crucial for pharmacological effect. That’s why it was performed modification of 2, 3-dissubstituted-1, 3-thiazolidin-4-ones to their 5-arylidene derivatives with using Knoevenagel condensation conditions.

Since methylene group in position 5 of such compounds is less reactive then such in 2-thioxo-4-thiazolidone (rhodanine) or 2, 4-thiazolidinedione cycles, the condensation was
performed in alcohol solution with excess of potassium tert-butylate as catalyst.

More than 50 different compounds were synthesized by means of different synthetic approaches. Structures of obtained compounds were validated by $^1$H NMR and elemental analyses. Studies of anticancer and antiviral activities were performed. 4-[2-(4-Methoxyphenyl)-4-oxo-1,3-thiazolan-3-yl]benzoic acid and ethyl 4-[2-(4-bromophenyl)-4-oxo-1,3-thiazolan-3-ylmethylcarboxamido]benzoate posses specific influence on renal cancer and they are perspective candidates for further optimizations; 2,3-di-(4-chlorophenyl)-5-[4-nitrophynylmethylidene]-1,3-thiazolan-4-one possess a moderate antiviral activities against Cowpox strains. Pharmacological screening for several compounds is in progress.
Synthesis and anticancer activity of novel non-condensed 4-thiazolidinones with benzothiazole and benzothiazol-2-one moieties

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Using modern technologies like virtual and high-throughput screening, combinatorial chemistry, and molecular modeling, it was established, that 4-thiazolidinones possess a high affinity to the PPAR-receptors family. Advanced studies of PPARγ-receptors developed the idea, that PPARγ agonists, including thiazolidinediones, have perspective in treatment of some cancer types. Moreover, binding inhibitors of antiapoptic proteins Bel-XL and BH3, which promote normalization of natural cell death, and inhibitors of TNF-α binding to TNFRe-1, as well as inhibitors of translation initiation, which cause cell cycle arrest in G1 phase via partial depletion of intercellular Ca²⁺ stores, were identified among 4-thiazolidinones.

The main goal of our research was synthesis of benzothiazole and benzothiazol-2-one containing 4-thiazolidinones as perspective anticancer molecules.

Synthesis of rhodanine derivatives 1 is based on reaction between thiocarbonyl-bis-thioglycolic acid and benzothiazol-2-yl-hydrazine or (2-oxo-benzothiazol-3-yl)-acetic acid hydrazide in ethanol-water medium. The condensation of 1 with aromatic aldehydes or isatin following conditions of Knoevenagel condensation yields the group of 5-ylidenederivatives 2 and 3. Also intermediates 4 were synthesized by reaction of starting substances 1 with triethylorthoformate acetic anhydride medium and consequently were used for obtaining enamines 5.

![Chemical structures](image)

The compounds structures were elucidated by ¹H, NMR and mass-spectra analysis.

Sixteen of synthesized compounds were tested and most of them displayed antitumor activity on leukemia, melanoma, lung, colon, CNS, ovarian, renal, prostate and breast cancers cell lines. The most efficient antitumor agent – Les-3166 was found to be active with average lgGl50 and lgTGl values: -5.38 and -4.45 respectively and exhibited all cancer cell lines in the NCI60 human tumor cell line anticancer drug screen (Shoemaker 2006). One should note, that mentioned compound showed no toxicity in Nontumored Animal Toxicity Assays.

Investigation of Polyethylene Terephthalate Packaging Material on Substances Migrating to Pharmaceutical Formulations

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For the last 10 years are widely used packed materials based on polyethylene terephthalate (PET) which can be used without the off-cuts. Bottles from PET are used for packing of all types of liquids. Packing samples from PET were tested in accordance with the common sanitary-hygienic requirements to packaging for soft and liquid products and pharmaceuticals.

Operating in Ukraine and in the EU sanitary norms does not give recommendations on sanitary or chemical estimation of polyethylene terephthalate in utensils contacting food or pharmaceuticals.

Aim of our investigation was establishing of nature and amount of compounds migrating from PET packaging to stored products and pharmaceutical formulations. These data in future will give occasion to assessment of polyethylene terephthalate safety using as packaging material and creation appropriate norms and regulations.

The program of our researches of PAT included the followings points:
1. Study of physical and chemical-technological properties of polymer:
   - identification of wares material;
   - assessment of wares thermo stability;
   - organoleptic assessment of wares material;
   - assessment of purity and chemical neutrality of wares material.
2. Sanitary-hygienic and toxicological study of packaging material composition on requirements to such parameters:
   - absence of highly toxic substances;
   - absence of cumulating and specific action on human organism (carcinogenic, mutagenic, allergenic);
   - chemical stability and absence of interaction with stored product.

From PET-containers in food, drinks, and pharmaceuticals can migrate such controlled chemicals compounds: formaldehyde, acetaldehyde, methanol, dimethyl terephthalate, ethylene glycol, and also cations of zinc, lead, arsenic compounds and like other.

For investigation of migration of listed compounds from PET bottles were used such modelling environments which imitate food products and pharmaceuticals: distilled water, 2 % solution of lemon acid, 20 % and 40 % solutions of ethanol, 10 % solution of sodium carbonate, glycerine, vaseline oil, dillseed oil, sunflower oil, 10 % solution of sodium chloride, and 40 % solution of glucose. 0.5 L of each modelling solutions were flooded in freshly-manufactured PET-bottles on 10 days at a temperature 20 °C. After infusing in all modelling liquids were determined concentration of listed substances by gas chromatography and atomic absorption spectroscopy.

Our investigation shown that aldehydes migrates from PET bottles to modelling liquids in amounts 0.007–0.019 mg/l – for formaldehyde and 0.036–0.108 mg/l – for acetaldehyde. Aldehydes migrate better to alcohols and natural oils. The same behaviour demonstrates ethers of phthalates. But heavy metals vice-versa better migrate to acidic and alkaline solutions.
Pharmaceutical Care Of Patients with Chronic Diseases

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Implementation of pharmaceutical care (PhC), as a modern part of treatment process is one of the ways in improvement of pharmacotherapy effectiveness and quality in chronic ill patients. Patients with chronic diseases such as hypertension, bronchial asthma, and diabetes mellitus have to take numerous medications. This leads to development of a large number of drug-related problems (DRP); requiring professional solving through PhC. Often among the causes of DRP is lack of patient’s knowledge about the disease and its management.

The study aimed to evaluate the influence of knowledge of patients with type 2 diabetes mellitus (T2DM) about the disease and its management on the occurrence of DRP. The investigation was performed in out-patient setting of Lviv city clinical hospital No4. A standardized questionnaire was developed, questions of which were used as an algorithm for obtaining of necessary information in evaluation of patients’ knowledge. All included to the study patients (n=100) were casually divided in two equivalent groups: group A (n=50), age 644±8.93, T2DM duration 9.02±6.74; received PhC during the interview and group B (n=50), age 616±8.89, T2DM duration 697±5.63, who filled in questionnaires without clinical pharmacist. Patients were inquired once.

It was assessed that the main factors in development of DRP in out-patients with T2DM were: lack of knowledge about T2DM (in 2/3 of questioned patients); violation of non-medical measures (diet – more than 20%; regular physical activity in 86% and 54% in both groups); irregular self-monitoring (blood glucose monitoring – more than 20%; body weight – almost 50% in both groups; foot assessment – 30% and 80% in both groups; blood pressure – almost 50% in both groups); non-rational use of hypoglycaemic medications (lack of knowledge about these medications was reported in 60% of cases; more than 10% of questioned patients didn’t know the name of used medications; almost half of patients used prescribed medicines incorrectly); 1/3 of patients were non-compliant.

Analysis of non-compliance episodes revealed that missing of the dose depends upon the type of prescribed hypoglycaemic drug. In contrast with patients using oral medications those using insulin never missed the dose (P<0.01; χ2-distribution). Among patients using oral hypoglycaemic drugs was estimated connection between active substance and frequency of non-compliance (P<0.01; χ2-distribution).

All mentioned factors allowed identifying the type and assessing the number of DRP in both groups. A total number of estimated DRP was 79 and 129, respectively in group A and group B. Approximately 60% of DRP were existing, connected with patient (in 70 and 120 cases, respectively).

Thus, lack of knowledge about T2DM and its management as well as reluctance to follow physicians’ and pharmacists’ recommendation confirmed an importance and necessity of PhC for out-patients with T2DM.
Test-System for Immunochromatographic Detection of ALFA–AMANITIN in Biological Liquids of Poisoned Persons

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Deadly poisonous mushroom Amanita phalloides (death cap, toadstool) produces two classes of similar in structure toxic bicyclic hepta- and octa-peptides (phallotoxins and amanitins respectively). Both oligopeptides have hapten properties.

Octapeptides are the most toxic component of A. phalloides toxin (venom). Amanitins irreversible conjugate with hepatocytes and destroy all protective system of the organism. All deaths of poisoned by mushrooms persons are attributed with alfa-amanitin toxic action. Presence of this oligopeptide in blood or urine indicates the dangerous intoxication by A. phalloides mushroom. The first day of illness is decisive for urgent treatment administration.

We studied mechanism of immune response of poisoned human organism and proposed test-system for detection of alfa-amanitine in biological liquids of poisoned persons. The immuno-chromatographic test is based on reaction of complementary linkage of alfa-amanitin with specific antibodies of rats that immobilised on surface of latex micro particles. Formed sandwich-kind complex has specific colour.

Proposed immuno-chromatographic system is a lateral flow test strip containing sample pad, conjugate pad, membrane, and absorption pad, assembled in plastic housing. Volume of investigated sample is ~ 0.2 ml (4-5 drops) of blood or urine. Conjugation of globulins with antigen take place on surface of nitrocellulose membrane with two zones – linkages (which changes colour after interaction) and indication of positive reaction (changes colour after wetting by blood serum). Time of biological fluid sample analysis is average 30-40 minutes.

Previously we studied toxicodynamics of alfa-amanitin applying high-performance liquid chromatography. We investigated distribution of alfa-amanitin in blood and urine of poisoned persons at time of aggressive clinical behaviour.

Maximal concentration of alfa-amanitin in blood is 102.7 ng/ml (5 cases) through 8-12 hours after mushroom ingestion with food. During the second day of intoxication alfa-amanitin concentration changed from 78.2–64.9 ng/ml to 23.5–17.2 ng/ml. The toxin concentration in blood rapidly decreases during 48 hours after ingestion. In blood toxin may be determined not later three days.

Our previous research showed that alfa-amanitin absent in urine during the first day of intoxication. The alfa-amanitin was determined in urine in concentrations 0.9–1.4 ng/ml only at two persons through 24 hours after poisoning. Maximal concentration of alfa-amanitin in urine was observed through 36-48 hours from a moment poisoning (in concentrations of 3.5 – 8.4 ng/ml). But after 60-72 hours from the poisoning moment alfa-amanitin in urine of victims does not detects. Remind that it is the heaviest status of poisoned person. Therefore, it is necessary to detect native poison in victim’s blood or urine during 12-24 hours after ingestion, when appear only first signs of intoxication.

The developed test-system allows with confidence to detect alfa-amanitin presence in biological liquids during three day from the moment of poisoning with sensitivity 50 ng/ml. Such sensitivity is good for blood examination but not enough for urine.
Role of L-CANAVALINE and L-ARGININE in Cytoprotective Mechanisms of the Action of Opioid Peptide – DALARGIN


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Background: Endogenous opioid peptides localized in gastric mucosa, manifest their cytoprotective action in response to stress factors (Gyires, Mullner, Ronai, 2001). But research on the role of NO-synthase system in the mechanisms of cytoprotection under the action of synthetic leu-enkephalin cannot be considered exhausting. For this reason, purpose of the work was to study processes of gastro protection affected by the injection of NO-synthase precursor L-arginine or iNOS blockage simultaneous with dalargin action.

Material and methods: Investigation was conducted on 38 Wistar line rats in acute experiment. Ulcerogenic lesions of GM were modeled by injecting adrenaline in the dose of 2.0 mg/kg. Changes of lipoperoxidation processes in GM were determined by the contents of MDA and NO, and by the activity of antioxidant protection enzymes –SOD and catalase. Dalargin was injected in the dose of 0.1 mg/kg, L-arginine – 300 mg/kg, L-canavaline; selective blocker of iNOS (100 mg/kg) evaluation of structural lesions in GM was conducted by the method of planimetry with the use of scoring system and histological investigations.

Results: Dalargin action, at the background of ulcerogenic impact of adrenaline, caused decrease in the area of GM lesions by 32% (p<0.05) and improvement of its qualitative characteristics – by 50%. At that, alterations of MDA and NO contents were insignificant (by 10-12 %), SOD activity enhanced by 48% (p<0.05), and catalase activity reduced by 70% (p<0.05). Action of dalargin with a simultaneous blockage of iNOS by a selective blocker L-canavaline resulted in a pronounced decrease of the area of ulcerogenic lesions – to 2-3% (p<0.01) and positive 90% changes in the character of lesions. MDA content decreased by 15%, SOD activity reduced by 40%, catalase activity – by 43%, and NO content decreased by 33% (p<0.05). Due to the effect of injected substrate for NO-synthase – L-arginine and dalargin – versus the action of dalargin, the area of structural lesions in GM decreased by 18%, and character of the disorders was evaluated 20 scores. At that, MDA content increased (by 41%), NO concentration decreased (by 57%), activity of antioxidant defense enzymes diminished, namely, SOD activity – by 57% (p<0.05) and catalase activity – by 64% (p<0.05).

Summary: Both L-arginine injection and blockage of iNOS with a selective blocker L-canavaline simultaneous with dalargin effect enhanced cytoprotection processes. But changes of lipoperoxidation processes, NO content and activity of antioxidant defense enzymes are evidence of the different roles of eNOS and iNOS in the protective processes of gastric mucosa.

Adaptation Changes of Antioxidant Defense System and System of Nitric Oxide in Central Nervous System in Rats under Complex Regional Pain Syndrome Development

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Complex regional pain syndrome is one of the most common postoperative and post-traumatic complications and nowadays has no adequate pathogenic remedies for treatment. Development of complex regional pain syndrome is based on cascade of combined changes in which shifts in antioxidant defense system and nitric oxide signal system play important role.

Materials and methods
Rat ischiadic nerve axonotomy was used as an experimental model of acute regional pain syndrome development. The study involved 10 Winstar male rats and 5 intact male rats formed control group. Neurological status, parameters of prooxidant-antioxidant metabolism and NO metabolites were estimated in brain tissue homogenate, spinal cord homogenate, erythrocyte hemolysate and blood plasma. The samples were taken on the 3d day after the onset of pathological process.

Results
Plasma MDA levels in rats with causalgia averaged 41.87 μmoll/g protein, in the group of intact animals–30.71 μmoll/g protein. Catalase activity in brain tissue and spinal cord homogenates increased while compared with control group. These shifts were coherent with shifts in glutathione-depended component of antioxidant system: level of reduced glutathione increased in brain and spinal cord tissue in experiment animals. NO concentration in brain tissue homogenate of experimental rats was 1.11±0.02 μmoll/g protein (control–0.93±0.02 μmoll/g protein), in spinal cord homogenate: 3.86±0.05 μmoll/g protein in intact animals, 8.25±0.05 μmoll/g protein in rats with complex regional pain syndrome.

Conclusion
Development of complex regional pain syndrome is accompanied by significant changes in prooxidative-antioxidative homeostasis in brain and spinal cord tissues. Components of nitric oxide system play important role in adaptation metabolic shift onset. At the same time these changes are very similar both at the segmental level (spinal cord) and in brain tissue. This peculiarity testifies integrative response of central nervous system metabolism during the adaptation to intensive pain signaling.
Neuroprotective Effect of Cardiac Glycosides on the Model of Focal Cerebral Ischemia


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The large number of failures of neuroprotective (NP) drug trials suggests that most of the current therapeutic targets can not give the appropriate and early defence during the ischemic damage of brain tissue. Recently, much attention has been focused on the maintenance of cell ionic balance at the early stage of ischemic cascade activation. Cardiac glycosides can provide important NP effect of Na+/K+-ATPase inhibition but nowadays haven’t been used for the NP purpose and their local and systemic therapeutic influence haven’t been studied.

Materials and methods
The study involved 15 Winstar male rats. Animals were divided into 3 groups with 5 rats in each. Temporary stroke was induced by occlusion of the right middle cerebral artery. I group included 5 untreated rats with focal brain ischemia, II group – 5 rats with focal brain ischemia which were treated with digoxine (0.75 mg/kg). At 48 h after the focal ischemia blood and brain tissue samples were obtained. Control group was formed from 5 intact male rats. Parameters of prooxidant-antioxidant metabolism status and nitric oxide metabolites were estimated in erythrocyte hemolysate, blood plasma and brain tissue homogenate. Results were analysed and differences tested statistically by Student’s t-test.

Results
In I group activities of lipid peroxidation were higher in the comparison with the treated rats as well as glutathione cycle enzymes activity and GSH content reduced. The highest concentration NOx-compounds was estimated in the I group, whereas L-arginine concentration in untreated animals was reduced in plasma and increased in erythrocyte hemolysate in the comparison with the treated and intact rats.

Conclusion
Administration of digoxine at the early onset of the animal model of ischemic stroke has inhibitive influence on the prooxidative cascade development and nitric oxide hyperproduction both at local and systemic levels. Consequently, ATP-preserving effect of Na+/K+-ATPase blocking can be a useful target for the therapeutic inhibition ischemic cascade damage of brain tissue, which probably can prolong cell surviving time period before the revascularisation and reduce reperfusion oxidative damage.
Institute of Cell Biology, National Academy of Sciences of Ukraine
Studying of interaction between CoA Synthase and scaffold P-bodies protein RCD-8

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CoA Synthase (CoASy) was recently cloned in our laboratory as an enzyme which mediates last two steps of CoA biosynthesis. It is a 62kDa protein which has – N-terminal regulatory domain and two enzymatic domains which is responsible for its activities: 4-phospho-pantethein adenylyltransferase and dephospho-CoA kinase. It was shown that N-terminal domain is responsible for CoASy interaction with the mitochondria outer membrane. Since intracellular level of CoA is not steady and is regulated by various extracellular stimuli we proposed that activity of CoASy may be regulated through it interaction with different regulatory proteins. To address this we performed immunoprecipitation experiments for pulling down CoASy protein complexes from HEK-293 cells, which were resolved by SDS-PAGE, silver stained and specific bands were analyzed by Matrix-assisted laser desorption/ionization (MALDI). By this we were able to identify RCD-8 as a novel binding partner of CoASy.

RCD-8 is scaffold protein of processing bodies (PB). These cellular structures are involved in the cytoplasmic processing of mRNAs and have critical roles in mRNA degradation and post-transcriptional gene silencing. Previously we had shown that interaction between CoASy and RCD-8 is changeable and depend on various conditions. Thus, osmotic and oxidative stresses strongly reduce formation of such complexes. We conducted cell fractionation experiments to examine RCD-8 distribution. We showed that it is localize on mitochondria during normal condition, and after stress treatment it was distributed to the cytoplasm. Taking into consideration, that RCD-8 is necessary for the formation of processing bodies, we proposed that CoASy serve as repository of RCD-8 on mitochondria and in this way can regulate mRNA life-span.

The aim of current work is studying of interaction between RCD-8 and CoASy. First, we want to map interaction site/domain on RCD-8 surface. We suggested existence a steric competition between CoASy and other proteins of processing bodies for docking with RCD-8. According to this we generated stable cell lines over expressing different parts of RCD-8. We showed that CoASy interact with RCD-8 in the same site, which is responsible for localisation of this protein in PB.

Next we want to determine distribution of these complexes in different cell structures (PB, stress granules, mitochondria) in cells upon different stress conditions. For this purpose fragment encoding N-terminal part of RCD-8 was amplified and cloned in the expressing bacterial vectors pET24a and pET42a. Purified proteins served as antigens for mice immunization and generating polyclonal antibodies against RCD-8. In future antibodies will be used in experiments for CoASy/RCD-8 co-immunoprecipitation assays and confocal microscopy.

Studying of interaction between CoASy and RCD-8 will shed light on link between CoA biosynthesis and regulation of gene expression through mRNA degradation.
GLOBAL ALLIANCE FOR LIFE SCIENCES

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INTERGENE PRE-TERM BIRTH CENTRAL AND EASTERN EUROPEAN SCIENTIFIC NETWORK MEETING

PECS, HUNGARY, OCTOBER 3, 2008
MORNING SESSION

08:00 – 08:30 Review “Pre-meeting” Home Work (Calvin J. Hobel, MD)
   1) WHO Handbook for Guideline Development
   2) Key papers on the Genetics of Pre-term Birth (PTB) and Intra-Uterine Growth Restriction (IUGR).
   3) Characteristics of the Eastern European Partners (Questionnaire)

08:30 – 09:00 Overview of the Genetic Association Studies (Ram Menon, Ph.D)

09:00 – 09:30 Introduction of the INTERGENE RECOOP Central and Eastern European Local or National Networks
   Czech Republic
   Hungary
   Slovakia
   Romania
   Ukraine

09:30 – 10:00 Questions from Eastern and Central Europe Members (ECEM) During Coffee Break

10:00 - 10:30 Introduction to the Development of Guidelines (Hobel, Menon & Merialdi)
   1) Rationale
   2) Hypotheses
   3) Objectives

10:30 - 12:00 Study Design (Hobel, Menon & Merialdi)
   1) Proposed Study Design
      a) Subject Recruitment
      b) Maternal, Paternal & Infant Phenotype
      c) Sample Collection
      d) Sample Processing
      e) Sample Storage
      f) Study Facility Requirements
   2) Development of Data Base (Vari)

12:00 – 12:30 Questions from ECEM’s

12:30 – 13:30 LUNCH
AFTERNOON SESSION

13:30 – 15:30 Project & Grant Management (Vari, Hobel, Menon & Merialdi)

1) Project Management Team #1
2) Development of Study Teams #2
3) Grant Writing Team #3
4) Executive Committee

15:30 – 16:00 Questions and Coffee Break with ECEM’s

16:00 – 16:30 Discussion and Recommendations for forming

Teams # 1, #2, #3 and

Executive Committee

16:30 – 17:00 Next Steps – Group Discussion with Dr. Sandor Vari reference EU Grant Application Process

18:00 Bus transportation to Zagreb for those will attend the Bridges in Life Sciences Second Annual Scientific Meeting of the Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium to be held on October 4, 2008 at Hotel International Zagreb (Miramarska 24, Zagreb 10000, Croatia, T: + 385 1 610 8800 F: + 385 1 610 8800 http://www.hotel-international.hr ).

19:00 Dinner
Guidelines for submission of abstract for the Bridges in Life Sciences Second Annual Review Meeting

Submission Deadline: July 28, 2008

The RECOOP HST Scientific Advisory Board invites abstracts for oral presentation on October 4, 2008 in Zagreb, Croatia and publication in the Bridges in Life Sciences Second Annual Review of the Regional Cooperation for Health, Science and Technology Consortium (Volume 2), slated for publication in September 2008. The abstracts typically evaluated by two or more reviewers are members of the RECOOP HST Scientific Advisory Board. These abstracts cover subjects related to

Proteomics/Genomics in Basic and Clinical Research
Pharmaceutical Research
Translational Research
Clinical Research
Human Development

From each member organizations the Scientific Advisory Board will select maximum three abstracts for oral presentation.

General Guidelines

Please send one copy of the abstract via e-mail in word format (maximum 450 words) to Sandor G. Vari, MD General Manager (vari@cshs.org) cc. to Calvin Hobel, MD, Ph.D (HobelC@cshs.org) Chairman of the Advisory Board and Kristin Martinez (Kristin.Martinez@cshs.org) Program Coordinator of the Research Networking of the RECOOP HST Consortium. Receipt of abstracts will be acknowledged by Sandor G. Vari, MD.

Abstracts requirements

Text, explanatory notes, and references should be typed single spaced on A4 (210 -297 mm) format paper. Margins: left 20 mm and right 15 mm, top – bottom 15 mm. Use Times Roman; type size 11 points.

Title should be bold but not italics, Times Roman; type size 12 points. Could not exceed 24 words, author's information includes complete mailing and E-mail addresses, and a short statement noting professional title and institutional affiliation.

Tables and figures should be no larger than 6 cm high and 9 cm wide. All words such as axis designations and line labels should be bold but not italics; in Times Roman; type size between 8 and 10 points, and one figure and one table per abstract. If you are sending tables and figures it should be incorporated into the text and should not take more then 30% of the A4 page.

Notes like sponsors are allowed and maximum three references. Follow Style B of Manual of Style, 14th ed., University of Chicago Press, 1993. Number notes sequentially and list at end of text. For text citations, use author/date style (Smith 1980). Include a page number for quotations. List full citations, alphabetically by author, in the references; include year of publication, title, publication, and publisher or volume, issue, and page numbers. For example: