Abstracts
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Oral Presentations

Cancer Research & Prevention

Anticancer potentials of a novel Streptomyces antibiotic landomycin E

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Development of effective anticancer drugs with minimal side effects and capability of overcoming drug resistance in tumor cells remain the crucial aims in cancer treatment. Landomycin E (LE) is a novel antibiotic of angucycline family produced by *Streptomyces globisporus* 1912 strain growing in soybean medium. It was found that several tumor cell lines were sensitive to LE pro-apoptotic action that was mediated by the reactive oxygen species (ROS). In this study, we addressed the molecular mechanisms of LE action towards human Jurkat T cell leukemia line *in vitro* and its antitumor action towards murine NK/Ly lymphoma *in vivo*. Phosphatidyl serine externalization and intensive membrane blebbing (early apoptotic events) were observed as early as 1 h after LE (2 μg/ml) treatment of Jurkat T cells, while nucleus fragmentation (late apoptotic events revealed by DAPI staining) appeared only in 12 h after the start of LE (2 μg/ml) action. N-acetylcysteine (NAC) was used to block ROS action and reveal ROS-dependent signaling pathways induced by LE. It was shown that LE (2 μg/ml, 24 h) led to splitting of anti-apoptotic PARP-1 and DFF45 proteins involved in DNA reparation. Cleavage of these proteins is mediated by active caspase-7 and caspase-3 whose levels were increased under LE action. Pre-treatment of target cells with NAC (5 mM, 30 min) completely inhibited activation of the effector caspases-3,-6,-7 and PARP-1/DFF45 cleavage, which indicates that these processes are ROS-dependent. Thus, mitochondria can play an important role at late stages of LE-induced apoptosis. This was also confirmed by a release of cytochrome c from mitochondria to cytoplasm. However, there were no significant changes in the levels of pro-apoptotic protein Bax and anti-apoptotic protein Bcl-xL involved in mitochondria-induced apoptosis. NAC pre-treatment also had no effect on their levels in target cells. To check if ROS are involved in initiator stages of apoptosis induced by LE, Western-blot analysis was performed with antibodies to initiator caspases-2,-8,-9. It was found that LE (2 μg/ml, 24 h) induced cleavage of all tested initiator caspases, however, caspase-8 was the only enzyme whose activation was not blocked by NAC. We suggest that caspase-8 that is involved in receptor-mediated apoptosis, can play a crucial role in apoptosis induced by LE in cancer cells. Thus, LE may realize its cytotoxic effects via receptor-mediated apoptosis signaling pathway. We found in the *in vivo* studies that LE (1 mg per kg) significantly increased a lifespan of NK/Ly lymphoma-bearing mice from 14 days after tumor inoculation, in control group of animals, to 21 days in the LE-treated animals, comparing with 30 days under doxorubicin treatment. This correlated with an increase in number of dead tumor cells and decrease in number of living lymphoma cells. In conclusion, we demonstrated that landomycin E is a potent anticancer drug, which is effective, both *in vitro* and *in vivo*, and caspase-8 may be a principal molecular target during its action towards tumor cells.

Role of surface sialilation in the clearance of apoptotic cells

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In our previous works we proved that exposure of subterminal galactose and mannose residues of glycocalyx glycoproteins (GP) via their desialilation is the intrinsic feature of apoptotic cells (Bilyy, et al., 2003, 2007; 2008) and can be effectively used for detection of apoptotic cells (Bilyy, 2009). At the same time, exposure of desialated proteins on the surface is one of the markers for phagocytes to eliminate the cell. Failure in the elimination of apoptotic cells result in the leakage of apoptotic cells content to intercellular milieu (during it conversion to secondary necrotic cells) and accompanying inflammation. Chronic deficiency of cell clearance leads to the immune response against self antigens and development of autoimmune disorders. In vitro, several signals have been identified as mediators of apoptotic cell recognition and clearance. However, the distinct mechanisms of apoptotic cell clearance are not fully deciphered to date. The aim of current work was to study sialidase activity during apoptosis and influence this activity with the aim to facilitate apoptotic cell clearance. In the current work we studied sialidase activity, responsible for such desialilation. We have developed know how assay for fluorescent vital study of enzymatic sialidase activity and proved the increase of sialidase activity on the surface of apoptotic cells, as early as 2 h after the onset of apoptosis. Sialidase activity was attributable to the surface of apoptotic (Anexin V positive and PI negative) cells and was blocked by neuraminidase inhibitor DANA, as was detected by confocal imaging. To localize the sialidase activity in the cell we performed cell fractionation of viable and apoptotic cells and detected sialidase activity by spectrofluorometric assay for 4-MUNA cleavage. Sialidase activity was found to be increase at apoptosis in the plasma membrane fraction. By isolation of plasma membrane fractions and by subsequent separation of peripheral and integral membrane proteins we localized neuraminidase activity as characteristic feature of integral proteins' fraction of plasma membrane. By treatment of cellular lysates with caspases 3, 7 and 8 and by inducing apoptosis in the presence of pan-caspase inhibitor as well as of specific inhibitors of caspase 3, 6, 8 and 9 we have demonstrated caspase-dependent mechanism for sialidase activation at apoptosis for both receptor-mediated (FasL) apoptosis induction pathway or mitochondrial pathway (induced by UV-B irradiation). By artificial desialilation of cells we were able to significantly increase their clearance by human macrophages. While the induction of apoptosis in the presence of sialidase inhibitor led to the inefficient clearance of apoptotic cells lacking desialilated glycotops on their surface. Thus we claim that sialidase activity is increased in the plasma membranes of apoptotic cells, its activation is caspase-dependent and its action lead to the formation of desialilated glycotops, which are important markers for the clearance of apoptotic cell by macrophages.

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CHI3L1 stimulates proliferation and migration of glioma cells

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Human genome encodes six proteins of family 18 glycosyl hydrolases, two active chitinases and four chitinase-like lectins (chi-lectins) lacking catalytic activity. CHI3L1 is the most investigated protein among human chi-lectins. It has molecular mass of about 40 kDa and is N-glycosylated at Asn^{60} (two residues of β(1,4)-N-acetyl-D-glucosamine, NAG). N-terminal amino acids of CHI3L1 mature form are tyrosine, lysine and leucine (Y, K, L) that gives rise to its alternative name "YKL-40". CHI3L1 protein sequence has a cluster of basic residues (GRRDKQH, Gly^{143}–Arg–Arg–Asp–Lys–Gln–His^{149}), similar to other heparin-binding proteins, while there is no conclusive evidence of its binding to heparin. Most of growth factors contain heparin-binding sites, which as a rule serve as a connector between two proteins, for instance, in case of FGF and FGFR. Crystallization studies failed to reveal the CHI3L1 binding of sulfated oligosaccharide units within its ligand-binding groove. However, it was shown that CHI3L1 could modulate the activity of basic fibroblast growth factor (bFGF). Also, there are recent findings proposing that CHI3L1 acts through cell membrane receptor syndecan-1. Syndecans bear most of heparan sulfate residues on the cell surface and are involved in angiogenesis and invasion. CHI3L1 stimulates the association of syndecan with a_Vb_3 integrin, and treatment with heparinase or chondroitinase removes this effect. Recombinant His-tagged CHI3L1 protein expressed in [a] prokaryotic system was purified by affinity chromatography using Ni-NTA agarose. Native CHI3L1 was purified by affinity chromatography from conditioned medium of MG-63 human osteosarcoma cell line using heparin-sepharose. We investigated CHI3L1-induced proliferation and migration on several glioma cell lines. MTT test showed that both, recombinant and native CHI3L1 stimulate proliferation of U373 and U87 cells. Scratch test revealed CHI3L1 stimulation effect on U87 but not on U251 migration. Glioblastoma, the most common intracranial malignancy is the aggressive brain tumor with short median survival time (12 months), frequent recurrence and significant drug resistance. Through four stages of progression, tumor cells cumulate genetic abnormalities and, as a result, have aberrant signaling networks. Glioblastomas are characterized by high invasion potential and can escape anti-angiogenic therapy. *CHI3L1* is overexpressed in glioblastomas in comparison to low-grade gliomas and normal brain, and a correlation between high expression of *CHI3L1* and short survival of patients is observed. CHI3L1 serving as a migration and proliferation factor for glioma cells can play significant role in tumor spreading and recurrence.

Cardiovascular Diseases & Prevention

N-cadherin deletion results in embryos heart malformations and embryonic death

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The structural integrity of the heart is necessary for its function and is maintained by the end-to-end connection between the myocytes called the intercalated disc. Cell adhesion molecules are critically involved in these interactions, in particular cadherins, the Ca^{2+} - dependent proteins family with homophiles type of interaction. In cardomyocytes adherent junction is presented only by N-Cadherin. Classical cadherins are transmembrane proteins. Extracellularly, they mediate specific cell-cell adhesion, while intracellularly they interact with catenins and through them with actin cytoskeleton. The catenins family includes α-, β- and γ- Catenin. Cadherins and catenins are structural compounds of adherent junction. In this study we focused on the analysis of N-Cadherin and α- and β-Catenin function during heart development using conditional knock-out approach. For this, we provided morphological and immunological analyses of heart tissue after N-Cadherin, αE-Catenin and β- Catenin missing at different terms of gestation. For determination of N-cadherin function at cardiogenesis we used 10- and 5-day embryos. The morphology of embryos was studied by whole mount embryo examination at E9.5 or E10.5. Mutant embryos were delayed and exhibited delays are in development comparable with wild type embryos and severe heart malformation. Neuronal and somatic defects evident for N-cadherin constitutive knockout (Radice 1997) have not been observed suggesting normal N-cadherin function in those tissues. The heart looked ballooning, looping morphogenesis was not completed, and extraembryonic circulatory system was poorly developed in the N-cadherin CKO embryos. Analysis of embryos at term of gestations more than 10, 5 days revealed complete maceration of mutant embryo. Transverse sections through the heart region of mutant embryos and control embryos at term of gestations E 10,5 demonstrated the expressive violations of cardiogenesis after N-Cadherin ablation, namely: thinner wall of myocardium compared to control, violation of heart tissue forming (intercellular connections). N-cadherin mutant embryos at E9.5 were immunostained with N-cadherin and β-catenin antibody. N-cadherin was missing from the heart tissue; there was also no detectable β-catenin expression in heart myocardium. These data suggest that N-Catherin plays a critical role in the regulation of β-catenin expression during early cardiogenesis. It is also possible, that N-cadherin affects the distribution of β-catenin, especially during the formation of intercalated disks. In our investigation we have shown that ablation of N-cadherin in the developing heart results in severe cardiac malformations and has lethality effect. Our data suggest a critical role of N-cadherin in the regulation of early cardiogenesis. Overall, our findings have extended the understanding of the role of cell adhesion components in early heart development and in adult heart.

Twins can help to prevent atherosclerosis disease. Findings of International twin study 2009

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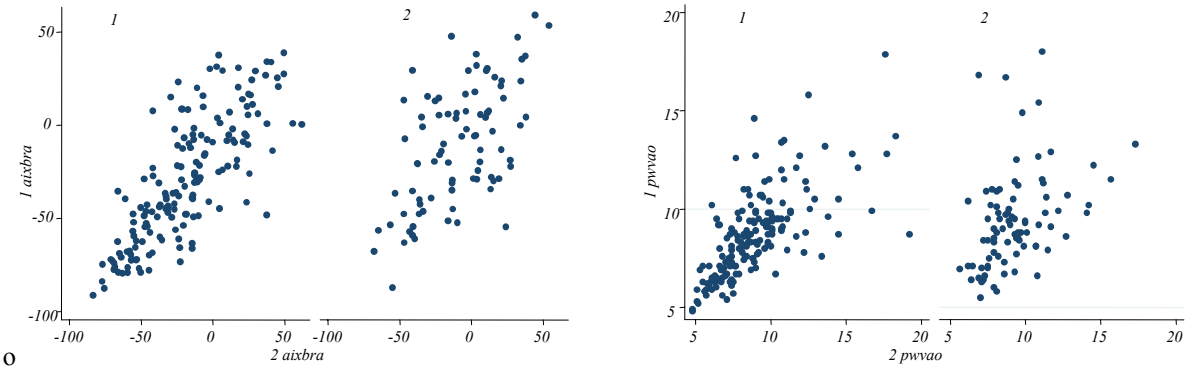
Objective: Atherosclerosis process is a pathophysiological condition in which the artery wall thickness is the result of a chronic inflammatory response in the walls of arteries which tends to be slowly progressive over decades and usually remains asymptomatic. Twin studies by comparing identical with non-identical twins produce information on the relative contribution of genes and environment, and how the two interact. **Purpose**: To estimate heritability and environmental effects on arterial stiffness using a twin sample. **Methods and Materials**: 126 Italian (66 MZ, 60 DZ), 48 American (45 MZ, 3 DZ) and 82 Hungarian (60 MZ, 22 DZ) twin pairs were included in the study as part of International twin study 2009. TensioMed Arteriograph was used to measure the arterial stiffness parameters. **Results**: Based on 512 samples (342 MZ, 170 DZ; mean age 50.9±15.2 years), mean Aixbra and PWVao indicated -16,4±32,3% and 9,008±2,4 m/s. Age-adjusted intraclass correlation of Aixbra and PWVao were 0.65 (95% CI, 0.55 to 0.72) and 0.46 (95% CI, 0.33 to 0.57) in MZ, 0.42 (95% CI, 0.24 to 0.57) and 0.28 (95% CI, 0.08 to 0.47) in DZ pairs; heritability 0.45 (95% CI, 0.12 to 0.71) and 0.42 (95% CI, 0.02 to 0.57) (Figures 1 and 2). Shared environmental effect of AIxbra and PWVao indicated 0.20 (95% CI, 0.0 to 0.49) and 0.04 (95% CI, 0.0 to 0.38), unshared environmental effects indicated 0.35 (95% CI, 0.28 to 0.45) and 0.54 (95% CI, 0.43 to 0.67) adjusted by age, respectively.



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Conclusions: Arterial stiffness parameters are moderately heritable. Due to the long-lasting process of atherosclerosis it can be detected in early stage by pulse wave analysis in order to be treated by appropriate therapy to prevent consequences of atherosclerosis like stroke, heart attack or peripheral vascular disease.

Mother and Child Health
Reproductive Health

Mathematical model enabling to differentiate between various modes of perinatal PCB exposure

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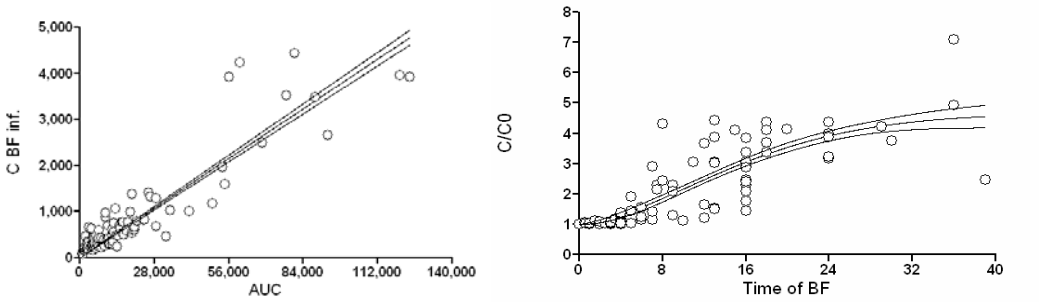
Introduction: The adverse effects of polychlorinated biphenyls (PCBs) on the developing organism may result from exposure prior to conception (either parent), during prenatal development or postnatally to the time of sexual maturation. The perinatal exposure consists of prenatal exposure due to passage of PCB through placenta and postnatal exposure through its uptake by breast or formula feeding. **Materials**: Blood samples were collected from newborns during years 2002-2004 at delivery from umbilical cord and at 6, 16 and 45 months of their age by venepuncture. The lipid adjusted serum concentration of the most abundant PCB congener #153 was used for modelling of PCB exposure. Methods: The time course of the measured PCB 153 concentration after birth of the child C(t) was described by the relationship $C(t) = C0 + \Delta C(t)$ where t is time, C0 is PCB 153 concentration (ng/g serum lipids) in cord blood serum and $\Delta C(t)$ is the concentration increase due to breast feeding until time tbf and normal food intake after weaning. As a best fit a linear one compartment model has been chosen on the basis of Akaike's Information Criterion with unit amplification. **Results**: The correlation between Cbf, inf and AUC was very high. The usefulness of the model can be demonstrated by computing for each child its PCB 153 serum concentration adjusted to cord serum PCB 153 concentration (C/C0) at the time of weaning.



Conclusions: Kinetics of PCB 153 in the serum of 103 infants was approximated using a „system model“ representing a novel approach compared to compartmental or physiologically based pharmacokinetic models. Duration of breast feeding and mean time were found to be correlated with PCB 153 concentrations in steady-state without normal food intake (Cbf, inf) and without breastfeeding (Cf, inf) and with both parameters adjusted to concentration at delivery (Cbf, inf /C0 and Cf, inf/C0).

The usefulness of the model was demonstrated by predicting of PCB 153 serum concentration at weaning adjusted to cord serum PCB 153 concentration (C/C0) from breast feeding duration. For approximation a modified Weibull function was used.

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Kynurenic acid concentration in the perinatal period

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Background and aim: The essential amino acid tryptophan is metabolized in the brain by two major pathways, through either the kynurenine or methoxyindole pathways. Kynurenic acid (KYNA), synthesized from L-kynurenine in reactions mediated by kynurenine aminotransferases in astrocytes, glial cells and neurons, is a metabolite of the kynurenine pathway. KYNA is one of the few known endogenous NMDA (N-methyl-D-aspartate) receptor antagonists with neuroprotective properties. Our aim was to determine the concentration of KYNA in the umbilical vein and arteries of term neonates. **Methods**: Umbilical venous and arterial blood samples were taken from twenty-four healthy term infants immediately after birth as well as by peripheral venipuncture on the fourth postnatal day (D4). Mean (+/-SD) birth weight and gestational age were 3445.0 (+/-499.3) g and 39.0 (+/-0.9) weeks, respectively. Serum concentrations of KYNA were measured by high performance liquid chromatography. **Results**: KYNA level in the umbilical vein was significantly ($p=0.022$) higher than in the umbilical arteries and in D4 venous blood, whereas the arterial and D4 samples did not differ significantly. There was no significant difference for KYNA neither in the umbilical arterial and venous samples nor in D4 samples between newborns delivered spontaneously and by cesaerean section. The KYNA concentration in the umbilical vein was significantly ($p=0.044$) higher in female newborns than in males. **Conclusions**: The higher KYNA concentration in the umbilical vein suggests that in normal term newborns KYNA level is not elevated during delivery and on the fourth postnatal day. The increased umbilical venous KYNA level can be of placental or maternal origin. Our findings may help to understand the neuroprotective and neurotoxic mechanisms in the infant brain during the perinatal period.

Inflammatory & Immune Response

Melatonin – novel cytoprotective and healing modulator of upper gastrointestinal tract by angiogenesis impact

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Recent data indicated that risk for premalignant Barrett's esophagus (BE) increases by 30 to 50% per decade of life in patients with gastroesophageal reflux disease (GERD). Modern day data revealed that widespread use of prolonged acid suppression is associated with side effects, as well known dismotility, dysbiosis in the upper gastrointestinal (GI) tract, hypergastrinemia etc. It is well known that melatonin (MT) possesses GI resistance to luminal damaging agents, improved motor dysfunction and reveled antineoplastic effect. Previously we have shown that disturbances of oral and esophageal mucosa (OM and EM, respectively) cytoprotection were followed by disorders in endogenous defence signalling pathways for inflammation, re-epithelialization, and formation of granulation tissue, interaction between the various cells and matrix, and apoptic cell recognition. However, the role of MT on angiogenesis signalling on mechanisms of maintaining OM and EM integrity and healing has not yet demonstrated. Materials: several series were used on rats after sialoadenectomy (SAE) *vs* rats with intact salivary glands (ISG) without/with MT (20 mg/kg/ip) treatment by water-immersion restraint stress (WRS) by Takagi, 1964; the acute injury and healing process of OM and EM were monitored at various time points: after WRS induction immediately and after 24 and 48 hr of WRS induction; estimation of injury, inflammation and hyperplasia *via* histological score index (HSI); VEGF, EGF, IL-1β, TNFα by ELISA. Results: WRS-induced non-erosive OM lesions and stress-associated esophagitis, constantly increased HSI in 150% in compare to MT treated rats; in SAE rats HIS was 2,5-folds time more than in ISG rats. MT decreased expression of VEGF and inflammatory mediators including IL-1β, TNFα, and increased EGF synthesis. At 24 hr&48 hr after WRS EM healing was delayed and defective response of EM basal layer indicated *vs* MT-treated ISG rats. In SAE rats pro-inflammatory interleukins were increased twice than in ISG rats; was shown MT treatment accompanied decreased synthesis of IL-1β, TNFα during hyposalivation and accelerated OM and EM healing *vs* SAE rats without MT. We concluded that MT increased resistance of epithelial barrier of upper GI tract and accelerated the healing of OM and EM lesions via increased expression of EGF and decreased of VEGF and inflammatory mediators, suggesting that it exhibit mucosal repair through the activation of angiogenesis signaling to induce proliferation activity and accompanied anti-inflammatory effects.

C60 fullerenes modify protein tyrosine phosphorylation patterns in normal and transformed T cells treated with apoptosis-inducing agents

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In recent years there has been increasing interest in the possible application of carbon nanospheres, known as fullerenes, in biomedicine. C_{60} fullerenes are nanodimensional molecules, which due to their small size and hydrophobicity can incorporate into cell membranes and specifically interact with cellular proteins, thereby exerting a variety of biological effects at relatively low concentrations. Nevertheless, effects of C_{60} fullerenes on normal and transformed cells as well as mechanisms of C_{60} fullerenes interaction with the cell structural components have been poorly studied. The aim of the present work was to study the effects of pristine C_{60} fullerenes on viability of normal and transformed T-lymphocytes treated with different apoptosis-inducing agents. Since phosphorylation of proteins on tyrosine residues plays a crucial role in the control of cellular signaling networks, the phosphotyrosine status of these cells was also investigated. Primary cultures of rat thymocytes and human T lymphoma Jurkat cells were used in the experiments. Cell viability was assessed by the MTT reduction assay. Protein phosphotyrosine patterns were analyzed by Western-blot analysis using monoclonal anti-phosphotyrosine antibodies. Colloidal solutions of C_{60} fullerenes were prepared in Technical University of Ilmenau (Germany). Preincubation of thymocytes with fullerenes (10^{-5}M) for 1 h significantly reduced cytotoxic effects of such agents as staurosporine (0.01 μM), cytosine arabinoside (10 μM) and hydrogen peroxide (50 μM). After 24 h incubation viability of thymocytes was increased by 25±9%, 16±7% and 19±6%, respectively. By contrast, C_{60} fullerenes did not protect human Jurkat T lymphoma cells from death induced by these agents. Next, investigation of phosphotyrosine status of T cells demonstrated, that the main immunoreactive bands detected with anti-phosphotyrosine antibodies correspond to proteins with M_r 17, 30, 50, 72 and 100 kDa in thymocytes and 17, 26, 30, 50, 55, 70, 90 kDa in Jurkat cells. In both cell types the intensity of phosphorylation of almost all phosphotyrosine-containing proteins was decreased after incubation with all the apoptosis-inducing agents studied. The lowest level of protein tyrosine phosphorylation was detected after 12 and 24 h incubation with investigated agents in both cell types. The effects of these cell death inducers on protein tyrosine phosphorylation were modifided in the presence of C_{60} fullerenes. In conclusion, the selectivity of C_{60} fullerenes effects in normal and transformed T-cells might be helpful for the development of the complex approaches to therapy of T-lymphomas.

Nanotechnology

Identification of novel tumor-associated antigens of medullary breast carcinoma by modified SEREX-approach

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Tumors elicit humoral and cellular immune response in the host organism. Autoantibodies specific to proteins that are mutated, misfolded, improperly glycosylated, overexpressed, truncated, or aberrantly localized in tumor cells have been detected in cancer patient sera. These autoantibodies or their aberrant targets can be utilized as molecular signatures of tumor genesis, thus being excellent candidates for cancer immunotherapy and diagnostics. Our aim was to identify and characterize novel tumor-associated antigens (TAA) of medullary breast carcinoma (MBC) which is heavily infiltrated by lymphocytes, indicating the possible presence of specific antigens on the surface of tumor cells, applying SEREX methodology (Serological expression of recombinantly expressed clones). For this purpose we modified SEREX approach to create a cDNA library depleted of immunoglobulin (IgG) clones, because heavy infiltration of this tumor by lymphocytes impedes the use of standard SEREX methodology for the search of TAA, due to very high percentage of IgG positive clones in generated library. Screening of depleted cDNA library from MBC tumor with autologous sera allowed us to identify 59 positive clones corresponding to 41 different genes, 9 of which were represented by several clones – up to 6. According to involvement of protein products of these genes in different biological processes we have categorized them in following groups: transcription, translation, cell signaling, cell adhesion, protein folding and others. It is noteworthy that 14 from identified clones have been already identified by SEREX techniques during screening of cDNA libraries from different tumors types, including breast carcinomas. Based on literature data analysis we found that protein products of 5 genes have immunomodulatory properties such as modulation of functional activity of macrophages, cell migration, activation of T-cells by regulation of the IL-1 pathway and regulation of cytokines expression. We cannot exclude that expression of these proteins in medullary breast carcinoma can cause such heavy lymphocytic infiltration of this type of tumor and relatively good prognosis for MBC patients. Further investigation of identified genes and protein products can help to understand the molecular mechanisms of breast cancerogenesis and to select antigens which can be used for immunotherapy as targets for creation of anti-tumor vaccines or the panel of TAAs to devise diagnostic tests that provide better sensitivity and specificity than single biomarkers alone.

Novel synthetic vehicles and nanoscale drug delivery systems overcoming cell membrane

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Novel telechelic, comb-like and branched oligoelectrolytes of tailored molecular weight, narrow molecular weight distribution, and functionality possessing controlled solubility and surface activity were developed in the lab of Lviv Polytechnic National University. The originality of the developed approaches is based on the synthesis of novel functional oligoperoxides with end or side ditertiary peroxide fragments and their subsequent using for initiation of block or graft copolymerization. That provides controlling the copolymer characteristics and behavior in water media of various polarities, namely: conformational state and size of the micelle-like zones, pH and temperature responsivity, untoxicity and biological compatibility or genuine physiological activity. As a result of the development of theoretical and experimental principles of the controlled synthesis of such oligoelectrolytes – surfactant novel telechelic, block and branched copolymers of vinyl acetate, vinyl alcohol, unsaturated acids, amines, and nonionic PEGylated monomers of desired macro- and microstructure and chain length as well as functional polymeric and polymer-mineral nanoparticles were synthesized and studied. There was shown by light scattering and SAXS techniques that size of the nanoscale drug delivery systems is in the range 20 – 100nm depending on oligoelectrolyte nature and content. Such novel functional oligoelectrolytes and nanoparticles possess ability to immobilize low molecular weight physiologically active substances (biocides, antibiotics including anticancer ones) via mechanisms of solubilization, intermolecular complex formation, covalent binding etc and to form nanoscale water drug delivery systems. They can be labeled with luminescent, MRI and X-ray detectable markers. The availability of reactive functional fragments in their structures provides irreversible binding antibodies, lectins or saccharide-containing fragments possessing specific interaction with cell membranes. The toxicity and genuine biological activity studied in the lab of Institute of Cell Biology witness their strong dependence on the molecular weight and molecular weight distribution as well as functionality and surface activity. Testing of oligoelectrolytes and nanoparticles *in vitro* and *in vivo* showed very low toxicity some of them and allowed to select the most promising ones as carriers for antimicrobial and anticancer drug delivery systems. Water based systems consisting of novel oligoelectrolytes carriers and immobilized chloramphenicol or ampicillin were successfully tested on microbial and fungi cultures. Study of anticancer drug (doxorubicin) delivery systems testified to their overcoming cell membranes and natural biological barriers and as a result high efficiency of the action on some tumor cells *in vitro* and *in vivo* and their low toxicity at the same time. This provides significant lowering the amount of anticancer drug. The study of the novel developed drug delivery systems are on the stage of the patenting.

Cell Biology

PPAR-α agonist BAY PP1 attenuates renal fibrosis in rats

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Renal fibrosis is the final common pathway of most progressive renal diseases. Effective treatment options are limited. There are no data on the role of peroxisome proliferator-activated receptor-α (PPAR-α) in the development and progression of kidney fibrosis. Therefore, we have examined the effect of two PPAR-α agonists, fenofibrate and BAY PP1, in a rat model of tubulointerstitial fibrosis: the unilateral urethral obstruction (UUO). Compared to unobstructed kidneys, five days after obstruction renal PPAR-α mRNA expression was reduced by 94%. Compared to vehicle treated rats with UUO, BAY PP1 significantly ameliorated the renal cortical expression of collagen type I, III, IV and fibronectin. The number of proliferating tubulointerstitial cells (mitotic figures, PCNA positive cells) and of PDGFR-β/PCNA double-positive cells was significantly lower in BAY PP1 treated rats. Treatment with fenofibrate had no effect on these parameters. The infiltration with monocytes/macrophages was not reduced by either treatment. In vitro, BAY PP1 had no direct effect on the proliferation or expression of fibrosis markers in rat renal fibroblasts. Conversely, rat tubular cells treated with BAY PP1 produced less collagen, fibronectin, TGF-β_1 and the conditioned media of these cells reduced proliferation of fibroblasts. In conclusion, the PPAR-α agonist BAY PP1, but not fenofibrate, reduced renal fibrosis in rats with UUO by affecting the cross-talk between tubular cells and fibroblasts. These data suggest that novel PPAR-α agonists could be an important treatment option in the early stages of renal fibrosis.

Kinetic model of the effects of extracellular pH and [K+] on the inactivation of the Kv1.3 potassium channel

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Kv1.3 is a voltage-gated potassium channel, which plays an important role in the membrane potential regulation of human T cells. During prolonged depolarization Kv1.3 enters a non-conducting state by the slow P/C type inactivation, whose exact mechanism is yet to be determined. Previous studies of our lab show that both the pH and the potassium concentration of the extracellular space ($[K^+]_e$) influence the kinetics of inactivation. According to these previous results acidic pH accelerates the inactivation in the presence of low $[K^+]_e$, while decelerates it in the presence of high $[K^+]_e$.
The aim of the present study was to asses whether the proposed kinetic models were able to explain the experimental results and therefore determine the model which is the most suitable to describe the inactivation gating of the Kv1.3 channel. To examine the models the author's own computer programme was used, which is able to generate simulated recordings from a given kinetic model. In this study whole cell patch-clamp measurements were modelled, 1500 channels per run were simulated. 10 runs were analyzed for each parameter set. Inactivation kinetics of the simulated traces was determined using exponential curve fits performed from 90% of the amplitude to the onset of the steady-state part of the curve. The results of the simulations show that a model containing only two open microstates and one inactivation pathway from both open microstates is not sufficient to describe the experimental results accurately. By contrast, the introduction of a new binary parameter and with this the duplication of the inactivation pathway leads to a good approximation of the experimental results. Based on these results it is likely that a kinetic model in which the speed of inactivation is the function of the binding of a sole potassium ion into the cavity of the channel, as proposed by earlier, does not fit the experiments. The probable structure of microstates corresponds to a model in which the conducting pore can have two different conformations with different conduction and inactivation properties depending on the $[K^+]_e$. The simulation provides the basis for future experiments verifying the model and leading to better understanding of the P/C type inactivation mechanism. The software that was developed for this study can be used to stochastically simulate the kinetics of any ion channel. Since ion channels are necessary regulatory elements of the almost all cells of a human body and play an important role in the pathogenesis of several diseases, e.g. long QT syndrome, the better understanding of their gating kinetics, which can be achieved by this simulation, could lead to results with clear clinical significance.

Stat5 deficiency influences survival and plasticity of hippocampal neurons affecting erythropoietin and growth hormone signaling

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Signal transducers and activators of transcription Stat5 represent an important downstream signaling pathway activated by cytokine type I receptors. Upon binding to their membrane receptors, growth factors such as erythropoietin (EPO) and growth hormone (GH) activate Janus kinase 2 (JAK2) and cause activation of Stat5 inducing expression of genes that regulate cell survival, proliferation and differentiation. Stat5 deficiency is characterized by a dwarf phenotype, anemia, and high perinatal mortality due to ineffective hematopoiesis. Recently Stat5 has been suggested to play a role in neuronal migration and axon guidance in the developing brain. Stat5 is activated by the neuroprotective and neurotrophic cytokines, including erythropoietin (EPO) and growth hormone (GH). To directly test whether activation of Stat5 in neurons is essential for EPO actions, we examined the neuroprotective and neurotrophic effects of EPO in hippocampal neuronal cultures isolated from Stat5-/- and Stat5+/+ mouse fetuses after acute abrogation of JAK2/Stat phosphorylation by the newly available pathway-specific inhibitor, cucurbitacin I. Since Stat5 has been shown to be crucial for intracellular signalling and the somatotrophic effects of GH, we also studied its effects in the Stat5-/- and Stat5+/+ neurons. Our data obtained in hippocampal neuronal cultures from Stat5a/b-knockout mouse fetuses suggest a dissociation of the intracellular pathway mediating the protective effect of EPO against glutamate toxicity from that needed for its neurotrophic activity. Both pretreatment and post-treatment with EPO counteracted glutamate-induced cell death in Stat5+/+ and Stat5-/- neurons. Acute pharmacological inhibition of JAK2/Stat signaling had no effect on EPO neuroprotection, whereas inhibition of phosphatidylinositol-3' kinase (PI3K)/Akt pathway abolished the protective effect of EPO in both Stat5+/+ and Stat5-/- neurons. GH effectively protected Stat5+/+ cells against glutamate toxicity but had no effect in Stat5-/- neurons or in Stat5+/+ neurons treated with JAK2/Stat or PI3K inhibitor. EPO and GH stimulated neurite outgrowth and branching of Stat5+/+ neurons through activation of PI3K/Akt signaling but had no trophic effect in Stat5-/- cells. We conclude that in hippocampal neurons Stat5 is not required for neuroprotection by EPO but along with Akt is essential for its neurotrophic activity. Both Stat5 and Akt are needed for neuroprotective and neurotrophic signaling induced by GH in neurons.

Drug Development & Pharmaceutical Research

New one-pot synthetic method of biologically active 2-substituted-4-thiazolidinones

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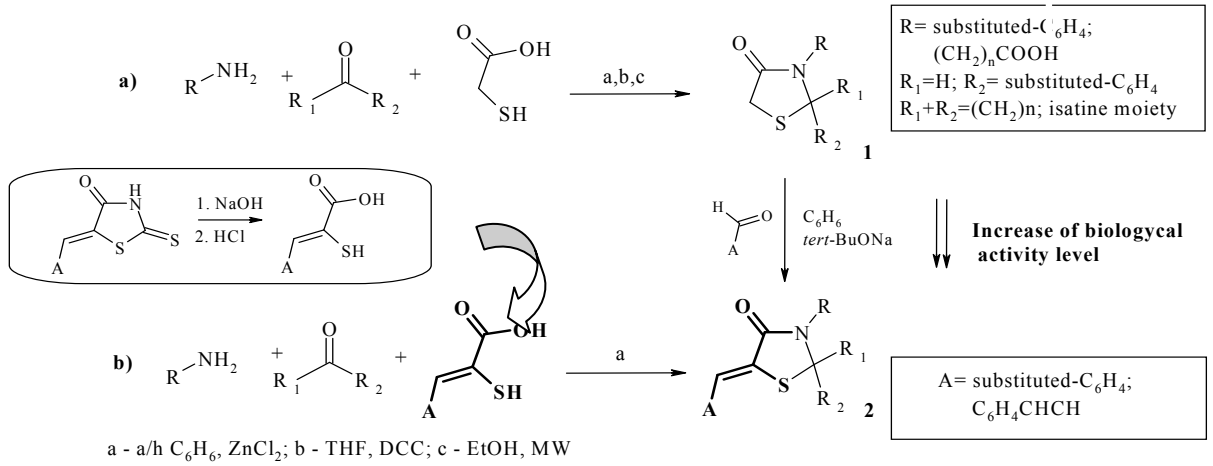
Design of "small molecules" as innovative biological active compound base on well-known scaffolds is one of most commonly employed approach in drug discovery. Among 4-azolidinone derivatives 2-aryl(heteryl) substituted 4-thiazolidinone attract attention of scientists as sources of antimicrobial, antimycobacterial, antiviral and anticancer compounds. Nowadays "one-pot reactions" of heterocyclic systems synthesis are effective method in medicinal che-mistry. Synthesis of 2,3-disubstituted derivatives of 4-thiazolidinone involves a one-pot three-component condensation of primary amine, oxo-compound and mercaptoacetic acid (scheme **a**) and describe for different condition/medium and catalysts. The row of new 4-thiazolidinone derivatives (compounds **1**) base on amino-acids or primary aromatic amines; aromatic aldehydes/cyclic ketones or isatine was synthesized according mentioned reaction. For synthesis of compounds with carboxylic acids moieties in position 3 of core heterocycle the MAOS (microwave assisted organic synthesis) method involved.



a - a/h C_6H_6, $ZnCl_2$; b - THF, DCC; c - EtOH, MW

Optimization of obtaining substances was carrying out via condensation reaction with aromatic aldehydes that allowed us obtained 5-ilydene derivatives (compounds **2**). Direction of chemical modification was substantiate by our previously investigation about critical role of presence or nature of moiety in position 5 for mentioned compound biological activity level. However, the general applications of the described processes are limited as the reactions are requiring the expenditure and have low yield according to low reactivity of CH group. We focused on developing new variant of one-pot three-component reaction (scheme **b**) which allowed us to obtain 5-ylidene derivatives of compounds **1** in one stage. In this case instead of mercaptoacetic acid we used 2-mercapto-3-arylacrylic acids obtained with high yields via alkali hydrolysis of appropriate 5-arylidene rhodanines.The all chemical reactions, purity and structure of new compound were determined by TLC and spectral data. Biological activity screening was realized according international programs of NIH (Bethesda, USA): ***DTP*** of NCI for anticancer activity, ***TAACF*** for anti-tuberculosis activity and ***AACF*** for antiviral activity of NIAID. The screening data allowed us to identify the hit-compound and show the increase of activity level after structure modification of compounds **1**.

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 thiazolidon 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 has been carried out on white rats with the weight of 70-80 g. Experimental immunodeficiency modeling was made by subcutaneous introduction of cyclophosphan in a doze of 10 mg/kg of weight during 10 days. The immune system functional status was conducted on the 12^{th} day of experimental therapy with the use of morphological, laboratory and immunological methods. 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 non-toxic lead-compounds which are characterized by high immunomodulating activity was chosen. Studied thiazolidon derivates possessed high activity in treating of cyclophosphan immunodeficiency comparing to the standard therapy (using polyoxidonium). It was confirmed, that there using leads to increasing of immune system basic morphological parameters (body weight, thymus and spleen mass and mass index), activity of phagocytosis, CD3, CD4, CD16, CD22 cells levels in animals with immunodeficiency. Also, increasing of IgA, IgM and IgG quantity was observed. Highly active lead-compound (LES 5849) among thiazolidon derivatives based on immunotropic activity was identified. The data obtained let us to enhance present spectrum of means – potential drugs with immunomodulating properties.

Combinatorial biosynthesis as a strategy of new biologically active compounds creation

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Actinomycetes are producers of variety antibiotics and other secondary metabolites. Biosynthetic gene clusters from several antitumor pathways in actinomycetes are presently being characterized and expressed in order to generate novel drugs. Several methylated and glycosylated antitumor-drug derivatives have been produced that show a relaxed substrate specificity for secondary-metabolic enzymes, which opens up the possibility of generating novel drugs by genetic manipulation. *Streptomyces nogalater* Lv65 is a producer of a cytotoxic antibiotic nogalamycin. This antibiotic was reported to be markedly effective against a variety of gram-positive bacteria and also found to be active against several rodent tumor models. To understand the regulation of nogalamycin biosynthesis and to facilitate design of novel anthraciclines we have cloned and characterized several genes that code for enzymes involved in nogalamycin biosynthesis (*snogD, snogZ, snogE , snogY, snogL* and *snogM)* and regulation (*snorA)*. In our experiments the transcriptional regulator of nogalamycin biosynthesis encoded by *snorA* gene has been inactivated within the chromosome of *S. nogalater* Lv65. The *snorA* gene was replaced with the mutated allele that was generated by insertion of spectinomycin resistance gene cassette *aadA* at a position adjacent to *snorA* start codon. The obtained mutant strain did not produce nogalamycin and its intermediates. Loss of nogalamycin production caused significant changes in morphology of the *snorA* deficient strain. Introduction of *snorA* gene, encoding a putative antibiotic regulatory protein, restored nogalamycin production in the mutant strain. We also decided to express *snorA* gene in the wild type *S. nogalater* strain in a multicopy plasmid. Finally, it was shown that the presence of extra copies of *snorA* caused increasing in nogalamycin production compared to that by the wild type. To elucidate the role of *snogD, snogZ, snogE* (a putative glycosyltransferases), *snogL* (a putative O-methyltransferase) genes in nogalamycin biosynthesis, there disruption was performed within the chromosome of *S. nogalater* Lv65 strain. We succeeded in generation of *snogD, snogZ, snogE* and *snogL* disruption mutants and verified them by Southern-blot hybridization. Using the TLC chromatography new hybrid compounds, probably amethylated and aglycosylated nogalamycins were observed. In our studies on O-methylation in nogalamycin biosyntheses, we were interested in the expression of *snogY, snogL* and *snogM* genes in heterologous hosts. This strategy is based on the relaxed substrate specificity of secondary-metabolic enzymes and the fact that structurally related polyketide drugs share part of their biosynthetic pathways. In order to caring out such experiments the putative O-methyltransferases were cloned into pKC1218E plasmid under the control of the erythromycin promoter. The obtained vectors were transferred into *S. echinatus* strain (aranciamycin producer) by conjugation from *Escherichia coli*. Using the TLC chromatography it was shown that the expression of *snogM* gene in *S. echinatus* leads to the production of a new hybrid compound, probably O-methylated aranciamycin.

Genomics

Hepatic gene expression in Prague Hereditary Hypercholesterolemic (PHHC) rat – a model of polygenic hypercholesterolemia

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Most of the hypercholesterolemic patients have a hypercholesterolemia of polygenic origin and the genes involved in are not well characterized yet. Unfortunately, there are almost no animal models that could be used for study of polygenic hypercholesterolemia. Prague Hereditary Hypercholesterolemic (PHHC) rat may be an exception – PHHC rats develop polygenic hypercholesterolemia (characterized by accumulation of cholesterol in nonHDL lipoproteins) after feeding cholesterol. **Objectives**: To determine differences in hepatic gene expression in the response of PHHC rats and control Wistar rats to the cholesterol in the diet. **Methods**: Male PHHC and Wistar rats were fed chow (C) and 1% cholesterol (CHOL) diet for three weeks. Hepatic gene expression was evaluated using Affymetrix GeneChip arrays. **Results**: On CHOL diet, cholesterol and triglycerides accumulated in the liver of both PHHC and Wistar rats; cholesterolemia rose significantly only in PHHC rats. Cholesterol feeding resulted in down regulation of several genes of lipid metabolism – most of the affected genes were genes involved in cholesterol biosynthesis. Surprisingly, no significant differences in the response of gene expression to dietary cholesterol were found between both strains (in spite of the fact that using microarrays we were able to detect expression of more than 6 500 genes in the liver). On the other hand, we identified several genes that were expressed differently between PHHC and Wistar rats independently of the diet. Among those genes, *Aldh1a7*, *Yc2*, and *Apof* genes were markedly up regulated and *Ugt2b*, *Cdh17*, *Ltc4s*, and *Slc6a6* genes distinctly down regulated in PHHC rats. **Conclusions**: The response of gene expression to dietary cholesterol was affected in PHHC and Wistar rats in the same way. The development of hypercholesterolemia in PHHC rats involves genes whose exact role in lipid metabolism remains to be determined.

Over expression of eEF1 subunits in human lung carcinomas.

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The impressive list of different translational components whose mRNA or protein levels appear higher in cancerous tissues as compared with non-malignant ones clearly demonstrates a link between the translational apparatus and cancer progression. In particular, the increase in expression of one or another subunit of the translation elongation factor 1, comprising eEF1A1/2, eEF1Bα, eEF1Bβ and eEF1Bγ components, in different types of cancer was reported. Unfortunately, almost all previous data concerning the expression level of a single eEF1H subunit were based on examination of the mRNA expression. Very narrow information about the cancer-related changes in corresponding proteins amount is available so any functional consequences of the known elevation of the level of one or another eEF1B mRNAs in cancerogenesis remained unidentified. We have investigated the expression of mRNAs coding for eEF1A1, eEF1A2, eEF1Bα, eEF1Bβ and eEF1Bγ components of eEF1H, and the level of corresponding proteins in 25 human lung cancer specimens by Northern blot and Western blot analysis. The increase in expression of one or another subunit of the eEF1H complex in lung cancer specimens was observed in 44% cases at the mRNA level and in 52% cases at protein level. Surprisingly, a significant increase of the protein components of eEF1H and elevated level of mRNAs coding for these components did not coincide in the majority of tumor specimens. We have concluded that the cancer-related increase in the level of mRNA coding for translation components is not coupled with the increase in the level of corresponding proteins in lung cancer. Thus, the elevation of the eF1H subunits level seems to be controlled by translational or post-translational regulation while the mRNA rise has no direct effect on the eF1H proteins level and may have additional regulatory consequences remained to be studied. Moreover, the increases in the amount of different protein components of the eEF1H complex were found to be unbalanced, suggesting a specific cancer-related role of individual eEF1H subunits rather than of the eEF1H complex as a whole. Thus, the stability and regulation of eEF1H complex may be one of the factors playing an important role in lung carcinogenesis.

The adaptor protein Ruk/CIN85 is involved in the regulation of hypoxia-induced gene expression

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Adaptor proteins, which link protein-binding partners together and stimulate formation of signalling complexes, play an important role in the regulation of intracellular signalling pathways. The adaptor/scaffold protein Ruk (also known as CIN85, SETA and CD2BP3) is a member of a distinct, evolutionary conserved family of SH3-containing proteins. Ruk/CIN85 was implicated to play a role during carcinogenesis by influencing a number of processes such as cell adhesion, motility and apoptosis. Though intratumoral hypoxia is one of the driving forces in cancer progression and a lot of genes involved in carcinogenesis are induced by hypoxia, there are no data concerning the involvement of Ruk/CIN85 in the regulation of hypoxia-dependent gene expression during carcinogenesis. The present study is focused on determining the role of Ruk/CIN85 in hypoxia-dependent gene regulation using MCF-7 breast adenocarcinoma cells as a model.

The effects of hypoxia on Ruk/CIN85 expression as well as effects of Ruk/CIN85 over expression on the hypoxia-dependent gene regulation were examined. By using Western blot analysis with both monoclonal and polyclonal antibodies against Ruk/CIN85, no significant changes in its expression under different oxygen concentrations were detected. However, it was found that in MCF-7 cells, which either stably or transiently over express Ruk/CIN85, hypoxia-inducible factor-1α (HIF-1α) induction by hypoxia was significantly higher as compared both to mock-transfected cells. Next, MCF-7 cells were cotransfected with a luciferase reporter gene construct containing three copies of the hypoxia-responsive element (HRE) from erythropoietin gene in front of SV40 promoter and Ruk/CIN85 expression vector. It was shown that in the presence of Ruk/CIN85 luciferase activity was induced both under mild hypoxia and normoxia. It was also shown that Ruk/CIN85 interfered with the proline hydroxylation-dependent HIF-1α protein destabilization. The Ruk/CIN85-dependent stimulation of HIF-1α was partially blocked by the PI3K/Akt inhibitor LY294002 but not the MEK inhibitor U0126. Further, upregulation of Ruk/CIN85 increased cellular sensitivity to IGF-1-induced and HIF-1 mediated proliferation.

In conclusion, our data show that Ruk/CIN85 is involved in modulation of the effects of hypoxia in breast adenocarcinoma cells. This work was supported by grants from the Ministry of Education and Research of Ukraine (№170-2009 and № Ф 28.4/041-2009).

Proteomics

Cysteinyl peptide enrichment in combination with iTRAQ as a tool for discovering low abundance protein biomarkers

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Since proteomics was proven to be capable of characterizing a large number of differences in both protein quality and quantity, it has been applied in various areas of biomedicine, including the discovery of potential diagnostic biomarkers. However, the enormous complexity of clinical samples is one of the major stumbling blocks in biomarker discovery efforts. Numerous earlier projects aimed at biomarker discovery reported altered concentrations mostly in high abundance proteins. Unfortunately, these changes are often ambiguous and insignificant, whereas the truly interesting low abundance proteins remain undetected. In order to provide a deeper insight into the proteome of clinical samples, several fractionation and separation strategies emerged. Here we describe a method that combines the fractionation power based on cysteinyl peptide enrichment (CPE) with the robustness of iTRAQ quantification. First, we used liquid scintigraphy to optimize the protocol by employing radioactive ^{35}S cysteine-labeled iTRAQ peptides. This allowed us to fine-tune individual parameters during the workflow in order to ensure maximum efficacy of the capturing process. The optimized protocol was then applied onto an iTRAQ-labeled BSA digest to assess the effect of CPE fractionation on iTRAQ quantitation, which was proven not to be influenced by the fractionation process. The final phase of the experiment involved application of the protocol on a complex clinical sample. We analyzed amniotic fluid samples obtained from patients with preterm premature rupture of membranes (PPROM) with intraamniotic infection (n=2) as well as from patients with PPROM with ruled out intraamniotic infection (n=2). Respective samples were combined into two pooled samples and subjected to immunoaffinity depletion in order to remove ballast proteins with no diagnostic potential and unmask lower abundance proteins. These were digested with trypsin and the resulting peptides were subjected to the optimized iTRAQ/CPE protocol. Peptides from individual fractions were then analyzed using LC-MALDI-TOF/TOF. First, MS spectra of intact peptides were acquired and based on these data; suitable peptides were selected, fragmented, and analyzed in MSMS mode. The resulting MSMS spectra allowed both protein identification and quantification. In total, we identified 242 proteins at 95% confidence. We proved this method to be able to uncover interesting differences among the samples. Therefore, a clinical proteomics study incorporating this method is currently under way.

ITSN2 adaptor protein: functional comparison and impact on early development

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Endocytosis is a vital cellular process that is mediated by machinery of proteins organized into functional complexes on multimodular scaffolds. One of such undermembrane scaffolds are proteins of intersectin (ITSN) family. Whereas most analyses are focused on ITSN1, participating also in cytoskeleton rearrangements, cell signaling and survival, little is known about ITSN2 high expression level of which predicts recurrence of breast cancer after chemotherapy. For better understanding of intersectin function we focused our research on ITSN2. The results on colocalization of intersectins and endocytic markers evidenced that both intersectins function in the same cellular compartments. The possibility of intersectins oligomerization was examined and not detected. Furthermore, investigation of ITSN2 interactions revealed that majority of protein partners of ITSN2 and ITSN1 are common – dynamin 1, SOS1, Sj, N-WASP, c-CBL – but the specificities in binding could be observed. Interaction with adaptor CIN85/Ruk was shown for ITSN1 but not for ITSN2. We found a new protein ITSN partner, Sema6A, implicated in axon guidance and endocytic adaptors of Reps family. Thus, ITSN1 and ITSN2 operate in common cell compartments providing similar but also meaningfully different interfaces for their protein partners from endocytosis, signaling and cytoskeleton rearrangements processes. Using Xenopus in vivo model and microinjection technique to study ITSN2 properties we observed the strongest effect of hyperpigmentation and gastrulation failure in case of over expression of ITSN2 long isoform C-terminal part (DH/PH-C2 domains). We showed that this isoform is present in Xenopus oocytes and embryos starting from 2-cell stage and could be involved in early development. DH/PH tandem is responsible for the observed phenotype as overexpressed alone it was sufficient to give a similar phenotype. Also we found that membrane-targeting by CAAX is not necessary for phenotype development but CAAX-tagged form showed more severe defects presumably due to targeting it to Cdc42. The distribution of DH/PH was more uniform while DH/PH-CAAX associated mainly with F-actin and cells formed extensive filopodia. This could explain the effect of hyperpigmentation as the pigment is located under the membrane and is connected with actin integrity. We demonstrated that the mechanism of DH/PH action involved activation of Cdc42. Cdc42 constitutively active produced strong hyperpigmentation resembling the effect of DH/PH-CAAX overexpression. Dominant negative Cdc42 rescued loss of cell-to-cell contacts in case of DH/PH overexpression and reduced the hyperpigmentation effect of DH/PH-CAAX. The results obtained suggest a possible role of ITSN2 as a participant (through Cdc42) of the coordinated changes in actin cytoskeleton during early embryonic development of vertebrates.

Metabolomics

Phosphatidylcholine-enriched diet ameliorates the endotoxin-induced inflammation in the hippocampus and the colon

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Background: Our laboratory has repeatedly shown that orally-administered phosphatidylcholine (PC) pretreatment exerts significant anti-inflammatory effects in experimental models of different pathologies. Peripheral endotoxin (ETX) release contributes to cytokine production and leukocyte accumulation, and induces generalized inflammation which affects the central nervous system also. Besides, it has been demonstrated that this reaction is leading to decreased hippocampal neurogenesis via an interleukin 1ß-dependent signal. Preventive approaches play increasing roles in modern medicine and functional foods offer potential means for the effective prevention of different pathological conditions. Therefore, the goal of our study was to examine the neuroprotective effect of PC-enriched diet in the hippocampus and the colon in a rodent model of ETX-induced systemic inflammation. **Materials and methods**: The experiments were performed on male CD rats (180-230g bw). Inflammation was induced by 2 mg/kg i.p. ETX; control animals received sterile saline in the same volume. The control group and one group of ETX-treated animals were nourished with standard laboratory chow, while another ETX-treated group received a special diet enriched with 1% PC for 5 days prior to the administration of ETX and thereafter during the 7-days observation period. The subgroups were sacrificed 3 hr, 1 day, 3 days or 7 days after the administration of ETX, respectively. The rats were treated with bromo-deoxyuridine (BrdU, 50 mg/kg/day) i.p. during the 7 days of observation. Tissue biopsies were taken from the hippocampus and the ascendant colon and immunohistochemistry was used to visualize the BrdU- and doublecortin-positive neuroprogenitor cells and the Iba1-positive microglia. Biopsies from the colon were examined after hematoxylin-eosin staining as well. Furthermore, the activities of pro-inflammatory myeloperoxidase (MPO) and xanthine-oxidoreductase (XOR) enzymes were also determined. **Results**: The ETX-caused inflammatory challenge decreased the neurogenesis in the hippocampus and led to an accumulation of microglia. PC pretreatment prevented these changes and also reduced the colonic activities of MPO and XOR which are considered as markers of inflammatory process. **Conclusion**: Oral PC pretreatment provided protection against ETX-induced peripheral inflammation and seems to be able to prevent the inflammation-linked decrease of neurogenesis in the central nervous system.

Poster Presentations

Cancer Research & Prevention

Hazardous second hand smoke exposure in hospitals

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Objective: Exposure to secondhand smoke (SHS) is a serious threat to public health, and a significant cause of lung cancer and heart disease among non-smokers. Hungarian hospitals have been declared smoke free since 2005. However, compliance with this law may not be 100 percent. The purpose of this study was to assess the level of compliance with the smoke-free hospital law by measuring levels of indoor air pollution in different indoor areas of a large public hospital in Hungary [1, 2]. **Methods:** The TSI SidePak AM510 Personal Aerosol Monitor was used to measure the concentration of particulate matter less than 2.5 microns in diameter ($PM_{2.5}$) observed in the ambient air of 117 locations sampled, containing 16 health care and 6 non-health care (other) departments, in a large public hospital in Hungary. The air monitoring was done between January and April 2009. **Results:** We observed evidence of smoking such as cigarette butts and ashtrays s in several indoor areas of the hospital. Air monitory revealed concentrations of respirable suspended particulates (RSPs) ranging from 1 to 336 μg/m^3 depending on the area sampled. Clinical departments averaged 83 μg/m^3 by average smoker density of 1,73 and the non-clinical departments averaged 37 μg/m^3 with average active smoker density of 1,71, respectively. In the rooms where evidence of smoking was not seen the mean $PM_{2.5}$ level was 6 μg/m^3. In areas where evidence of smoking was observed the mean $PM_{2.5}$ level was 138 μg/m^3. The highest levels of indoor air pollution were found in the Radiology (336 μg/m^3), Intensive Care Unit (230 μg/m^3) and Neonatology (163 μg/m^3), respectively (Fig.).

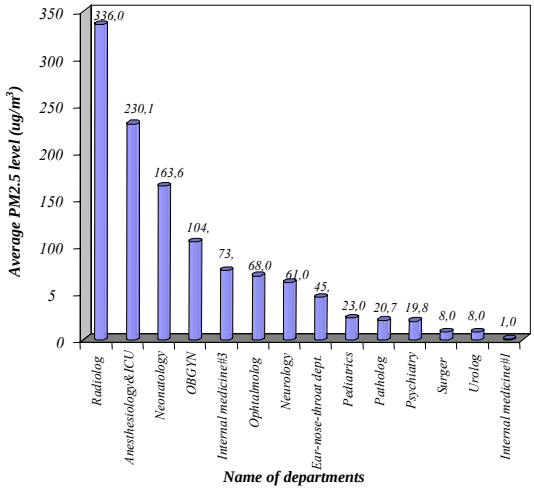

Fig. Average particle pollution by clinical departments

Conclusions: Conclusively, at least in one large public hospital it appears that compliance with the smoke-free law is incomplete resulting in high levels of indoor air pollution which pose a health risk to both patients, visitors and staff. It is clear that proper implementation and enforcement of the legislation that bans smoking in hospitals is imperative to protect the health of patients and staff alike.

1. World Health Organization. 2008. Report on the Global Tobacco Epidemic. In the MPOWER Package, 2008. Geneva, Switzerland: World Health Organization.

2. Tárnoki Á, Tárnoki D, Travers M, et al Tobacco smoke is a major source of indoor air pollution in Hungary's bars, restaurants and transportation venues. Clin. Exp. Med.J.2009;3(1):131-138.

Primary systemic therapy of breast cancer - our experiences

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Purpose: To identify breast cancer subtypes by immunohistochemistry likely to respond to neoadjuvant chemotherapy and to analyse the used chemotherapy regimen and the range of response rates. Compare the accuracies of physical examination (PE), breast ultrasound (US), and pathologic evaluation in tumor and lymph node assessment. **Methods**: Analysis of a prospectively collected clinical database was performed. Eighty-one patients were identified in our files who received neoadjuvant chemotherapy between 1998 and 2009. Prior to neoadjuvant therapy the diagnosis of carcinoma was established through core biopsy (NCB) and/or fine-needle aspiration biopsy (FNAB) of the primary tumor and palpable lymph node, if present. We used immunohistochemical profiles (ER, PgR, and HER2) of FNAB and additionally Ki67 and p53 of NCB and surgical breast specimens to subclassify the tumors. Pathological response rates were assessed following surgical removal of tumors by using the Chevallier classification. Tumor and lymph node measurements by PE and US were obtained before and after neoadjuvant chemotherapy. Concordance among different clinical measurements was assessed and compared with the tumor and lymph node staging by pathology. DFS and OS was measured in 68 cases from the date of definitive surgery to the date of last follow-up or death. **Results:** Pathological complete or near-complete remission (pCR = Chevallier I and II) was observed in 12 of 81 cases (14, 8 %). According to the preoperative characteristics of the 12 tumors achieving pCR 8 of the cases were triple negative, one of 12 was ER-/HER2+ and three of 12 ER+/HER2+. 20 of 81 patients received taxane based neoadjuvant chemotherapy, 27 of 81 anthracyclin based neoadjuvant chemotherapy, 32 of 81 taxane + anthracyclin regimen and 2 of 92 CMF regimen. In the taxane treated group of patients the pCR rate was 30 %, in the anthracyclin group 7% and in the taxane + anthracyclin treated group 12,5 %. After neoadjuvant chemotherapy, PE correlated better with pathology than US ($p = 0,017$ and $p = 0,037$). The decrease of tumor size after the second cycle of the chemotherapy was able to predict the pathological response diagnosed after the final cycle ($p = 0,001$). Concerning DFS, significant difference was observed between the Chevallier III and IV group ($p = 0,016$), and less events were observed in the pCR group (not significant). pCR was associated with significantly better OS ($p=0,033$). **Conclusions**: It seems that even limited, routinely used immunohistochemical profiling of tumors is able to predict the likelihood of pCR to neoadjuvant chemotherapy: Patients with triple negative and HER2-positive cancers are more likely to achieve pCR after neoadjuvant chemotherapy. Breast US is an accurate imaging study at baseline but PE correlates better with the pathology finding. US after the 2nd cycle of chemotherapy predicts the pathological response diagnosed after the final cycle.

Metastasis-regulatory microRNAs: one step further

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Despite the advancements, understanding of genetic programs and molecular mechanisms required for cancer metastasis are still incomplete. Genes that specifically regulate the process of metastasis are useful tools to elucidate molecular mechanisms and may become markers for anti-metastatic therapy. Recently, several non-coding regulatory RNA genes were identified, which play roles in various steps of metastasis. MicroRNAs are ideally suited to regulate tumor metastasis due to their capacity to coordinately repress numerous target genes, thereby potentially enabling their intervention at multiple steps of the invasion-metastasis cascade. In breast cancer, there tend to exist pro- (mir-10b, -21, -373, -518d, -520c) and anti-metastatic (mir-31, -34, -126, -200b, -205, -206, -335) microRNAs. We previously identified a microRNA, miR-31, whose expression correlates inversely with metastatic recurrence in human breast carcinomas. In this study we aimed to analyze the expression of these molecules: we compared their expression in primary breast carcinomas (without relapse: 23 cases, and with recurrence: 23 patients) and their corresponding distant metastatic sites (applicable: 40 samples). As the studies investigating this topic usually lack this data, because of sample unavailabilty, we are taking the identified microRNAs under inquisitory inspection.

Endocytic adaptors in traffic of latent membrane 2A of Epstein-Barr virus.

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Endocytosis is a fundamental process of membrane vesicle-dependent substrates uptake and intracellular trafficking. Large number of nutrient, growth factors, messengers and pathogens enter cells utilizing different types of endocytosis.
Intersectin 1 is an endocytic adaptor protein known to be crucial at the earliest stages of endocytosis. Moreover, it was shown to control RTK-signaling in concert with c-CBL, promote actin cytoskeleton nucleation in Cdc42- and NWASP-dependent manner. Often, different pathogens manipulate hosts mechanisms of endocytosis and signaling to enter cells and affect cell differentiation and proliferation. Epstein-Barr virus infects epithelial cells and B-lymphocytes. Viral latent membrane protein 2A (LMP2A) mRNA is frequently detected in peripheral blood B lymphocytes from healthy individuals and the protein is often present in tumor biopsies from EBV-associated malignancies. Here we report about interaction between viral protein LMP2A and endocytic adaptor intersectin1. The immunoprecipitation data evidence for the complex formation between LMP2A and ITSN1 in vivo in HEK 293, MCF-7 cells and in B-lymphocyte cell line CBMI. SH3-domains of ITSN1 are sufficient to precipitate LMP2A in vitro, thus it was supposed that ITSN1 binds -PXXP- motives of LMP2A. Moreover, another endocytic adaptor - amphyphisin1 was found to bind -PXXP- of LMP2A through its SH3 domain. Mutational analysis of LMP2A sequence evidences for at least two binding sites for ITSN1 and three for Amphiphysin. According to our data, intersectin1 interacts with both isoforms: LMP2A and -2B, while amphiphysin 1 binds only LMP2A. Amphyphisin1 binds two sites at the N-terminus of LMP2A that were known to mediate interaction of this viral protein with ubiquitin-ligases Nedd4-2 and Aip4. Thus, it is tempting to speculate that amphyphisin 1 competes with mentioned ubiqutin-ligases inhibiting LMP2A-mediated down regulation of Syk and Lyn. Analysis of subcellular distribution of LMP2A and ITSN1 in MCF7 cell line supported our data about these proteins interaction in vivo. Overlapping of the signals of LMP2A and ITSN1 is rather a rare event, LMP2A is co localized with ITSN1 only in clathrin-coated pits. According to immunofluorescence analysis we suggest that ITSN1 and LMP2A interact initially during LMP2A internalization on plasma membrane. Summarizing these data we can propose a model of clathin-mediated internalization of LMP2A which could be ITSN1-dependent. Investigation of ITSN1 – LMP2A interaction could provide important clues for understanding of LMP2A role in EBV cycle in epithelial cells during viral infection and EBV-dependent cancerogenesis.

Immunocytochemical analysis of subcellular localization and content of S6 kinase during cell cycle progression

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Introduction. The ribosomal protein S6 kinase is an important component of PI3K signal transduction pathway. Members of this signaling pathway, including S6K, have been shown to be either mutated or overexpressed in malignant tumors, therefore inhibition of some of them is supposed as one of approaches to suppress cancer growth. S6K plays a key role in the regulation of cell growth by stimulating protein synthesis when growth factors and nutrients are available. Its activation is initiated by phosphatidylinositide-3-OH kinase (PI3K)-mediated activation of downstream effector molecules, such as mTOR, PDK, AKT. There are several substrates of S6K, including rpS6, some translation and transcription factors and apoptosis related proteins. In mammalian cells two highly homologous forms of S6Ks are expressed, namely S6K1 and S6K2 that play both redundant and distinct functional roles. S6K1 and S6K2 have cytoplasmic and nuclear isoforms. It was shown, that activity of S6Ks increased during cell cycle. Selective inhibition of S6Ks with neutralizing antibodies led to proliferation delay. Besides, CCT (Chaperonin containing TCP1 complex) providing folding of important participants of mitosis (tubulin, cdc 20, PLK1 etc.) is the substrate of S6K. The goal of our work was to study subcellular localization and content of S6K1 and S6K2 during all stages of the cell cycle. **Materials and methods**. Human breast cancer cells MCF-7 were cultured on the cover glass slides until the cells reached 70% monolayer. Anti-S6K1 rabbit antibodies/anti-Ki-67 mouse antibodies and anti-S6K2 rabbit antibodies/anti-Ki-67 mouse antibodies mixtures were used to detect S6K1 and S6K2 content in proliferating cells. Furthermore, proliferating cells were detected using Hoechst 33258 that binds to DNA. The patterns were examined using fluorescent microscopy. The correlation of qualitative data was detected by tetrachoric test. **Results**. Our data show, that S6K1 and S6K2 are localized predominantly in the cytoplasm during interphase, however weak positive reaction was revealed in cell nuclei as well. It may be explained by a high level of protein synthesis at this stage of cell cycle. The population of studied cells demonstrated the same level of intracellular content of S6Ks. Immunocytochemical and immunofluorescent analyses revealed that the content of S6K1 and S6K2 increased during mitosis. Some increase of S6K1 and S6K2 content was observed in prophase. In metaphase, anaphase and telophase the immunofluorescent reaction becomes much more bright and prominent. The statistical analysis confirmed the correlation between increased S6K1, S6K2 content and activation of proliferation process. So, the increased level of S6K1 and S6K2 content has been revealed in proliferating cells that give additional grounds to study the influence of PI3K inhibitors on the growth of malignant cells

Synthesis and pharmacological screening of novel 4-thiazolidinones with heterocyclic moieties in molecules

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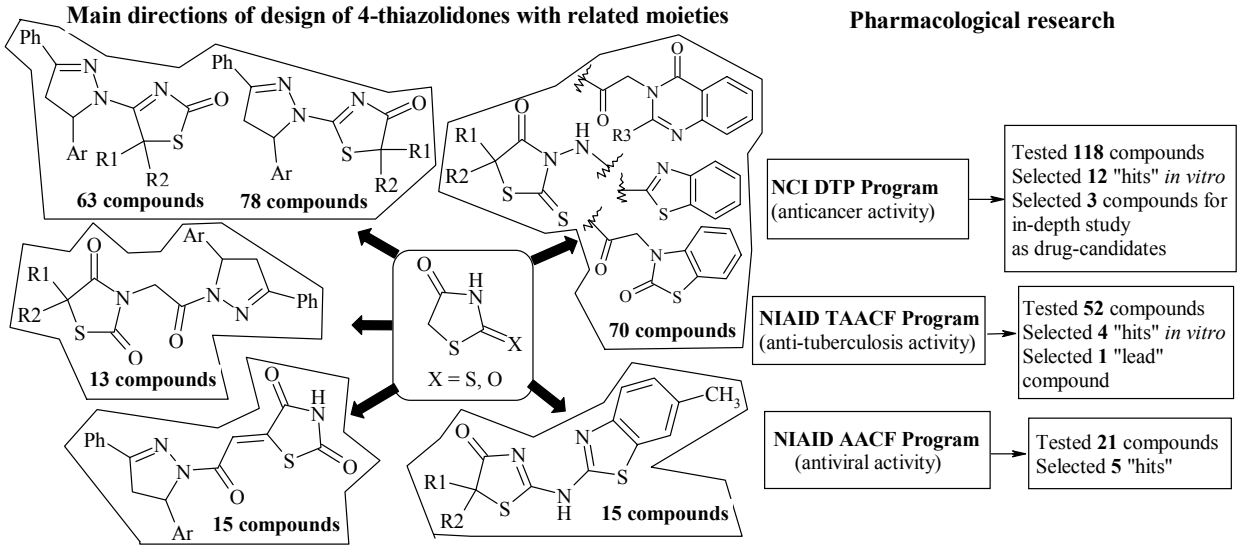
Systematic study of various 4-thiazolidinones allowed us to identify series of compounds as potential anticancer, antiviral and anti-tuberculosis agents. As we established the combination of thiazolidone and diazole or benzothiazole moieties in molecule is perspective direction for new "drug-like" structures design, considering significant biological potential of 4-thiazolidones and the affinity of diazole derivatives (including pyrazolines) to the following antitumor targets, such as cyclin-dependent kinase, heat shock protein (HSP90), vascular endothelial growth factor (VEGF) and P-glycoprotein, etc. We obtained the broad group of 4-thiazolidone derivatives based on chemical modification of thiazolidone cycle in positions 2, 3, 4 and 5. For realization of synthetic schemes the various types of reactions, such as Knoevenagel condensation, [2+3]-cyclocondensation, diazotization, reaction of N-alkylation, acylation, etc were used. Biological activity screening is conducted within the framework of scientific programs of National Institutes of Health (Bethesda, USA): DTP of NCI for anticancer activity, TAACF for anti-tuberculosis activity and AACF for antiviral activity of NIAID. Anticancer activity assays of more then 110 compounds allowed us to identify 3 lead-compounds, which currently are under in-depth in vivo studies according to decision of NCI Biological Evaluation Committee. As a result of anti-tuberculosis assays we identified one lead-compound which was selected for in-depth in vivo study. Antiviral activity screening allowed us to identify some “hit-compounds” which showed moderate or high activity against influenza virus, corona viruses and Tacaribe.



Docking studies have shown that the most probable biotarget for anticancer compounds is Bcl-X_L-BH3 protein complex. QSAR modeling resulted into the row of predictive linear regression models of “anticancer activity” / “molecular descriptors” relationships, which could be used for preliminary prediction of antitumor activity for structurally related compounds.

Lack of pttg-1 gene can cause myelodysplasia and autoimmune disorders

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Pituitary tumor transforming gene-1 is known to induce malignant transformation of pituitary cells and found to be over expressed in many types of tumors. *Pttg*-1 is also believed to be involved in activation of anaphase-checkpoint during the mitosis. Knockout of *pttg*-1 gene caused thrombocytopenia and disproportion of CD4/CD8 lymphocytes in blood. In human, *pttg*-1 gene is located in 5q35.1 (Entrez Gene, HGNC) or 5q33.3 (Ensembl) locus. Deletion of q arm of 5^{th} chromosome, in human leads to myelodysplasia 5q syndrome. This syndrome is characterized by myelodysplastic changing in blood cells concentration and development of autoimmune disorders. Patients with 5q syndrome often die from cancer. It is known that 5q-syndrome develops if deleted 5q31-33 locus, but the molecular explanation of the importance of 5q31-33 locus in development of 5q syndrome was not revealed. We showed that deletion of *pttg*-1 gene can induce myelodysplastic changes and autoimmune disorders in mice similar to 5q syndrome in human. In particular, we found that *pttg*-1 knockout in mice leads to decrease in erythrocytes, thrombocytes and neutrophils number in the blood. However, lack of this gene caused increase in erythrocytes degradation and elevation of bilirubin concentration in the blood, accompanied with compensatory 100% increase in erythrocytes production and doubling number of young erythrocytes in the blood. We also found that blood of *pttg*-1-knockout mice contain elevated level of anti-dsDNA antibodies, while amount of anti-ssDNA and antibodies against simple negatively charged compounds were not elevated. We discovered, that in *pttg*-1 knockout mice anti-dsDNA antibodies are increased owning to 200% elevation of low-affinity anti-dsDNA antibodies, while the level of high affinity anti-DNA antibodies was comparable to that in the blood of wild type mice. Investigation of cancer receptivity of pttg-1 knockout mice is in progress.

Summarizing, obtained by us results suggest the deletion of *pttg*-1 gene to cause autoimmune disorders and myelodysplasia symptoms in mice, similar to 5q syndrome in human. The obtained data drives at functional connection of *pttg*-1 deletion and development of 5q syndrome in human.

Novel expression platform of human arginase I as anticancer enzyme.

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To meet the expected practical needs, many foreign proteins of medical and biotechnological importance have to be expressed in a heterologous host. Often this is not a straightforward task. One of such examples is recombinant human liver arginase I (rhARGI). This enzyme is of great interest for several practical applications but is not currently commercially available due to the absence of efficient producers and purification protocols. First of all, rhARGI is potentially the best enzyme for the development of non-toxic, selective and efficient anticancer enzymotherapy based on single amino acid, arginine, deprivation. We recently developed a novel yeast-based expression system for secreted rhARGI and started the testing of different protocols for its purification to homogenous state. As a heterologous host for rhARGI expression we have chosen the methylotrophic yeast *Hansenula polymorpha,* which is known as an efficient expression platform for human and other eukaryotic proteins. As a eukaryotic host, *H. polymorpha* provides correct folding, little unwanted posttranslational recombinant protein modifications, like hyperglycosylation, little secretion of own proteins and produces virus-free products. We also designed a set of constitutive and regulatable promoters and vectors for stable, including multicopy, integration into *H. polymorpha* genome that do not rely on antibiotic-resistance markers (this is important for bio-safety reasons). Cultivation conditions for yeast producers were optimized that provide stable accumulation of the secreted rhARGI in the culture medium. Enzymatically active purified rhARGI preparations have been obtained on a laboratory scale. Testing of our rhARGI preparations *in vitro* on the models of cultured human cancerous cell lines (as hepatocarcinoma HepG2 sensitive to arginine deprivation) revealed that the enzyme produces strong antiproliferative and proapoptotic effect. Interestingly, rhARG1 appeared to be an efficient antitumor enzyme also in combination with canavanine, arginine analogue of plant origin known as antineoplastic agent.
Summarizing, our data suggest that rhARGI heterologously produced in yeast is an effective antitumor agent and can be further developed as an agent of novel combinational enzymotherapies.

Arginine deprivation induces autophagic process in SKOV3 human ovarian carcinoma cells

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It is known that some tumor cells have an elevated requirement for exogenous arginine (ARG) *in vitro* and *in vivo*, the reason for which is not clearly understood. This feature has been exploited in the development of anticancer ezymotherapy based on ARG deprivation. We hypothesized that upon amino acid restriction, the process of autophagic protein turnover may affect cells response and fate. To address this question, we developed a model of cultured SKOV3 human ovarian carcinoma cells. Using monodancylcadaverine (MDC), a classical fluorescent dye that selectively labels autophagosomes and autolysosomes, we revealed that ARG deprivation induces process of autophagic degradation in SKOV3 cell already after 30 min of starvation. Treatment with classic inhibitors of autophagy, 3-methyl adenine (3MA) and asparagine (Asn), abolished the fluorescent signal. We also monitored the appearance of autophagolysosomes, characteristic of the last stage of autophagic process, using immunofluorescent staining of the autophagosome proteins MAP LC3 and Beclin1 as well as lysosome membrane proteins LAMP1 and Golgin97. The colocalization of MAP LC3/LAMP1 and Beclin 1/Golgin 97 signals was observed already at 30 min of ARG deprivation, what is the strong evidence for induction of autophagy (Fig. 1A). Colocalization of these proteins was not detected upon treatment with different autophagy inhibitors (3MA, Asn, and chloroquinone (CQ)). We also observed accumulation of MAP LC3 protein upon treatment with autophagy inhibitors (Fig. 1B). SCOV3 cells were resistant to classical apoptosis upon ARG deprivation as suggested by the absence of the cleavage of caspase-3 into its activated 17 kDa fragment, even upon treatment with autophagy inhibitors (Fig. 1B).

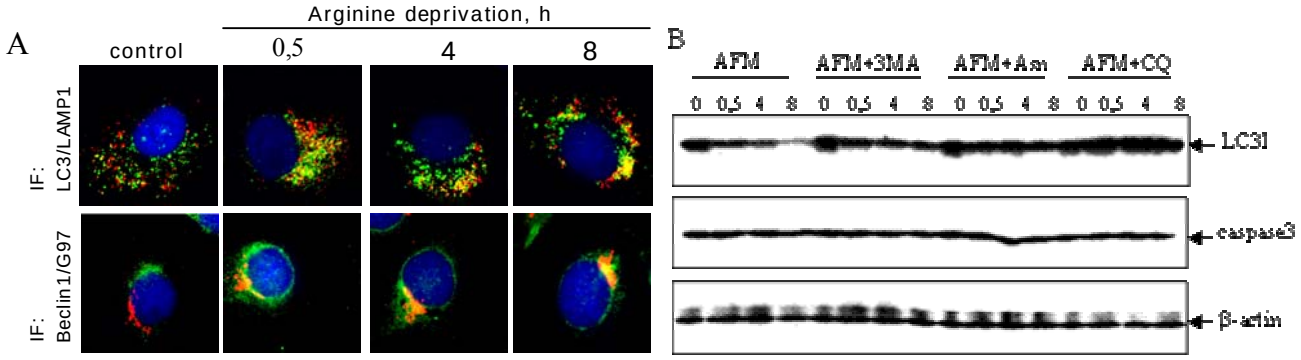

Fig.1. ARG depletion rapidly induces autophagy in SKOV3 cells:
A - Immunofluorescence staining of MAP LC3 and Beclin 1 (green fluorescence) as well as LAMP1 and Golgin 97(red fluorescence) in SKOV3 cells upon ARG starvation. Nuclei labeled with DAPI. **B** – Immunoblot of the lysates of SKOV3 cells incubated in ARG-free medium (AFM) using polyclonal MAP LC3 and caspase3 antibodies.

Our data suggest that single amino acid, ARG, deprivation strongly induces autophagic response in the ovarian carcinoma SKOV3 cells. Autophagy may serve as a protective mechanism upon such conditions providing the additional recycling of ARG from preexisting proteins. It remains to be elucidated whether there is a positive correlation between autophagy induction and survival of different tumor cells upon ARG deprivation. We suggest that ovarian carcinoma SKOV3 may serve as a suitable model for studying the role of autophagy modulation in anticancer therapy.

Over expression of adaptor protein Ruk/CIN85 decreases estrogen sensitivity and promotes resistance to TNF-α-induced apoptosis of breast adenocarcinoma MCF-7 cells

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Ruk/CIN85 is a SH3-containing adapter/scaffold protein that plays important roles in the regulation of homeostasis in normal cells and also can be involved in carcinogenesis by influencing a number of processes such as cell adhesion, motility and apoptosis. Our previous findings suggest that high levels of Ruk/CIN85 modulate EGF-dependent signalling and contribute to the conversion of breast adenocarcinoma MCF-7 cells into a more malignant phenotype. Since estrogens are critically involved in the development of breast cancer, it was the aim of the current study to compare sensitivity of MCF-7 cells with different levels of stable Ruk/CIN85 expression to β-estradiol treatment. Mock-transfected MCF-7 cells as well as sublines that stably over express either low (D4) or high (G4) levels of Ruk/CIN85 were treated with β-estradiol (20 and 100 pM). MCF-7 cell viability was analyzed by MTT-test after three days of β-estradiol stimulation. In concordance with previous data, β-estradiol in our study increased proliferative activity of MCF-7 cells in a concentration-dependent manner. However, β-estradiol sensitivity of cell sublines that stably over express Ruk/CIN85 was lower as compared to control MCF-7 cells. There are data that Ruk/CIN85 plays a role in control of apoptosis induced by TNF-α treatment. Therefore, another aim of the study was to investigate whether over expression of Ruk/CIN85 affected MCF-7 sensitivity to TNF-α-induced apoptosis. Control MCF-7 cells and three MCF-7 sublines with different levels of Ruk/CIN85 over expression (D4, G4, and G10) were cultured for 3 days in presence of TNF-α (5, 10, 50 ng/ml). Cell viability was analyzed by MTT-test. It was shown that TNF-α decreased viability of control as well as D4, G4, and G10 subline cells in a concentration-dependent manner but viability of Ruk/CIN85 over expressing cells was higher in comparison with control cells. Apoptotic cell death was determined by increase of cleaved PARP levels. For this purpose, control MCF-7 cells and D4, G4, G10 sublines were cultured in the presence of TNF-α (10 ng/ml) for three days. The cell lysates were analyzed by Western blotting using antibodies against cleaved and intact PARP. The results of Western blot analysis correlated with MTT test results: the highest accumulation of cleaved PARP was detected in control cells and the lowest - in G10 subline cells. Taken together, our data show that over expression of Ruk/CIN85 in MCF-7 cells is accompanied by attenuation of estrogen sensitivity and increased resistance to TNF-α-induced apoptosis.

The role of inositol requiring enzyme-1α signaling in brain tumor growth and gene expression

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Astrocytes represent the most abundant cell type in mammalian brain, and play an important role in the maintenance and regeneration of neuronal functions. Inadequate responses of astrocytes to ischemic conditions may contribute to the development of various pathologies of the nervous system. Hypoxia and glucose deprivation, which are essential features of ischemia and chemicals have been shown to induce a set of complex intracellular signaling events known as the Unfolded Protein Response (UPR). This adaptive response is activated upon accumulation of misfolded proteins in the endoplasmic reticulum (ER) and is mediated by three ER-resident sensors named PERK (PRK-like ER kinase), IRE1/ERN1 (Inositol Requiring Enzyme-1) and ATF6 (Activating Transcription Factor 6). Activation of the UPR tends to limit the *de novo* entry of proteins in the ER and facilitate both ER protein folding and degradation. Cells' ability to handle these stresses may therefore condition their intrinsic capacity to adapt for cell survival or, alternatively, to enter cell death programs through ER-associated machineries. IRE1 activation and signaling is a common molecular determinant linking hypoxia- and hypoglycemia- dependent responses to the up-regulation of several pro-angiogenic and neurotrophic factors, including vascular endothelial growth factor, fibroblast growth factor 2, hepatocyte growth factor, CSF1R, tissue factor, death receptor 6 and DATF1, in various cell types including glioma-derived cells. IRE1 is a single-spanning ER transmembrane protein that expresses both intrinsic Ser/Thr protein kinase and endoribonuclease activities in its cytosolic domain. Astrocytes subjected to ischemia trigger distinct signaling pathways contributing to angiogenic stimulation, neuronal stabilization and survival. The blockade of IRE1 ser/thr kinase and RNase activities is thought to either lead to a decrease in survival signals between astrocytes and neuronal cell, as suggested by transcriptomic analyses, or/and triggered cytotoxic signaling towards neuron-type cells. The blockade of IRE1 RNase activities precipitates U87 tumor cells to death after only a few passages in culture. We determined a set of genes which expression depends from IRE1 RNase activity and possibly involved in death processes. Thus, the evaluation of respective roles of the two intrinsic catalytic activities (kinase and endoribonuclease) of IRE1 in its signaling is very important in order to get chemical inhibitors that block IRE1 as a whole or only on its RNase activity.

Circadian and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase genes expression in glioma cells: effect of hypoxia and nutrient starvation

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Hypoxia and glucose deprivation, which are essential features of ischemia, have been shown to induce a set of complex intracellular signaling events known as the Unfolded Protein Response (UPR). This adaptive response is activated upon accumulation of misfolded proteins in the endoplasmic reticulum and is mediated by Inositol Requiring Enzyme-1 (IRE1) as well as by two others endoplasmic reticulum-stress sensors: PRK-like ER kinase (PERK) and Activating Transcription Factor 6 (ATF6). We studied effect of hypoxia and nutrient (glucose or glutamine) starvation on the expression of several circadian and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase genes in U87 glioma cells and its subline without IRE1 ser/thr kinase and ribonuclease activities using real time polymerase chain reaction. We have shown that the expression of Clock, BMal2 and Per3 was decreased in hypoxic conditions in glioma U87 cell line. However, expression of BMal1b, BMal1e, Per1 and casein kinase-1 epsilon circadian genes as well as 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 was increased in this line of glioma cells in hypoxic conditions. The blockade of IRE1 ser/thr kinase and ribonuclease activities is lead to an increase of most circadian genes (Clock, BMal1b, BMal1e, Per1, Per2, Per3 and casein kinase-1 epsilon) and PFKFB genes (PFKFB-3, PFKFB-4 and PFKFB-2), but to a decrease of BMal2 and casein kinase-1 delta. Glutamine or glucose deprivation conditions are lead to suppression of the expression of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 and 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-4 in glioma cells without IRE1 kinase and ribonuclease activities only. Expression of circadian genes casein kinase-1 epsilon, BMal1b and BMal1e was increased both in U87 glioma cells and in its IRE1-negative subline but expression of BMal2 was decreased in these cells. However, the expression of circadian genes Clock, BMal1b, BMal1e and Per1was increased only in the cells without kinase and ribonuclease activities in glutamine deprivation conditions. The blockade of IRE1 kinase and ribonuclease activities in U87 glioma cells was induced expression of Clock, Per1 and BMal1b under glucose deprivation conditions. Thus, the expression of different circadian genes is depended from inositol requiring enzyme-1 signaling as well as from nutrient (glucose or glutamine) starvation.

Cardiovascular Diseases & Prevention

F2 G20210A, *F5* G1691A, *MTHFR* C677T polymorphisms – involvement in stroke

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Stroke is considered to be a complex polygenic disorder arising from a wide number of gene–gene and gene–environment interactions. The *F2* G20210A and *F5* G1691A mutations are the most common genetic risk factors of venous thrombosis and ischemic stroke. *F2* G20210A is associated with higher plasma prothrombin concentrations and augmented thrombin generation. *F5* G1691A provokes a structural change of a factor V molecule and shifts the balance toward thrombosis in the clotting cascade. The *MTHFR* C677T gene variation is associated with 30-50% reduction of the enzyme activity and is the most common inherited cause of hyperhomocysteinemia, which increases the risk of ischemic stroke. Aim of the study was to establish possible involvement of the *F2* G20210A, *F5* G1691A, *MTHFR* C677T genes alleles into stroke development. Unrelated individuals from different regions of Ukraine were included in this study: case group - patients with ischemic stroke (n=183, men-95, women-88), average age – 64.6±9.1, control group I - individuals from the general population (n=100, men-50, women-50), average age – 30.2±7.6, control group II – healthy individuals elder than 65 years (n=88, men-35, women-53), average age – 73.9±6.4. No significant difference between the age of men and women within each group was observed. Blood samples for DNA analysis were obtained after informed consent. Genotyping was performed by PCR followed by RFLP analysis. The differences were considered significant at $P<0.05$ value of Fisher exact test. *F2* 20210A carriers frequency in the case group (4.6%) was significantly higher than in control group II (0%) and was higher in men (7.4%) comparing to women (1.1%) in the case group. Frequency of genotype with at least one risk allele of *MTHFR* 677T was higher in women from the case group (54%) than in women from control group II (41%). Frequency of combined genotype with at least one of the risk alleles of *F2* or *F5* or bearing two copies of the *MTHFR* 677T frequency in men from case group (20%) was higher than in men from control group II (5%). In women from case group (57%) frequency of the combined genotype with at least one of *F2, F5, MTHFR* genes risk alleles was higher than in women from control group II (41%). Our findings suggest a relevant role of *F2* 20210A, *F5* 1691A, *MTHFR* 677T alleles in stroke development.

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Vasculometry of upper and lower extremities in correlation with development of pathologic conditions like the diabetic foot

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We assume that the vascular apparatus of the lower limb did not evolve to adapt to leg mass and volume. The lower limb is greater in length and volume than the upper limb, and therefore the arteries should have a bigger diameter and cross-sectional area. During pathoanatomic autopsies on the Department of Pathology of Clinical Hospital Center Osijek we have taken segments of 1 cm of length from the subclavian, femoral, radial and tibial artery. Our sample contained segments from 51 bodies, 24 female and 27 male. We have measured leg and arm length and circumference. From these data the idealized limbs volume was calculated by geometric approximations to a cone fragment. The relation between idealized leg and arm volume and arterial cross-sectional area were calculated. For statistical analysis, Student's t-test was used. There is a slight difference between the diameter and cross-sectional area of subclavian and iliac (femoral) artery. Leg length was for 48.5% bigger than arm length and the difference in volume between upper and lower limb is significantly different. The foot has four to five times greater volume than the arm, and is vascularised by an arterial tree of similar diameter. This fact proves our hypothesis that the blood supply to the lower limbs compared to the mass of tissue is smaller.

Remote monitoring of single lead implantable defibrillator holders

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Lack of direct discrimination of atrial and ventricular rhythm disturbances is the major disadvantage of VVI-ICD. Remote monitoring is a goal of modern ICD follow-up.
The **aim** of this study is to analyze the usefulness and reliability of single lead implantable cardioverter defibrillators (VDD-CD) with home monitoring system (HM).
Patients and methods: 18 patients - 12 males, mean age: 58,9 ± 14,9 years, ejection fraction: 36,33±12% - underwent single-lead VDD-CD (Biotronik Lexos A+T) implantation between June 2007 and December 2009 in our clinic. All patients were monitored for 14,7 ± 8,3 months, mean clinical follow-up was 3,11 ± 2,29. HM events (supraventricular tachycardia (SVT), ventricular tachycardia (VT), ventricular fibrillation (VF)) were analyzed. **Results:** in about 44, 4% (n=8) of the patients 911 HM events (747 SVT, 116 VT, 48 VF) were detected. Antitachycardia pacing (ATP) was used in 195 and cardioversion (CV) in 83 cases. 93 ATPs were successful, 102 were inadequate. Out of 34 started CV with low energy following failed ATPs, 24 were successful, 9 were failed and 1 was aborted. The 9 failed CV occurred in 1 patient, and the reinitiation of the arrhythmia was stopped due to the repeated ATP or CV. 49 high energy shocks were initiated due to VF, and 13 of them were successful. 35 detected VF were terminated spontaneously, 1 shock was ineffective. **Conclusion**: The method of implantation of VDD-CD is equal with that of VVI-CD. Atrial sensing ensures a direct discrimination of atrial arrhythmias, decreases the number of false detection and therapy. In patients with atrio-ventricular block either AV conduction with long PR interval, or P wave triggered ventricular stimulation is possible. Remote monitoring allows early detection of arrhythmias and shortens physicians' reaction time from their development to therapy. The VDD-CD with HM option is a milestone in the follow-up of patients living with ICD.

Effects of postconditioning on kidney ischemia/reperfusion injury in hypercholesterolemic rats

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Background: Ischemia/reperfusion injury frequently threats the integrity of the organs during surgery. The protective effect of postconditioning (PK), the short repetitive ischemia/reperfusion cycles, applied in the beginning of reperfusion, has been improved the outcome in vital organs. Signaling cascades are induced by PK interfere in several points to preconditioning, which is blocked by metabolic diseases, such as insulin resistance and type 2 diabetes. The **aim** of our study was to compare the efficacy of PK after reperfusion injury of both kidneys in metabolically healthy and hypercholesterolemic rats. **Methods**: Male Wistar rats (n=30) were fed by either normal or high cholesterol (1.5%) containing chow for 8 weeks. Following pretreatment period, both groups were divided into two subgroups, where under general anesthesia both kidneys were exposed either to 45 min ischemia followed by 2 hours of reperfusion, or to additional 4x15 sec postconditioning cycles. Serum creatinine, carbamide, oxLDL levels, PMA-induced free radical production was determined before and after surgery. After euthanasia both kidneys were removed for TNF- alfa immunohistochemistry, and for PAS and HE staining. **Results:** Cholesterol feeding resulted in a significant elevation in serum cholesterol and triglyceride levels ($p<0.05$). In the control rats significant elevation was observed in free radical production ($p<0.01$), lipid peroxidation ($p<0.01$), and serum TNF-alfa levels ($p<0.05$), following ischaemia and reperfusion, which were markedly reduced by post conditioning. However, we did not reveal any beneficial effect of post conditioning in the cholesterol fed rats. Tissue TNF- alfa level was significantly higher in cholesterol fed, than in control animals, and this high level did not change in response to post conditioning. In healthy animals post conditioning caused a significant reduction ($p<0.05$) in tissue TNF- alfa level. **Conclusions:** PK proved to be a very effective defense against I/R in healthy animals, but it was ineffective in hypercholesterolemic ones.

Comparison of red wine, beer and vodka effects on oxidative stress and increase in arterial stiffness after normobaric oxygen breathing in healthy humans

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Introduction: We determined and compared acute effects of different alcoholic beverages on oxygen-induced increase in oxidative stress plasma marker and arterial stiffness in healthy humans. **Materials**: Ten males randomly consumed one of four tested beverages: red wine (RW), beer, vodka (0.32 g ethanol / kg body wt) and water as control. Every beverage was consumed once, a week apart, in a cross-over design. The volunteers breathed 100% normobaric O2 between 60th and 90th min of 3h study protocol. Plasma lipid hydro-peroxides (LOOH) and uric acid (UA) concentration, blood alcohol concentration (BAC) and arterial stiffness (evaluated as augmentation index, AIX) were measured before and 30, 60, 90, 120 and 180 min after beverage consumption. **Results**: Intake of all alcoholic beverages caused a similar increase of BAC. In contrast to that, only RW caused significant increase in plasma UA (34±4 vs. 15±3, -6±2 and -8±2 μmol/L for, beer, vodka and control, respectively). Exposure to oxygen resulted in increased plasma LOOH in all groups. However, in RW group this increase was lowest (1.1±0.5) in comparison to the control (2.5±0.4), beer (1.6±0.3) and vodka (2.1±0.5 μM/L H2O2). The oxygen-induced elevation in AIX was similarly reduced in all three alcoholic beverage groups relative to the control (13.7±2.6 vs. 3.4±1.3, 0.2±1.6 and 5.4±2.2 % for red wine, beer, and vodka, respectively). **Conclusion**: RW provided protection against oxygen-induced oxidative stress, in contrast to beer and vodka. This beneficial effect was mainly mediated by corresponding increases in plasma UA levels. All three alcoholic beverages provided similar protection against oxygen-induced increase in arterial stiffness, probably due to central vasodilatatory effect of alcohol itself.

Mother and Child Health
Reproductive Health

PAPP-A as an early marker for Fetal Growth

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Objective: Determination of pregnancy associated plasma protein (PAPP-A) and beta-hCG for bi-test evaluation in week 11w0d-13w6d may help in predicting an accelerate growth later in pregnancy and a Large for Gestational Age (LGA) or macrosomia at birth. **Method**: Prospective study on 193 patients who made the bistest between 11w0d-13w6d and weight at delivery. LGA was defined as a weight over 90 percentile for gestational age and macrosomia as a weight over 4000 grams at term (over 37 gestational weeks). Statistical correlation was made for Mom and values in MoM (calculated by PRICA software) and in mUI/mL, and the statistical power was evaluated by Mann-Whitney test is SPPS 12 ROC curves. **Results**: PAPP-A values were significantly increased in women who delivered both macrosmic and LGA babies (n=23/131, 1.12 MoM; p=0.036). The cutoff value for an increase for fetal accelerate growth was 5.41 mUI/mL. Association with other pregnancy complications as intrauterine growth retardation, small for gestational age, pretermbirth and gestational diabetes are under analasys.
Conclusion: Level of PAPP-A in maternal serum can be an early predictive factor of fetal growth.

Complex role of ultrasound and biochemical markers for prenatal diagnosis of chromosomal abnormalities

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Introduction: Prenatal study is used to identify major genetic and congenital abnormalities in a developing fetus. Prenatal karyotyping was performed in women with high risk of children's birth with chromosomal abnormalities. Actual problem for today remains to determine the most specific clinical and laboratory markers to the chromosome abnormalities. **Aim**: Establish communication between the deviations of indicators of ultrasound and biochemical examinations of pregnant women and established prenatal chromosomal abnormalities. **Materials and methods**: Cytogenetic analyses of the chromosomes from amniocyte *in vitro* culture were performed according to standard protocols. Ethidium bromide (10mcg/mL) was added simultaneously with colchicines in order to obtain high-quality chromosomes of early and middle mitotic stages. GTG and CBG banded chromosomes were analyzed at the 550 bands resolution level. Analyzed the frequency of detected chromosomal abnormalities depending on indications to the amniocentesis. **Results**: Prenatal study have been performed in pregnancies after genetic counselling in the next cases: changes in maternal serum markers during first/second-trimester; ultrasonic markers of congenital malformations; family history of stillbirth, recurrent spontaneous abortions, affected child with congenital birth abnormalities; advanced maternal age >35 year; previous child with a de novo chromosomal abnormality; presence of structural chromosome abnormality in one of the parents. 74 prenatal cytogenetic studies have been performed. In 14 amniocyte cultures (18,9%) numeral (9 cases) and structural (5 cases) changes of the karyotype were detected. In 11 cases unbalanced chromosomal abnormalities was observed: trisomy of 21-st and 18-th chromosome; simple and mosaic forms of gonosomal monosomy; additional marker chromosomes. Non-balanced derivative chromosomes were detected in two fetuses — 46,XY,der(4)(?::p16→q35)de novo and 46,XY,der(10), t(10;7)(q26,13; p21.2)pat. High level of unbalanced chromosomal abnormalities was confirmed in fetuses from pregnancies with ultrasound markers of congenital malformations – 16.6% and maternal serum markers – 13.3%. The highest percentage established prenatal unbalanced chromosomal abnormalities – 33.3%, observed deviation of ultrasound and biochemical markers simultaneously. **Conclusion**: Therefore, the combination of ultrasound and biochemical studies of pregnant women is most effective for establishing appropriate prenatal cytogenetic analysis.

Analysis of the potential role of Apolipoprotein E polymorphism in genetic predisposition to spontaneous abortion.

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Introduction: According to estimates, up to 20 % of pregnancies end in the first trimester by spontaneous abortion. There are many causes (negative influences of the environment, genetic determination, etc.) but a significant number still remains unexplained. The aim of this study is to investigate the role of variants in the gene for Apolipoprotein E in the genetic determination of spontaneous abortions. **Material and methods**: We collected DNA from 171 samples of spontaneous abortions and genotyped Apolipoprotein E by PCR-RFLP method. The frequencies were compared with known population sample of adults (N=653). **Results and discussion**: The frequencies of the Apolipoprotein E genotypes did not significantly differ from the frequencies in analyzed population study (Table). Out pilot study suggests that apolipoprotein E is not a major determinant of spontaneous abortions in Caucasians.

Table

	Numbers of carriers according the Apolipoprotein E genotype			
Genotype	Controls		Aborts	
	n	%	n	%
22	3	0,5	4	2,3
32	75	11,5	16	9,4
33	444	68,0	109	63,7
43	108	16,5	33	19,3
42	12	1,8	5	2,9
44	11	1,7	4	2,3

Pregnancy loss – "Split protocol" for successful next pregnancy

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Chromosome aberrations and infections come first as potential causes of pregnancy loss. The two hits theory is established acknowledging the role of infections in pregnancy loss. The first hit could have been triggered by patient's prior genitourinary system infection, while the second hit is triggered by the reactivation of latent EBV infection.
The presented results support this hypothesis, which encouraged us to propose the "Split protocol" for planning a successful next pregnancy. Couples (all together 442) having one, two, three, four or more pregnancy losses (PL) were analyzed in this retrospective case study. The majority of couples (265) had two (530) PL. From 312 samples of the aborted material, aberrant karyotype (trisomy, monosomy, aneuploidy, tetraploidy and others) was found in 21.8% of cases. Pathological analysis revealed hydropic degeneration in the majority of samples. Genitourinary infections prior to pregnancy were reported in 58% female and 41% male participants. These infections were most frequently caused by E. coli, Enterococcus, Streptococcus agalactie, Ureaplasma urealyticum and/or Mycoplasma and Chlamydia. Serological analysis on samples of 309 female and 293 male participants revealed the presence of EBV EA in 67 female and 22 male participants. Presence of IgM and/or IgA against CMV, HSV1/2, Rubella, Toxoplasmosis, HHV6, Parvo B19, RSV, Adenovirus and Varicella Zoster, as a result of reactivation of infection in pregnancy, was also noted. Forty-three percent of couples with aneuploidy in the aborted material (54) had positive serological results. Serum iron values and feritine values for 65 and 74 women respectively, indicate that they have suffered from sideropenia and low iron reserve. Urinary and/or genital infection and low iron reserve must be regulated before the next pregnancy. The effect of the reactivated infection must be excluded in next pregnancy.
Research of the genes involved in this process is the goal of the future “Split protocol project".

Evaluation of cervical, pharyngeal and anal HPV status of female sex-workers in Hungary

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Human papilloma virus (HPV) is the most common sexually transmitted infective agent known today. Sexually active women are the most affected, approximately half of them is infected at least once during their lifetime. Chances of being infected and transmitting the virus correlates with the number of sexual partners. Sexual intercourse is not even mandatory in terms of development of HPV infection, genital contact (skin to skin) is fair enough. The use of condom decreases the risk, but doesn't give full protection. When talking about sexually transmitted diseases (STDs), including HPV, we must focus our attention on prostitutes, who are mostly endangered. Switching partners is not without risk nowadays. Every fifth switch goes hand in hand with an HPV infection, which means that prostitutes and their partners are in greater jeopardy every day.

In malignant transformations of chronic HPV infections high risk HPV types are involved. Earlier HPV was only connected to cervical cancer, but latest data show, that HPV is the primary cause of more than 95% of cervical, 50% of genital (vagina, vulva, penis), 70% of anal and 20% of oro-pharyngeal cancers. A survey based prospective study was carried out screening the sexual habits of women prostitutes and their knowledge of HPV. Thorough STD screening was supplemented with HPV testing using cervical, anal and pharyngeal samples along with Pap smear screening. The questioned population had bare knowledge about HPV, the infection and its long distance consequences. It can be stated that almost every one of them uses condoms on a regular basis as protection against sexually transmitted diseases. We found HPV DNA positivity in 82,4% (14/17) of the cases. 11 HPV genotypes were identified, mostly HPV 16 and 33. 53% of HPV positive women (9/17) tested positive for high risk HPV, 23,5% (4/17) was positive for more than one type of HPV. High risk HPV positivity was proven as follows: 41,1% (7/17) of cervical, 17.7% of anal (3/17) and 11,8% (2/17) of pharyngeal samples. Screening, sampling, testing and evaluation are still under way.

HPV infection is in most cases symptomless and remains latent for months or even years, carrying the potential of malignant transformation. Since prostitutes are sources of infection and their partners are in danger of getting infected thus becoming a significant health hazard, routine STD screening among prostitutes (Chlamydia, Hepatitis-B, HIV, Syphilis, Gonorrhea – once in every 3 months) should include HPV testing as well.

Inflammatory & Immune Response

Anti-inflammatory and antioxidant effects of thiazolidin derivatives possessing dual cox/lox inhibition upon the heart tissue and gastric mucosa of rats

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Compounds that combine COX/LOX inhibition are potential new drugs to treat inflammation. Dual inhibitors, by acting on the two major arachidonic acid metabolic pathways – cyclooxygenase (COX) and lipooxygenase (LOX), possess a wide range of anti-inflammatory activity. Besides that, dual inhibitors appear to be almost exempt from gastric and cardiovascular toxicity, which is the most troublesome side effect of COX inhibitors.

The purpose of the research was to determine PGE_2, LTB_4 concentration and activity of the antioxidant protection system in heart tissue and gastric mucosa under prolonged application of thiazolidin derivatives possessing dual COX/LOX inhibition. {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-acetic acid – (agent 1) (10 mg/kg) and {2,5-Dioxo-3-[4-oxo-5-(3-phenyl-allylidene)-2-thioxo-thiazolidin-3-yl]-pyrrolidin-1-yl}-benzene-sulfonamide – (agent 2) (10 mg/kg) were introduced per os for 14 days. The concentrations of PGE_2 and LTB_4 were determined in homogenates of healthy rats' tissues using ELISA method after blockage of COX/LOX. It was shown that the content of both metabolites of arachidonic acid had the tendency to decrease, proving the inhibitory action of both agents. Injection of agents 1 and 2 considerably increased activity of the antioxidant protection system enzymes (catalase, SOD, GP, GR) in both investigated tissues. After dual inhibition by agents 1 and 2 MDA content in heart tissue was increased by 28% and 30% subsequently. MDA concentration remained unchanged gastric mucosa after action of these 2 types of inhibitors. COX/LOX dual inhibition led to considerable rise in NO concentration in gastric mucosa (by 40% and 22%). Morphological changes of gastric mucosa due to injection of agent 1 that are evidence of a higher degree of integrity of the mucous barrier components in comparison to the action of COX-2 inhibitor celecoxib, increased density of epithelial cells of the surface of mucosa, reduced degree of edema. Due to injection of agent 2, protective effect upon the status of mucous membrane was less manifested.

Enhancement of cytoprotective mechanisms under combined action of amaranth oil with blockage of inos in experimental colitis

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In ulcerogenic colitis lipoperoxidation processes and activity of NO-synthases intensify, and content of nitric oxide and proinflammatory cytokines in the mucous membrane of large intestine (MMLI) increases. Ulcerogenic colitis is treated with the drugs blocking iNOS, cyclooxigenase-2 and 5-lipooxigenase, and different oils containing considerable amounts of ω-3, ω-6 and ω-9 of unsaturated fatty acids. **Purpose** of the research was to study the effect of blockage of iNOS activity with aminoguanidine combined with injection of amaranth oil on the cytoprotective and metabolic processes under experimental ulcerogenic colitis. **Methods**. Ulcerogenic colitis in rats (n = 60) was modeled with 4% acetic acid. In the MMLI homogenates were determined: content of TBA products, activity of SOD, catalase, eNOS, iNOS, NO and concentration of L-arginine in the plasma of blood. **Results**. Injection of 4% acetic acid induced development of ulcerogenic defects, erosions, massive hemorrhages of the area of 77.2 ± 25.1 mm^2. Content of TBA products increased by 2.2-fold ($p < 0.001$), activity of SOD enhanced by 71 %, of catalase – by 54 % ($p < 0.01$), of general NOS – by 2.5-fold ($p < 0.001$), of eNOS – by 21 %, of iNOS – by 6.9-fold, content of NO in the MMLI increased by 64 % ($p < 0.05$). Concentration of L-arginine in the blood decreased by 51% ($p<0.001$). Due to iNOS blockage with aminoguanidine, the area of ulcerogenic lesions decreased to 69.6±19.7mm2. Activity of general NOS reduced by 41 % ($P < 0.01$), of iNOS – by 46 % ($P < 0.0$!). MDA content decreased by 28 % 9$p < 0.05$) and catalase activity diminished by 20%. Injection of amaranth oil caused reduction of the area of ulcerogenic lesions to 45.4 ± 20.83mm2. Activity of general NOS reduced by 52 % ($p < 0.01$), of iNOS – by 69 % ($p < 0.001$), NO content decreased by 29 %, and concentration of L-arginine in the blood increased by 23 %, contents of TBA products decreased by 35 % and catalase activity reduced by 27 %. In combined action of amaranth oil with the blockage of iNOS by aminoguanidine, area of MMLI lesions considerably decreased and was 38.8 ± 30.1mm2 ($p < 0.01$). Activity of general NOS diminished to the normal and activity of iNOS – by 73 % ($p < 0.001$). Content of MDA and catalase was at the level of intact animals. Thus, combined action of aminoguanidine with amaranth oil manifests pronounced cytoprotective and anti-inflammatory effects in ulcerogenic colitis that is associated with a steep decline in the activity of NO-synthases and indices of oxidative stress. Obtained results substantiate application of combined action of oils with high concentrations of unsaturated fatty acids and selective blockers of iNOS in treatment of inflammatory processes.

Peculiarities of apoptosis-induced DNA degradation against experimental esophagitis and its correction by melatonin

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Gastroesophageal reflux disease is one of the topical issues in medicine. Great interest in this issue is caused not only to increase of prevalence rate of the said disease, requiring thorough investigation of terminal exchange components, functional failure of which reduces resisting power of epithelial barrier of mucous coat of esophagus. We conducted the research of apoptosis-specific internucleosomal DNA degradation of cells of mucous coat of esophagus in rats against esophagitis simulation and its correction by melatonin. The research was conducted on sexually mature male rats. Experimental esophagitis was induced by means of dosed administration (using an external perfusion technique) of acidic and pepsic mix for days (an experimental group). Melatonin was administered (intra-abdominal administration in the dose of 20 mg/kg per day) for days in a separate group of experimental animals against perfusion of the acidic and pepsic mix. A fluid rich in cells of mucous coat of esophagus was used, after pre-treatment, for separation and cleaning of fragmented DNA using traditional DNA ladder technique (Herrmann, 1994). Fragmented DNA was visualized, after separation, on a transilluminator, and photographed. Conducted research is indicative of the fact that significant apoptosis-specific internucleosomal DNA degradation can be observed against experimental esophagitis model used by us. With the view to peculiarities of change of other parameters explored by us (ultrastructural changes, production profile of anti-inflammatory cytokines), and reference data, such pattern of changes in mucous coat of esophagus may be rated as a moderately grave condition. Death of epithelial esophageal cells occurred in individual animals among analyzed general population with experimental esophagitis by means of apoptosis, as well as necrosis. According to reference data, apoptosis is treated as a highly regulated active process, mostly depending on efficiency of oxidative energy-producing metabolism. Findings prove reasonability of use, under the present conditions, of melatonin that can universally reduce, according to reference data, level of tissue hypoxia, improve usage of lipid peroxidation products, stimulate production of NOS with further vasodilatation and improvement of local blood flow. Degree of manifestation of ultrastructural pro-apoptotic changes appeared certainly lower in the group of experimental animals for whom melatonin was used as a corrective medium. Positive effect of melatonin use against experimental gastroesophageal reflux disease model is apparently due to the ability of melatonin to provide for cell oxidation homeostasis and optimize local blood supply of epithelial cells of esophagus by means of attraction of metabolites of nitrogen oxide cycle.

The effect of rabeprazole on gastric acid and mucoid-electrolyte secretion in patients with duodenal ulcer

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Background and aim. Proton pump inhibitor (PPI) rabeprazole has been widely used in clinical practice due rapid suppression of gastric acid secretion. However, it is still little known about the mucoid-protecting action of rabeprazole. The aim of the study was to evaluate the effects of rabeprazole on gastric acidity and mucoid-electrolyte secretion in patients with duodenal ulcer after two weeks therapy with this PPI. **Methods**. 15 patients with duodenal ulcer (Helicobacter pylori-positive) were investigated. The size of ulcerous defect ranged from 0,5 to 1,5 cm. All patients received triple therapy that included the standard dose of rabeprazole (20 mg/day), clarythromycin and metronidazole for two weeks. The volume of gastric aspirate, hydrogen ion concentration, gastric acid output, pepsin concentration, N-acetylneuraminic acids (NANA) and Na ions concentrations in gastric juice and insoluble mucus were measured and compared before and after treatment with rabeprazole. **Results**. After two weeks, complete healing of ulcer was documented in 60 % of patients. Rabeprazole demonstrated inhibitory effect on gastric acidity by 72 %, volume secretion by 50 %, and pepsin concentration by 82 %. At the same time level of NANA and Na-ions increased by two times in gastric juice compared in insoluble mucus. **Conclusion**. Healing of the ulcerous defect after rabeprazole admission is due to effective lowering of acidity and peptic secretion. At the same time increased the frequency of duodenogastral reflux and mucous secretion. Future investigations are needed to determine influence of switching from function of high specialized parietal cells to the phylogenetically older mucous secretion in consequence of epithelial cells rebuilding.

Role of hemodynamic forces in the regulation of vasomotor tone of skeletal muscle venules

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Background: The roles of hemodynamic forces, such as pressure and wall shear stress have been well characterized in arterioles. It has been shown that smooth muscle and endothelial mechanisms (nitric oxide (NO) and dilator prostaglandins) contributes to the development of vasomotor responses and in certain conditions reactive oxygen species, as well. Much less is known regarding the importance of hemodynamic forces-induced regulation of vascular resistance in venules. **Methods and results**: Isolated rat gracilis muscle venules developed substantial tone in response to increases in intraluminal pressure (active diameter: ~260 μm, whereas passive diameter: ~360 μm at 10 mmHg). Presence of catalase or indomethacin reduced significantly the pressure-induced development of myogenic tone (control: 40 +/- 5 % vs. control+CAT: 60 +/- 11 % of PD, at 5 mmHg pressure). In addition, venules dilated as a function of intraluminal flow, which was augmented in the presence of the thromboxane A2 (TxA2) receptor antagonist SQ 29,548 or the TxA2 synthase inhibitor ozagrel. The selective cyclooxygenase (COX)-1 inhibitor SC 560 reduced, whereas the selective COX-2 inhibitor NS 398 enhanced flow-induced dilations. We have also found that H2O2 elicited concentration dependent constrictions (max.: 137 +/- 8 to 61 +/- 18 μm), which were inhibited by the presence of indomethacin or SQ 29,548. **Conclusion**: Skeletal muscle venules exhibit myogenic response, which is - in part - mediated by H_2O_2, which elicits the release of constrictor TxA_2. In addition, flow/shear stress dependent regulation of diameter is present in venules, which is mediated by simultaneous release of constrictor TxA_2, the dilator NO and PGs, with an overall effect of limited dilation. These responses and mediators are likely to have important roles in the multiple feedback regulation of wall shear stress and resistance in skeletal muscle venules.

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SNAC – The last step???

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Aim: We would like to present the biomechanical evaluation of the "scaphoid nonunion advanced collapse" – a not very well known, but severe arthrotic disease of the wrist – regarding each stage with its adequate treatment. In a case presentation we will discuss a patient with stage II SNAC deformity where we performed proximal row carpectomy. **Background:** Long-standing untreated scaphoid nonunion results in carpal collapse and subsequent arthrosis, which is termed "scaphoid nonunion advanced collapse". However, this is a progressive disease, the stage depends on which joint surface is involved, and regarding the stage we can choose the adequate therapy. At stage I SNAC deformity the proximal pole of the scaphoid is free of degenerative changes, the distal fragment of the scaphoid flexes and arthrosis arises only between this fragment and radial styloid. In stage II arthrosis occurs furthermore between the proximal fragment of the scaphoid and the head of the capitate, the severity depends on the amount of carpal instability. Further shift and collapse of the scaphoid results in an increasing load on the capitolunate joint with shear loading of the capitolunate cartilage resulting in arthrosis in the midcarpal joint which is termed stage III SNAC deformity. Regarding the different joint involvations, in stage I styloidectomy of the radius is recommended with scaphoid reconstruction – interpositional bone graft and screw fixation; in stage II proximal row carpectomy or four-corner fusion with scaphoid excision should be considered, and in stage III the procedure of choice is four-corner fusion with scaphoid excision or radiocarpal arthrodesis. **Materials and methods**: At the Department of Sports Surgery in the National Institute for Sports Medicine in our practice the most frequent stage of SNAC deformity was stage II, which correlates with the literature. Between 2005 and 2009 we performed radial styloidectomy in 7 cases, proximal row carpectomy in 9 cases and radiocarpal arthrodesis in 3 cases. We would like to present one case operated in august of 2008, emphasizing the clinical and radiological results after one and a half year. Collected data: range of motion, grip strength, VAS, DASH, satisfaction, radiological evaluation. **Conclusion:** The "scaphoid nonunion advanced collapse" can be divided in different stages and the treatment is based on the involved joint surfaces; the diagnosis of this disease itself is not an indication of the radiocarpal arthrosis – as we can see it unfortunately in the everyday practice.

Analysis of the K^+ current in human T cells in hypercholesterinaemic state

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It is well documented in the literature that the changing the cholesterol content of the cell membrane may influence the functioning of the membrane proteins, including that of the ion channels. In most of the relevant studies the cholesterol content of the membrane is altered artificially, using various forms of cyclodextrins. Our laboratory has reported previously that the increase of the cholesterol content of the cell membrane (*in vitro,* using cholesterol/cyclodextrin inclusion complex treatment) modified the biophysical parameters of the gating of Kvl. 3 K^+ ion channels in human T-lymphocytes.
As Kv1.3 channels have a pivotal role in the regulation T cell proliferation and function we addressed whether the changes described in the model experiments could be observed for Kvl.3 channels of T cells isolated from patients with hypercholesterinaemia.
T-lymphocytes were isolated from the peripheral blood of patients with cholesterol level considered normal (< 5.2 mmol/l) according to the laboratory standards (control group) and patients with hypercholesterinaemia („hc", the cholesterol level is the 1.5 – 2-fold of the normal one, n = 5). Patient samples ('hc' samples) were obtained form untreated (first-visit) outpatients of the Department of Medicine and they did not suffering from any additional other diseases.
The biophysical characteristics of the Kv1.3 ion channel were studied using the whole-cell configuration of the patch-clamp technique. We determined the kinetic (activation and inactivation kinetics, time constants for recovery from inactivation) and equilibrium parameters (voltage-dependence of steady-state activation and inactivation) characterizing the gating of Kvl. 3 channels. The expression level of the channels was characterized by current density (pA/pF) calculated as the peak current normalized to the membrane capacitance, the latter being proportional to the membrane surface area.
Our results indicate that the biophysical parameters of Kv1.3 gating are similar in the control group and in the 'hc' samples ($p= 0.05$). Thus, the increase of the serum cholesterol level does not result in such biophysical changes in Kv1.3 gating as those that we measured in model experiments using cholesterin/cyclodextrin inclusion complex treatments. On the other hand the current density measured in T cells isolated from the 'hc' sample (CD_{hc} =610 ± 98 pA/pF) was significantly higher than in the case of the control cells (CD_c = 398 ±10.8 pA/pF). This increase in the current density may reflect chronic inflammatory processes coupled with hypercholesterinaemia and the cytokines produced.

Nanotechnology

Inhibition of phosphate transporter NaPi2b function with specific antibodies

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Ovarian cancer is the most common gynecologic malignancy that usually becomes far advanced before it is diagnosed. So far, only few markers and antigens specific for ovarian cancer are known. MX35 antigen is one of them and it has been recently identified as sodium-dependent phosphate transporter NaPi2b (SLC34A2, NaPi3b, Npt2). NaPi2b belongs to the SLC34 family of sodium-dependent phosphate transporters which are involved in the transport of inorganic phosphate and the maintenance of phosphate homeostasis in human body.

Since the clinical trials with labeled MX35 antibody and its Fab2 fragments suggest their therapeutic potential in patients with ovarian cancer it is important to know how they affect NaPi2b phosphate transporter. The main objective of this work was to investigate the effect of MX35 monoclonal antibodies on NaPi2b protein expression and function.

For this purpose the expression level of NaPi2b protein was compared in stable cell lines expressing NaPi2b protein after incubation with MX35 MAbs in Western-blot analysis whereas phosphate uptake assay was applied to study NaPi2b phosphate transporting function. We used stable cell lines HEK293 expressing wild type WT_NaPi2b and mutant form of transporter T330V_NaPi2b. This mutant form is not recognized by MX35 MAb because of single amino acid substitution (T330V) in the major extracellular loop of NaPi2b phosphate transporter. WB analysis showed that incubation of cells expressing WT_NaPi2b with MX35 MAbs led to a significant reduction of NaPi2b protein while application of MX35 MAbs to cells expressing mutant T330V_NaPi2b had no effect on the NaPi2b protein level. The results of phosphate uptake assay indicated approximately 40% inhibition of inorganic phosphate transport in cells expressing WT_NaPi2b after incubation with MX35MAb but this antibody did not affect NaPi2b function in cells expressing mutant T330V_NaPi2b. The data obtained allowed us to suppose possible internalisation and further degradation of NaPi2b transporter initiated by MX35 MAb.

To summarize, we have shown specific inhibition of phosphate transporter NaPi2b function by MX35 monoclonal antibodies. This knowledge could be useful for the investigation of therapeutic potential of MX35 MAbs in the treatment of ovarian cancer patients and in the developing of new specific inhibitors for NaPi2b-mediated phosphate transport.

Effect of oligoperoxide coating of magnetic nanoparticles on the efficiency of stem cell labeling

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With the progress in regenerative medicine, magnetic resonance imaging (MRI) is a suitable noninvasive method for in vivo transplanted cell tracking in the host organism. Dextran-coated superparamagnetic iron oxide nanoparticles (e.g., Endorem®) are widely used for cell labeling in combination with transfection agents or at high iron concentrations, both of which can be cytotoxic. To overcome these drawbacks, the search for better coatings of superparamagnetic iron oxide nanoparticles is required.

Maghemite (γ-Fe2O3) nanoparticles (~ 12 nm) were obtained by the coprecipitation of Fe(II) and Fe(III) salts in alkaline medium which was followed by the controlled oxidation of magnetite to maghemite. Resulting nanoparticles were coated with oligoperoxide in an aqueous solution. Advantage of oligoperoxides consists in the possibility to initiate polymerization of another monomer grafting thus various functional biocompatible chains. In this report, poly(vinyl acetate-co-5-tert-(butylperoxy)-5-methylhex-1-en-3-yne-co-butyl acrylate-co-maleic anhydride) of low molecular weight (Mw =4,500) was selected as a coating. Carboxyl groups of the oligoperoxide formed by hydrolysis were of crucial importance for subsequent attachment of the oligomer on iron oxide surface. Oligoperoxide-coated γ-Fe2O3 nanoparticles were thoroughly characterized by ATR FT-IR spectroscopy, SAXS, QELS, AAS and elemental analysis. Coating had a marginal effect on the size and the morphology of the particles, however, it strongly affected efficiency of labeling of mesenchymal stem cells (MSCs) with the nanoparticles. Oligoperoxide-coated nanoparticles were compared with Endorem® (control) in terms of MSCs cytotoxicity, labeling efficiency and MRI detection limit.

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Enhanced antisense oligodeoxynucleotides delivery for prevention prion infections

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Prospects of disorders correction of humans and animals using antisense oligonucleotides have attracted much attention of scientific community as antisense technology is a powerful tool against many diseases which were incurable yet. One of those cases, for which there are no means of treatment and prevention are prion infections. The characteristic feature of prion infections is an accumulation of pathological form of prion in a brain and some other tissues, which is derived from the host-encoded cellular prion (PrP^C). It was ascertained that presence of PrP^C is absolutely necessary for illness development. Thus, targeting PrP^C has the potential to remove the substrate for the pathogenesis. Based on this, we decided to reduce the PrP^C expression level through the inhibition its mRNA translation using antisense oligonucleotides (asODNs).

For effective inhibition of PrP^C gene mRNA translation it was decided to select asODNs for 5'-nontranslating region, start codon and middle of open reading frame of the gene. Efficiency of application of asODNs substantially increases by using drug delivery systems, which protect therapeutic agent from degradation, continue his half-life time and prolong his action. We used cationic liposomes and synthetic poly dimethylamino-ethylmetacrylate (polyDMAEM) as carriers. PolyDMAEM is water-soluble cationic oligoelectrolite and it can efficiently bind negatively charged DNA through electrostatic interactions as it was shown by turbidimetric analysis. The potential cytostatic and cytotoxic effects of polyDMAEM were verified on the rat fibroblasts cell culture. It was shown that the most optimal doses are 0.1 and 1 mkmole/ml culture medium. After 4 hours of incubation L1210 cells with asODNs incorporated into liposomes all used asODNS decrease the PrP^C expression level approximately on 80 % ($r < 0,05$). Immunoblot analysis demonstrate that asODN complementary to the area of start codon of prion mRNA was less effective and decreased expression of PrP^C on 60-70 % ($r < 0,01$) by comparison to two other oligonucleotides that inhibited PrP^C expression on 75-90% ($r < 0,01$). If on 4^{th} h. of incubation L1210 cells with complex asODN and polyDMAEM the most effective was asODN complementary to the start codon then all three asODNs showed 95-98% ($r < 0,01$) efficiency in decreasing PrP^C expression level on 24^{th} h. Notably, it was found that polyDMAEM is able to decrease PrP^C content by itself.

This work demonstrates potential possibility of using asODN for prophylaxis and therapy of prion infections. As a result of carried out research it was synthesized nanocarrier of polymeric nature for delivery of nucleic acids. It was found that polyDMAEM is able to reduce expression level of cellular prion in L1210 cells independently.

Flavocytochrome b2 and recombinant *Hansenula polymorpha* cells, overproducing this enzyme, as perspective tools for chromate bioremediation

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In spite of the great interest to the study of biological role of chromium, as well as of toxic influence of Cr (VI)-species on living organisms, the molecular mechanisms of chromate bioremediation remain vague. Among possible mechanisms for chromate biodetoxification, a reductive pathway resulted in formation of the less toxic Cr (III)-species is suggested to be the most important. The yeast L-lactate: cytochrome c oxidoreductase (flavocytochrome b2, FC b2) has absolute specificity for L-lactate, although is non-selective with respect to the nature of electron acceptors. Such properties allow consider this enzyme as a potential candidate for reduction of chromate by living cells.

To elucidate this hypothesis, recombinant strain of thermotolerant methylotrophic yeast Hansenula polymorpha was used as a model organism possessing a six fold increased FC b2 enzymatic activity (up to 3 μmol min-1 mg-1 protein in cell-free extract) compared to the initial strain. The lyophilized recombinant cells, stored in dried state, as well as living yeast cells were tested in respect of their chromate reducing activity in vitro in the presence of L-lactate (as an electron donor for chromate reduction) and different low-molecular redox-active dyes: dichlorophenolindophenol (DCPIP), Methylene blue, Meldola blue, Nile blue facilitating electron transfer from the reduced form of the enzyme to chromate (as a final electron acceptor). It was shown that the highest chromate-reducing activity of the cells was achieved in the presence of DCPIP.

The ability to catch electrons from the reduced flavocytochrome b2 by chromate was also demonstrated on the model of purified enzyme immobilized on the surface of platinum electrode. It was clearly shown that with increasing concentration of Cr(VI) the peak of enzyme-mediated L-lactate oxidation is decreased, indicating Cr(VI)-dependent competition between reaction of chromate with reduced FC b2 and direct electron transfer from the enzyme to the electrode surface.

The perspectives of the application of the observed chromate-reducing ability of FC b2-overproducing recombinant cells of *H. polymorpha* for chromate bioremediation, as well as for construction of cells-based biosensor for chromate monitoring in environment are discussed.

Cell Biology

The effect of sildenafil versus erdostein on the monocrotaline induced pulmonary disease in the Wistar rat a morphopathological study

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Introduction. Monocrotaline (MCT) a pyrrolizidine alkaloid is a phytotoxin used experimentally to cause a pulmonary vascular syndrome in rats characterized by proliferative pulmonary vasculitis, pulmonary hypertension and cor pulmonale. Sildenafil (SIL), the phosphodiesterase 5 inhibitor, is an effective pulmonary vasodilator. Erdostein (ERD) is a mucolytic agent with antioxydative effects.
Aims. Compare the benefits of administering Sildenafil versus Erdostein to the MCT inoculated Wistar rats. **Methods**. Three groups of 15 rats each received subcutaneously 1 dose of MCT 60 mg/kg.The first group received SIL orally for 28 days, the second received ERD orally for 28 days, the third remained untreated. Sacrifices were made on the 14th and 28th day.Pulmonary tissue samples were processed using hematoxylin-eosin stain and immunohistochemistry techniques with active antibodies. **Results**. The same type of pulmonary lesions inflammatory interstitial and alveolar infiltrates, interstitial edema, remodeled pulmonary arterial vessels, was found in all three groups. Lesions were found to be less severe in the two treated groups vs non-treated group ($p<0.05$).The SIL treated group had less severe lesions compared to the ERD treated group ($p<0.05$). Mortality during the first 14 days reached: 1 in the SIL treated group, 5 in the ERD treated group and 4 in the non-treated group.
Conclusion. SIL and ERD are both effective in diminishing the severity and extent of the MCT induced pulmonary disease, with a direct positive effect on the vascular remodeling. SIL was more efficient than ERD and was the only one to positively influence mortality.

K+ current expression and proliferation of human T-lymphocytes induced by various stimuli

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Ion currents of human T-lymphocytes and specifically Kv1.3 potassium channel currents are crucially important for the triggering of an immune response. Resting T cells also express this channel, but as a result of stimulation (e.g. an immune reaction), the expression of the channel increases. In contrast to the in vivo activation of T cells, where the interaction with specialized antigen presenting cells triggers T cell proliferation, in vitro stimulation of the cells uses molecules that can act on specific signal pathways. Our aim was to investigate whether Kv1.3 channel expression in T-cells depends on the nature of the signaling pathway activated in vitro.

We used healthy human peripheral blood T-cells in our experiments. We monitored the division of the cells in a flow cytometer using CFSE (carboxyfluorescein succinimidyl ester) dilution assay. The cells were stimulated with a mitogenic lectin (phytohaemagglutinin: PHA); a combination of a diacylglycerol analog (phorbol 12-myristate 13-acetate: PMA) and a calcium ionophore (ionomycin); furthermore with antibodies affecting the TCR-CD3 complex (anti-CD3 by itself; anti-CD3 and anti-CD28 used in combination). Five days after application of the stimulus we measured the proportion of dividing cells, change in cell size and cellular granulation. Subsequently we measured the Kv1.3 ion currents through the T-cell membrane as well as biophysical parameters of this channel (e.g. activation and inactivation kinetics and voltage dependence of conductance), using the whole-cell patch-clamp technique.

Our flow cytometric measurements indicated that as the cells divided, cell size and amount of granulation increased after all treatments. Our patch-clamp experiments showed that there are significant changes in Kv1.3 ion currents at +50 mV depolarization after both PHA and anti-CD3+anti-CD28 stimulation. Inactivation kinetics changed only as a result of anti-CD3+anti-CD28, which could be a sign of posttranslational modulation of the channel. Similarly, increase in the current density was only significant in case of anti-CD3+anti-CD28 treatment. We can conclude that for measurement of Kv1.3 currents (e.g. for ion channel pharmacology), PHA treatment is sufficient but for the simulation of in vivo T-cell activation the most effective method is stimulation with anti-CD3+anti-CD28.

Formaldehyde dehydrogenase from the methylotrophic yeast *Hansenula polymorpha* as bioanalytical instrument for assay of toxic formaldehyde

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Formaldehyde (FA) is a highly reactive compound that has a toxic effect on all organisms due to a non-specific reactivity with proteins and nucleic acids. FA reacts as an electrophile with the side chains of arginine and lysine and the amino groups of RNA and DNA and causes protein–protein, protein–DNA, and DNA–DNA cross-links. This substance is a hazardous air pollutant and prolonged exposure to formaldehyde FA can cause serious health effects. FA has been connected to cancer deaths; recent observations show that factory workers who had been exposed to high FA levels are at increased risk for leukaemia. At home, off-gassing of FA over time from pressed wood products can also result in health hazards. Indoor, non-industrial exposure to chemical hazards can occur continuously at low levels, contributing to symptoms such as headaches, fatigue, and upper respiratory and eye irritation. Effective detection of chemicals in the environment requires simple, rapid, sensitive and selective analytical methods. Such devices could continuously monitor surrounding media and give warnings about the level of toxic chemicals in workplaces, factories, and homes, even when they are present in extremely low concentrations. All organisms employ certain metabolic pathways of FA detoxification, involving glutathione-dependent FA oxidation by Formaldehyde dehydrogenase (FdDH). FdDH, a key enzyme of FA metabolism in microorganisms, is proposed for bioanalytical purposes. We suggest using FdDH, isolated from the gene-engineered recombinant strain Tf 11-6 of methylotrophic yeast *H. polymorpha* for the enzymatic assay of FA. The method is based on the photometric detection of a colored product, formazan, formed from nitrotetrazolium blue in a reaction coupled with FdDH-catalyzed oxidation of FA. The reliability of the developed analytical approach was tested on real samples of waste waters, pharmaceuticals, FA-containing industrial products, and vaccines. The comparison of formaldehyde FA content values obtained by biosensors (enzyme and cells-based), enzymatic methods and two routinely used chemical ones (chromotropic acid and 3-methyl-2-benzothiazolinone hydrazone) showed a good correlation between these approaches. Using nanosized matrix as a carrier for FdDH immobilization will allow increase a local enzymes concentration, enhance a stability of the protein and, probably, increase catalytic activity of the enzyme. We suppose to apply FdDH bound with nanoparticles in biosensors on FA in air. We carried out immobilization of FdDH on magnetic nanoparticles (γ-Fe_2O_3) and biocompatible poly(2-hydroxyethylmethacrylate-co-40%ethylene dimethacrylate)/P(HEMA-EDMA)/microspheres using carboxylic groups for covalent biofunctionlization of matrix. The bioanalytical properties of such FdDH–modified materials were studied.

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Enhanced antioxidant formula based on selenium-enriched biomass of the yeast *Phaffia rhodozyma*

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The red yeast *P. rhodozyma* is able to produce astaxanthin as a final product of carotenoid biosynthesis. The properties of astaxanthin as antioxidant, anticancer agent, and coloring agent are well-known. Aim of this work was to combine this powerful antioxidant with selenium in *P. rhodozyma* biomass. We suggest that this formula will play a potential role in human and animal's health by controlling free radical and antioxidant balance, thus protecting cells from damage caused by ROS. Moreover, yeast biomass can efficiently accumulate selenium from growth medium and transfer inorganic form of Se to organic, this is considered to be more bioavailable and suitable for dietary application. Wild type strain *Xanthophyllomyces dendrorhous* (*P. rhodozyma*) NRRL Y-10921 was cultivated in sucrose medium, supplemented (if necessary) by sodium selenite to the final concentration 5 μg/L. On 6th day of cultivation, astaxanthin content was measured after treatment of the cells by DMSO and extraction by a mixture of hexane:ethylacetate (1:1), and, finally, measuring the absorbance of an organic phase at 480 nm. The determination of total selenium content in the cells was performed using atomic absorption spectrometer. Cells for analysis were prepared using acid-hydrogen peroxide mineralization. Male Wistar rats with an initial body weight of approximately 120 g were divided into 2 groups. The first group was intact animals and for another we used tetrachloromethane (TCM) for intoxication. Groups were divided to 4 subgroups which differ by the feeding: 1 - got standard combined feeding (SCF); 2 - SCF with 4% of P. rhodozyma biomass which consisted astaxanthin 370 μg/g of dry weight; 3 - SCF with 4% of yeast biomass which contained 2 μg of organic selenium; 4 - SCF with 5 μg of sodium selenite. Animals were killed on 16th day of the experiments and their livers and blood were taken to assay enzymes activities and oxidative modification of lipids. Injection of TCM resulted in increase of activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum and liver in comparison with intact animals. Activity of ALT and AST were decreased to 11 and 14 % for animals treated by TCM, which were fed by SCF - containing yeast biomass. The activity of enzymes for the subgroup, which also were treated by TCM and were fed by selenium-enriched biomass was decreased in 47 and 9 %. Activity of catalase in liver was not changed in subgroups influenced by TCM, while activity of glutathione peroxidase was decreased to 44, 33 and 55 % for 2, 3, 4 subgroups, respectively. TCM also activated peroxidative modification of lipids. The addition of the yeast biomass to SCF resulted in the decrease of conjugated dienes (CDs) content to 16 % and malondialdehyde (MDA) to 20 %, while selenium-enriched biomass provoked decreasing CDs to 4-fold and MDA - to 24 %, respectively. The addition of sodium selenite did not result in positive influence against oxidative stress. We have demonstrated that the formula, containing selenium-enriched biomass of astaxanthin-synthesizing yeast *P. rhodozyma* can be used as an effective biologically active additive with a highly extensive antioxidant potential and can be applied in various human and animal diets.

Auto-antibodies in human milk and blood serum: characteristics of biological activity

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Antibodies against DNA, nucleoprotein complexes, as well as enzymes participating in metabolism of nucleic acids, have been described in patients with a variety of autoimmune diseases and certain infections. Increasing evidence exists that the same auto-antibodies can also hydrolyze their specific antigens. Antibodies possessing catalytic activity have been called catalytically active antibodies or abzymes. They belong to a new group of physiologically active substances with dual characteristics: they represent a pool of canonical autoantibodies and possess catalytic activity. Proteolytic and DNA-hydrolyzing auto-antibodies are of special value among them. An increase in their activity correlates with clinical manifestations of the autoimmune disorders, disease severity, and the rate of progressing disability. Abzymes are crucial for immune homeostasis regulation. They can be of practical value for the development of modern immunodiagnostic tools and immunotherapy schedules. With a few exceptions, blood serum of normal human donors (men and women) usually does not contain catalytic antibodies. During pregnancy and immediately after delivery (i.e. at the beginning of lactation), female organism is frequently characterized by an immune status similar to that in patients with autoimmune diseases. In addition, lactation is associated with an appearance of catalytically active antibodies with DNAse, RNase, ATPase, amylolytic, protein kinase and lipid kinase activities present in breast milk. These data suggest that abzymes play an important role in the innate and adaptive immunity. Our study addressed the characterization of antigen specificity and catalytic activity of antibodies of blood serum and milk of healthy human donors and patients with some autoimmune pathology. Besides, we estimated the effect of these antibodies towards growth and survival of cultured mammalian cells. It was shown that different samples of immunoglobulins isolated from colostrum of healthy women and blood serum of some patients with multiple sclerosis, induced cell death *in vitro*. However, in some cases they stimulated proliferation of the studied target cells. Functional activity of antibodies toward mammalian cells could be linked to their antigen specificity and catalytic activity. Anti-DNA sIgA isolated from human colostrum with nuclease activity, possessed different extent of cytotoxic activity towards Namalwa, Jurkat, L1210 and L929 cell lines. We firstly found anti-histone sIgA- and IgG-antibodies capable of catalyzing a hydrolysis of linker histone H1 in colostrum of healthy woman and blood serum of some patients with autoimmune diseases - multiple sclerosis or systemic lupus erythematosus. Anti-histone IgGs were internalized into Jurkat T cells and L929 transformed fibroblasts, and also stimulated proliferation of human Jurkat T-cells.

Summarizing, we found that functional characteristics of immunoglobulins isolated from human colostrum and blood serum depend on individual peculiarities of humoral immunity. These characteristics can be of diagnostic and prognostic value, namely for complex detection of early autoimmune disorders linked to pregnancy and delivery.

Metabolic enzyme CoA synthase is tyrosine phosphorylated and is a part of signaling network in the cell

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In the recent years the mechanisms which underlie complex interplay between metabolic pathways and fundamental processes in multicellular organisms, such as proliferation, differentiation and others, are starting to emerge. Some of the metabolic molecules and enzymes are shown to play an active role in the regulation of cell behavior by acting on different regulatory cellular systems including signal transduction. **Objectives***:* we studied relationships between an enzyme involved in de novo Coenzyme A biosynthesis – CoA Synthase and the signaling proteins in mammalian cells. **Methods***:* Metabolic enzyme CoA synthase (CoASy) mediates two final stages of *de novo* biosynthesis of coenzyme A. This protein possesses multiple proline-rich and phosphorylated tyrosine-based motifs which according to *in silico* predictions could mediate its interactions with SH2/SH3 domains of different signaling proteins. Based on these data we hypothesize that CoASy has some unknown functional relationships with components of cellular signaling. We tested this prediction of bioinformatics experimentally in pull down analysis of tissue extracts with a number of recombinant GST-SH2 and GST-SH3 domains of different signaling molecules and confirmed several protein-protein interactions by further co-immunoprecipitation. Namely, existence of CoASy complexes with signaling proteins – protein tyrosine phosphatase Shp2, p85α regulatory subunit of PI3K and Shc protein was shown in mammalian cells. Importantly, formation of complexes between CoASy and the listed proteins is sensitive to availability of growth factors indicating the regulatory nature of observed interactions. Furthermore, the tyrosine phosphorylation of CoASy *in vivo* which regulates CoASy interaction with SH2 domains of these proteins was revealed. Involvement of c-Src kinase in CoASy phosphorylation *in vivo* and its dephosphorylation by Shp2PTP *in vitro* were demonstrated. Further studies of physiological relevance of CoASy interactions with signaling proteins using siRNA technology revealed that CoASy knockdown leads to decreased phosphorylation of substrates of PDK and Akt protein kinases. Altogether our data indicate the existence of an unexplored physiological link between CoASy as a part of CoA biosynthetic pathway and signal transduction pathways in mammalian cells. The mechanisms and physiological significance of the observed phenomena are under investigation.

Effects of fluid resuscitation methods on the pro- and anti-inflammatory cytokines and expression of adhesion molecules after burn injury

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Objective: Fluid resuscitation management can influence inflammatory response after burn injury. The aim of this study was to analyze the effects of two fluid resuscitation methods on the cytokine production and on the expression of the leukocyte surface markers.

Methods: Thirty patients were included in this prospective randomized study with burn injury affecting more than 20 % of the body surface area. Fluid resuscitation was guided by hourly urine output (HUO, n = 15) or by intrathoracic blood volume index (ITBVI, n = 15). Blood samples were taken on admission and on the next five consecutive mornings. Concentrations of IL-1ß, IL-6, IL-8, IL-10, IL-12p70 and TNF-a were measured in phorbol myristate-acetate stimulated and non-stimulated samples. Leukocyte surface marker expressions (CD11a, CD11b, CD14, CD18, CD49d, CD97) were also determined.

Results: In the ITBVI group IL-6 levels on days 2-3 and IL-6/IL-10 ratios on days 2-3, and the IL-8/IL-10 ratios on days 3-5 were significantly higher than in HUO group ($p < 0.05$). In the HUO group IL-10 levels were significantly higher ($p < 0.05$) on days 4 and 5. Granulocyte CD11a levels on day 2, CD11b levels days 4-6, lymphocyte CD11a on days 5-6, CD11b on days 3-6, CD49d on days 2-6 and CD97 on day 6 and monocyte CD11a, CD11b, CD18 levels on days 4-6, CD14 levels on days 3-5 were significantly higher in the HUO group ($p < 0.05$).

Conclusions: Our study suggests that ITBVI guided fluid resuscitation of burned patients suppresses the shift towards anti-inflammatory imbalance and the expression of leukocyte surface markers more than HUO guided resuscitation.

Expression of endothelial selectin ligands on leukocytes following repeated dive in SCUBA divers

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Leukocyte cell surface adhesion molecule CD11b, decorated with CD15s, plays a critical role in regulation of β_2 integrin functions during neutrophil endothelial transmigration. Hyperbaric oxygenation reduces neutrophil-endothelial cell adhesion which is mediated by Mac-1 (CD11b/CD18) β_2-integrin. This study investigated expression of CD15 and CD15s on leukocytes, following repeated trimix (a mixture of oxygen, helium and nitrogen) dives in two series: in study I, 7 divers performed 6 consecutive dives from 55-80 m (total dive time varied from 65-75 min), while in study II, 7 divers performed 3 consecutive dives from 63-65 m (total dive time varied from 59-83 min). More intense dive profile was used in study II as can be seen from longer total dive time. 5 divers took part in both studies. CD15 and CD15s were determined before and after the 1st and the last dive in study I and before and after the 1st and the last dive in study II. Leukocyte subpopulations were not elevated after both dives in study I. Only CD15+CD15s+ granulocytes were significantly decreased after the 1st dive ($p = 0.006$). In study II, monocyte proportion was increased ($p = 0.014$) and lymphocyte decreased ($p = 0.020$) within total leukocyte population, and CD15s+ monocytes and CD14+CD15s+ granulocytes were elevated ($p = 0.019$, and $p=0.018$, respectively) after the 1st dive in the study II. CD15+CD14+ granulocytes were decreased after the 1st and the last dive ($p = 0.048$ and 0.017, respectively), while CD15s+ granulocytes were decreased only after the last dive of study II ($p = 0.006$). The current findings of decreased endothelial selectin ligand CD15s expression on CD15+ granulocytes after certain dives point the role of this subpopulation in the endothelial damage prevention.

Drug Development & Pharmaceutical Research

Development of an *in vitro-in vivo* correlation for poorly soluble drugs

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For poorly soluble substances, both the dissolution and the diffusion kinetics are limited by the reduced solubility, which is the reason for which both the in vivo and in vitro assaying use surface active agents (saa) which amplify and accelerate both phenomena. The surface-active agents have fundamentally different behavior at levels before and after the critical micelle concentration, and a chaotic behavior around that concentration. Furthermore, due to accumulation at interfaces, there is a superficial excess of surface-active agents and the critical micelle concentration is reached faster than in the case of solutions. For pharmaceutical products containing poorly soluble active substances, the dissolution methods stipulated in pharmacopoeias and used by the manufacturers are mandatory tests for quality control. The aim looked for by producers is to fully and rapidly dissolve the active ingredients. Following this unnecessary "quality" pharmaceutical companies are frequently using non-physiological pHs and very large amounts of surface-active agents Such tests are not predicting the way in which the given product will behave in vivo. Therefore, it is possible for products which in compendial conditions yield similar in vitro release profiles to behave essentially different in vivo.
The present study concerned development of in vitro dissolution methods, using optimal concentrations of surfactants able to obtain reliable results concerning in vivo dissolution and bioavailability. Ketoconazole and nimesulide were chosen as test products. The dissolution studies were performed by using USP apparatus 2 (paddles). Polysorbate 80 (Tween 80) in the concentration range 0-2,5% was used in combination with a phosphate buffer solution to increase the solubility of the tested drugs. Pharmacokinetic data were obtained from as a single-dose, randomized, two-treatments, two-periods, two-sequence cross-over bioequivalence study, performed in accordance with Good Clinical Practice regulations. Venous blood samples were collected through a catheter up to 24 hours after drug administration for both compounds. The active substances were extracted from plasma samples by means of a liquid-liquid extraction method, and analyzed using a fully validated HPLC method, with UV detection. The *in vitro* dissolution profiles were compared with the in-vivo pharmacokinetic data, by using the FDA Level A correlation recommended method (fraction of drug dissolved versus fraction absorbed calculated using Wagner Nelson formula). The surface-active agent concentration 0.5 %, i.e. two-three fold lesser than that used in official methods was found as optimum. A new correlation method was tested, by substituting the absorbed drug fraction with the eliminated one. This alternative method proved to provide a better correlation between in vitro dissolution and in vivo pharmacokinetic data.

In vitro studies regarding the interactions of some ruthenium (II) fluoroquinolones complexes and some plasmatic proteins

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The study on the interaction of small molecules (usually called ligands) with DNA and plasmatic proteins has been the focus of many recent works. Investigating the interaction of drugs with these endogenous biomolecules has a great significance for disease defense and drug development.
Numerous biological experiments have demonstrated that DNA is the primary intracellular target of anticancer drugs, due to the interaction between small molecules and DNA, which cause DNA damage in tumor cells, blocking their division and resulting in cancer cell death.
Of these studies, the interaction of transition metal complexes with DNA and plasmatic proteins has gained much attention.
The present paper presents the DNA-binding properties and the plasmatic proteins interactions (namely human serum albumin-HSA and transferrin) of some ruthenium (II) complexes with ofloxacin and norfloxacin.
In this regard we investigated *in vitro* the interactions of the ligands studied with double stranded calf thymus DNA through fluorescence emission spectrophotometry.
We also performed fluorescence studies for evaluating the HSA-binding properties of our complexes, while the transferrin interactions were assessed through UV absorption spectroscopy.
Our results showed that the studied complexes developed concentration-dependent interactions with the DNA, HSA and transferrin.

Docking studies of 4-thiazolidone derivatives as potential COX-2-pathway blockers in oncogenesis

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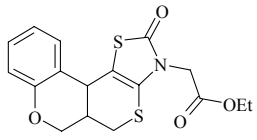
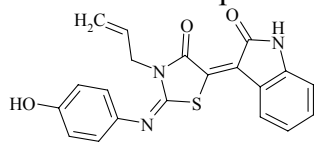
Last years COX-2 and arachidonic acid metabolites expression was found in human colon cancer cells, human breast cancer cells, mouse melanoma, human fibrosarcoma, invasive lung adenocarcinoma cases, human colorectal carcinoma, gastric carcinoma and human skin epidermal cancer cells. 4-Thiazolidinone derivatives are well-known class of substances that is related with a wide spectrum of biological activity and usage as pharmacological agents. This class of compounds possesses hypoglycaemic, anti-inflammatory, choleretic, antitumor, diuretic, immunostimulant, and other activities. Recently, attention has been paid to the antitumor activity of thiazolidinone derivatives. It has been known that 4-thiazolidinones can behave as COX-2-inhibitors by showing affinity to COX-2 enzyme. This determines novel direction in search of new anticancer agents among molecules containing 4-thiazolidinone moiety. Our aim was to conduct molecular docking of some 4-thiazolidinone and related heterocyclic derivatives into COX-2 active site for purposeful search of COX-2 inhibitors as potential anticancer agents. Docking studies were conducted with OpenEye Scientific Software program package that include Fred Receptor, Vida, Flipper, Babel3, Omega2 and Fred2. Chrystallographic models of COX-2 were obtained from Protein Data Bank (www.rcsb.org), particularly medels 6COX (COX-2 in complex with selective inhibitor SC-558 at a resolution of 2,8 Å) and 1CX2 (COX-2 in complex with selective inhibitor SC-558 at a resolution of 3,0 Å). 145 compounds among 4-thiazolidinone and related heterocyclic derivatives, which possessed in vitro anticancer activity, were selected for docking studies from in-house library, as well as few known selective COX-2 inhibitors such as Celecoxib, Etoricoxib, Rofecoxib, Valdecoxib, Meloxicam and non-selective COX inhibitor Aspirin. The molecular docking included the following stages: [a)] generation R-, S- and cys-, trans-isomers of ligands using program Flipper (obtained 461 isomers of studied compounds), [b)] 3D optimization of isomers using program HyperChem 7.5 (www.hyper.com) (molecular mechanics method MM+ with following semi-empirical quantum-mechanical method PM3), [c)] conformers generation (Omega2) and [d)] 3D molecular docking (Fred2). Obtained seven scoring functions values (Chemgauss2, Chemscore, PLP, Screenscore, Shapegauss, Zapbind and Consensus) were used for *in silico* estimation of COX-2-compound binding. Consensus (cumulative) scoring function ranking allowed us to select 20 compounds, which can prospectively be selective COX-2 inhibitors at the level of celecoxib, for in-depth pharmacological studies and templates for synthesis of various related analogues. Compounds Les-942 and Les-1009, as the first in compound ranking, to our opinion could be the most promising structures for the optimization of the COX-2 inhibitors search with anticancer profile.

Les-942 (1CX2)





Les-1009 (6COX)

Preliminary docking studies of in-house library could be considered as a first stage of long-term project dedicated to rational design of selective COX-2-inhibitors.

A radial distribution function (RDF) approach to the QSAR study of thiazolidine analogs

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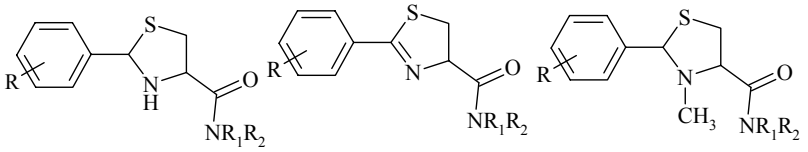
The prediction of anticancer activity of newly synthesized compounds provides a novel lead for the drug-development process. In this study, RDF descriptors were used to predict the antiproliferative activity for thiazolidine analogs. A series of 28 compounds was studied with their activity IC_{50} values (concentration which inhibited cell growth by 50%) being examined in a SK-MEL-188 human melanoma cell line [1].



I **II** **III**

R = H, 3,4,5-OCH_3, 3,4-OCH_3, 3,4-OCH_2O-, $NHCOCH_3$, NH_2

R_1 = Alkyl, Enyl, OCH_3

R_2 = H, CH_3

Firstly we carried out geometry optimization of each compound, using the quantum chemical semi-empirical method PM3 included in HyperChem 7.5 [2]. The DRAGON computer software, web version 3.0 was used to calculate 150 RDF molecular descriptors for each compound. The atomic masses, van der Waals volumes, Sanderson electronegativities and polarizabilities were used as bond weightings. All statistical analyses were carried out using BuildQSAR program [3]. GA-MLR algorithm was used for the QSAR models construction. Two-, three-, four- and five-variables linear regressions were built. They were validated by calculating Q^2 values from "leave-one-out" (LOO) testiness, known as cross–validation and can be considered a measure of the predictive power of a regression. Correlation coefficients r, the Fischer ratio (F) and the standard deviations (s) are of higher quality for the models. The most important variables in the two-variables equations are the atomic masses and van der Waals volumes. The negative contribution to the IC_{50} values (meaning the increasing of activity) RDF020*m* descriptors give while the positive contributions are supplied with RDF120*m* and RDF120*v* descriptors. This corresponds to a radius of 2.0 to 12.0 Å. In this sense a spherical molecular volume could have certain restrictions to the addition of bulky substituents. The r values are in the range of 0.82-0.76, the values of Q^2 are 0.529-0.498 while the Fischer ratio lies between 25.786 and 17.590.

Three-, four- and five-variables models include also weighted by atomic polarizabilities and electronegativities being of a significant contribution into the IC_{50} value. Most correlation coefficients were higher than 0.85. One of the compounds presents large residual and should be considered as an outlier. The outlier number represented only a 3.57% of the whole data. The RDF descriptors can be used for predicting the anticancer activity of new chemicals, thus contributing to the design and development of safe drugs. The linear regression models developed are easily calculated and suitable for the rapid prediction of activity, and cross-validations of the final models support this claim.

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3.de Olivera D. B., Gaudio A. C. Quant. Struct. – Act. Relat. 19, № 6 (2000), 599-601.

Synthesis of novel 4-thiazolidinones with (3,5-diaryl-4,5-dihydropyrazol-1-yl)-ethanone moieties in molecules and evaluation of their antitumor activity

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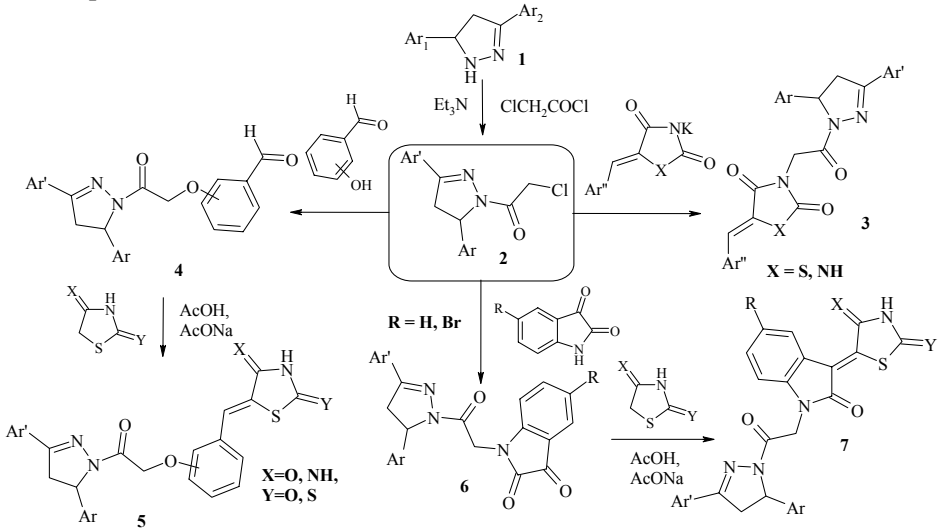
Investigation of 4-thiazolidinones antitumor activity is actual and perspective tendency in medicinal chemistry. The mechanisms of antitumor activity of 4-thiazolidinones and related heterocycles can be associated to their affinity to anticancer biotargets such as phosphatase of regenerating liver (PRL-3), nonmembrane protein tyrosine phosphatase (SHP-2), JNK-stimulating phosphatase-1 (JSP-1), tumor necrosis factor TNFα, and anti-apoptotic biocomplex Bcl-X_L-BH3 etc. Among 4-thiazolidones the integrin $\alpha_v\beta_3$ antagonists and inhibitors of necroptosis have been established. Combination of thiazolidine template with pyrazoline moiety is a perspective approach for drug-like molecules design (Havrylyuk, 2009), considering that pyrazoline derivatives have a wide spectrum of pharmacological activities.

The purpose of our work was the synthesis of new 4-thiazolidinones with pyrazoline fragments for pharmacological screening. 3,5-Diaryl-4,5-dihydropyrazoles **1** easily react with chloroacetyl chloride yielding derivatives **2**. Compounds **2** were tested as alkylating agent in the reaction with of 5-arylidene-2,4-azolidinones potassium salts, thus the corresponding derivatives **3** have been obtained. It is known, that nature of arylidene moiety in position 5 of azolidinone cycle has a critical influence on the antitumor activity (Lesyk, 2004). Therefore another focus of our research was the synthesis of [2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethoxy]benzaldehydes **4** and 1-[2-(3,5-diaryl-4,5-dihydropyrazol-1-yl)-2-oxoethyl]-1*H*-indole-2,3-diones **6**. The Knoevenagel reaction of **4, 6** with several 4-thiazolidinones yielded the group of new 5-arylidenederivatives **5, 7.** The structures of compounds were confirmed by ^{1}H NMR spectra.



Antitumor activity screening of the synthesized derivatives has shown their moderate activity with high selectivity to individual cell-lines of lung, renal, ovarian and CNS cancers.

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D. Havrylyuk, B. Zimenkovsky, O. Vasylenko et al. 2009. Synthesis of novel thiazolone-based compounds containing pyrazoline moiety and evaluation of their anticancer activity. *Europ. J. Med. Chem.* 44(4): 1396–1404.

Phytochemical research of *Hedera helix* leaves

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Hedera helix L. (English ivy, Common ivy) is an evergreen dioecious woody liana, one of 15 species of the genus *Hedera*, Araliaceae family. Whole or cut, dried leaves of *Hedera helix* L. from vegetative and reproductive forms are used as medical plant material. The major types of biologically active compounds, that are responsible for the medicinal use of *H.helix,* are triterpene saponins with hederagenine or oleanolic acid as aglycones (α- and β-hederin, hederacosides A-J, glycosides L-1, L-2a, L-2b, L-3, L-4a, L-4b, L-6a, L-6b, L-6c, L-7a, L-7b, L-8a and hederoside B).

H.helix is traditionally used in the folk and official medicine, homeopathy and cosmetology. The German Comission E confirms *Hedera* leaves use as the treatment for catarrhs of the respiratory passages and for symptoms of chronic inflammatory bronchial conditions.

The aim of our research was detailed phytochemical investigation of *H.helix* leaves. We separated and identified rutin, caffeic, chlorogenic, isochlorogenic and rosmarinic acids by several methods of chromatography (CC, PC, TLC). The structures of pure substances were determined by the methods of spectral analyses (UV, MASS, H^1- NMR (COSY, HSQC, HMBC) and C^{13}-NMR).

Some elements and amino acids were determined in the plant material by spectrophotometric method. *Hedera* may be considered a perspective material for manufacturing of phytosubstances with potential immunological and antioxidant activity, due to high concentrations of Ca, Mg, Mn, Fe, Zn, Cu. High concentrations of proline, asparagine, γ-aminobutyric and glutaminic acids seem to become perspective for use of *Hedera* leaves as reparative, detoxicant, antioxidant remedies and ones with positive influence on central nervous system.

The results of phytochemical researches prove the perspective and the possibility of wide use of *Hedera* phytopharmaceuticals in medicine and cosmetology and stimulate pharmaceutical science for further investigations for development of new qualitative, effective and competitive medicines.

RDF and 3D-MORSE descriptors in thiazolidine derivatives anticancer activity prediction

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Thiazolidine derivatives represent a well-known class of patented drugs and substances at different stages of research, which possess various biological activities. Recently, attention has been paid to the antitumor activity of thiazolidine derivatives as novel potential anticancer agents. Quantitative structure–activity relationships (QSAR) have been broadly used for some years mainly in medical research. This methodology makes use of the molecular descriptors offering valuable and simple information about the structure of the molecules which is used later in the elaboration of the predictive models. The employment of this methodology allows cost savings by reducing the laboratory resources needed, and the time required to create and investigate new drugs with certain desired biological activity. A data set of 17 thiazolidine derivatives, which possess the most significant antitumor activity on two renal tumour cell lines 780-0 and UO-31, was used. Primary anticancer assays were performed according to the US NCI protocol. The Radial Distribution Function and 3D-MORSE descriptors for the given compounds were calculated using DRAGON software on the (x,y,z)-atomic coordinates of the minimal energy conformations determined by the AM1 method in Hyperchem 7.01 Evaluation Package. Descriptors with constant or near constant values inside each group were discarded. Mathematical QSAR models were obtained by means of Multiply Linear Regression technique as implemented in BuildQsar software. Quality of the obtained models was determined by examining the following data: correlation coefficient (R) and Fisher's statistic (F). Robustness of the obtained models was examined by Leave-One-Out (LOO) cross-validation technique, which characterises by cross-validation coefficient (Q^2). The best three-dimensional QSAR models for two different tumour cell lines are given below together with the statistical parameters of the regression:

UO-31 = -36.79**RDF115m** - 3.61**RDF045e** +51.78**RDF145p** +151.31
R = 0.97, F = 69.10, Q^2 = 0.888;
UO-31 = +381.23**Mor30u** +17.48**Mor03m** -191.88**Mor25p** +194.92
R=0.797, F=7.553, Q^2=0.325;
786-O = +30.49**RDF055m** +22.33**RDF105m** -21.23**RDF100e** -87.63
R=0.923, F=24.848, Q^2=0.787;
786-O = -71.33**Mor16u** +230.46**Mor24u** -607.92**Mor28v** +161.24
R=0.945, F=36.278, Q^2=0.821.

Variables in the obtained models encode specific 3D structural information about the size and arrangement of particular atoms or atom groups which presence induces anticancer effect. To posses significant antitumor activity on renal tumour cell line UO-31 optimal size of thiazolidine derivatives must be equal to 2.5-14.5 A° and for line 786-O – 4-15.5 A°. Also we can make an assumption about different mechanisms of action on different tumor cell lines. Obtained QSAR models possess high predictive ability and quality and may be used for virtual screening of potential anticancer drug candidates.

The possibility of modern evidential pharmaceutical care elaboration in Ukraine

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WHO strategy in relation to antimicrobial drugs argues their rational use as: cost-effective, with a maximum clinical effect, minimal toxicity and with account of resistance. GCP offers accounting of according group of these drugs to infectious agents and diseases, sensibility to certain antimicrobial drugs. Until today in Ukraine, with the exception of the State formulary of drugs, first release (2009), level of clinical effect evidence, particularly antimicrobial drugs, was not practically considered. We consider that modern pharmaceutical care that conduces to the improvement of pharmacotherapy quality must be based on the priority – high evidence of the given information about drugs. In a similar aspect a problem is examined for the first time in Ukraine.

Aim of this stage of research was to prove the possibility of elaboration the modern evidence-based pharmaceutical care in Ukraine. For realization of research's tasks the following methods were used: modern informative search, statistical, analytical; principles of evidence (to evaluate the quality of information). On the basis of using the high-quality world databases (SIGN, NICE, Cochrane Collaboration), and also most authoritative pharmaceuticals formulary (BNF, WMF), we analyzed 22 antimicrobial drugs (9 different pharmacotherapeutical groups) with the best clinical efficiency (evidence level A, B) at gynecological pathology (sexual Chlamydia infection, gonorrhea during pregnancy, post-natal endocentric). Instructions for medicines contain a number of indications, including for use in gynecology. However, the evidence of clinical efficacy of antimicrobial drugs (level A, B), especially in gynecology, available today, only for single illnesses. Instead, responsibility for use of certain antimicrobial drug, in particular, during pregnancy, fully, by the instruction for medicines, relies on a doctor who hasn't, usually, access to the high-quality information, and sometimes doesn't know about its existence. Therefore, he makes clinical decision which is based only on his experience, considerably lowering the level of evidence.

Modern pharmaceutical care requires the use of evidence-based information about drug's clinical efficiency, which is possible only with high-quality computer database using. The evidence of high truth level clinical efficacy, in the moment of research, has only specific indications or illnesses, instead of all marked in instruction for medicines. The elaboration of modern evidential pharmaceutical care is possible in Ukraine and can reduce pharmacotherapy medical errors because of its influence on the clinician's decision.

Traditional honey food product abbamele – chemical characterisation and antioxidant activities

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Sardinian *abbamele* is a typical product originally obtained from the recuperation of honey from the combs, but without information about its useful properties as well as used honey types in the production. On the other hand, a honey in general is an excellent nutritional food with health benefits. It has been used for the treatment of flu and common cold, healing of wounds and burns, as anti-microbial agent as well as the source of natural antioxidants. Therefore, five *abbamele* samples were obtained. The long thermal treatment applied in *abbamele* production caused very high (1007.0 - 4405.8 mg/kg) HMF content (HPLC-DAD), while glucose and fructose amounts were quite similar to the honey ones (HPLC-RI). Thermally derived furan derivatives and terpenes were abundant among the headspace volatiles (HS-SPME), particularly limonene (0.5 - 76.0%) that probably originated from citrus rinds addition during abbamele production. GC and GC-MS analyses of ultrasonic solvent extracts revealed HMF predominance as well as the compounds originated from the honey (if/when existing) such as methyl syringate (up to 49.2%), marker of *Asphodelus microcarpus* honey previously determined by HPLC-DAD (chemical traceability of *A. microcarpus* honey is of great importance since melissopalynology does not allow the unambiguous determination of its botanical origin). However, it was necessary to confirm its predominance in volatile compounds of *Asphodelus* honeys. Therefore, GC and GC-MS analyses were performed on *Asphodel* honey ultrasonic extracts (USE). Methyl syringate was the major compound ranging from 50.8 to 87.0%. High isophorone percentage (up to 30.9%) determined by HS-SPME followed by minor percentage of 4-ketoisophorone and norisoprenoides in one *abbamele* sample indicated *Arbutus unedo* L. honey use in the production. HPLC-DAD analysis confirmed the presence of specific honey markers: two samples showed high methyl syringate concentrations (150.4 - 120.1 mg/kg) while homogentisic acid and other specific markers of *A. unedo* honey were found in one sample. The compared GC-MS and HPLC-DAD data proved to be useful to obtain information about the use of specific honeys in *abbamele* production and to verify citrus addition. Total antioxidant activity (FRAP assay) of the samples ranged between 13.3 and 71.2 mmol Fe^{2+}/kg, while antiradical activity (DPPH assay) ranged between 3.8 and 23.3 mmol TEAC/kg. Antioxidant values were linearly correlated with total phenols amount (1297.8 - 4469.5 GAE mg/kg) determined by Folin-Ciocalteau method. However, because of the strong correlation between total polyphenols content and abbamele antioxidant activity, the total phenol amount is an interesting aspect, even affected from the contribution of Maillard reaction products (antioxidant activity of the honey is greatly influenced by its botanical origin as well as heat treatment). Such high antioxidant values indicate a good potential in the scope of food pharmacy in comparison to the honey. Particularly, *Asphodelus* honey showed average antiradical activity value of 0.7 mmol of TEAC/kg, whereas the antioxidant activity ranged between 3.0 and 5.7 mmol of Fe^{2+}/kg.

Genomics

Results of molecular genetic analysis of SMN1 and NAIP gene deletions in the high risk of SMA families

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The Spinal muscular atrophy (SMA) (I-Werdning-Hoffmann disease; II-intermediate form; III-Kugelberg-Welander disease) vary according to the age of onset and the rate of degenerative changes of spinal cord motor neurons progression, are connected with the Survival Motor Neuron gene (SMN1) mutation. Two highly homologous copies of Survival Motor Neuron gene: telomeric gene (SMN1) and centromeric gene (SMN2) are defined in this genome areal. Deletions of the SMN1 gene appear to be directly involved in SMA and are detected in over 95 % of SMA patients. The differences in exons 7 and 8 sequences are used to detect the genes in DNA analysis.

The NAIP gene lies in the region adjacent to the SMN1 gene and is present in the SMA region with multiple pseudogenes, which apparently arise independently of the inverted duplication. The neuronal apoptosis inhibitory protein gene has been postulated to have neuroprotective effect and acts as a negative regulator of motor neuron apoptosis.

DNA of the patients with the clinical diagnosis of SMA was extracted from blood leucocytes and PCR was conducted. The amplified products were analysed by RFLP with endonuclease DraI and DdeI for exon 7 and exon 8 of SMN1 correspondingly. Deletion analysis of exon 5 of NAIP gene was conducted (detection of exon 5 of NAIP has been taken as evidence for the existence of at least one active copy of the gene).

The DNA bank of 63 probands with SMA clinical features and 82 family members' DNA samples has been collected. Among the probands homozygous deletion of SMN1 exons 7 and 8 has been found in 23 (36,5 %) cases. Homozygous deletion of SMN1 exon 7 only has been identified in 2 (3,2 %) probands. The detection of homozygous deletion of SMN1 exons 7 and 8 enables to verify the diagnosis of SNA in 39,7 % of affected individuals. The absence of these genetic defects does not rule out the diagnosis of SMA.

The homozygous deletion of NAIP gene was detected in 11 patients with deletions SMN1 exons 7 and 8 and the deletion rate was higher in SMA type I patients than that in SMA type II or III.

These findings could be of relevance concerning a potential role of the NAIP gene as a modifying factor in the pathogenesis of SMA.

The further research should be aimed at the detection of heterozygous deletion carriage and the possible gene conversion which can be conducted using quantitative molecular genetic analysis.

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The essence of the spontaneous point mutations: quantum-chemical seeking for means of the general structural principles

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Nowadays the occurrence of spontaneous transitions is explained by two physico-chemical mechanisms – the first is a formation of mismatched (wobble) base pairs in the center of recognition of highly replicative DNA-polymerases ; the second is considered in the framework of tautomeric hypothesis that suggests the formation of the base pairs involving rare (imino and enol) tautomers in the recognition centre [1]. Thus, the advantage of the first mechanism lies in the fact that the wobble pairs Gua·Thy and Ade·Cyt are registered experimentally and incorporated satisfactorily into the double helix of DNA. The advantage of the second mechanism is quasiisomorphism of the complementary base pairs which invoke imino and enol tautomers (or protonated species) to the Watson-Crick base pairs but its shortcoming is that such [however, these] mispairs invoking rare tautomers are not experimentally registered. Analyzing the literature we come to a conclusion that these two mechanisms are regarded as alternative [2].

In the present work we attempt for the first time to prove that the aforementioned approaches to the basic nature of spontaneous transitions actually are interrelated, so we proposed a new mechanism of spontaneous transitions appearance. We hypothesize that they emerge due to the transformation of the wobble DNA base pairs Gua·Thy and Ade·Cyt into the pairs invoking the mutagenic tautomers Gua*·Thy and Ade·Cyt* accordingly (the mutagenic tautomers unlike the canonical are designated with an asterisk).

Moreover we proved the existence of a new, unknown before, molecular mechanism of the spontaneous transitions during DNA biosynthesis, namely – the tautomerisation of the bases induced by the center of DNA base pairs recognition of the replicative DNA-polymerases resulting in the formation of the mispairs Gua*·Thy and Ade·Cyt*.

The object of our research are molecular structures – wobble DNA base pairs, DNA base pairs invoking the rare tautomers and the transition states of their reciprocal transformation. The subject of our investigation is physico-chemical mechanism of the transformation of the wobble DNA base pairs into the pairs invoking rare tautomers of the isolated bases or, hypothetically , in the center of recognition of Watson-Crick DNA base pairs by the replicative DNA-polymerases. The method of research – quantum-chemical modeling on the MP2/6-311++G(2df,pd)//B3LYP/6-311++G(d,p) level of theory.

For the first time a new physicochemical mechanism has been proposed and grounded in order to understand the origin of the spontaneous transitions. It is based on the tautomerization, induced by the interaction of the DNA-polymerase recognition center with the canonic nucleotide bases, of the pyrimidine bases in wobble base pairs Gua·Thy and Ade·Cyt which transit into the pairs Gua*·Thy and Ade·Cyt* accordingly.

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The expression of cell cycle and several other genes in embryonic kidney cell line HEK293 is controlled by ZXDC transcription factor signaling

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The zinc finger X-linked duplicated type C (ZXDC) protein is the founding member of ZXD family proteins. The other two members, ZXDA and ZXDB, are retrogenes derived from ZXDC and are located on the short arm of the X-chromosome. The transcriptional factor ZXDC contains ten zinc fingers and a strong transcriptional activation domain. Previously was shown that transcription factor ZXDC in complex with ZXDA is participating in the regulation of major histocompatibility complex class II genes expression as the class II trans-activator. In this work we created subline of embryonic kidney cell line HEK293 which over express transcription factor ZXDC for identification of genes, transcription of which is depended from ZXDC signaling. Overexpression of transcription factor ZXDC was confirmed by polymerase chain reaction and Western blot analysis. Cells stable transfected with beta-gal was used as control. Using microarray analysis we have shown that overexpression of transcription factor ZXDC significantly changes the level of expression of large group of genes which control different processes in the cells. Thus, we identified several genes which participate in the control of cell cycle and which expression significantly increases in subline of embryonic kidney cell line HEK293 which over express transcription factor ZXDC: CDC14 homolog A (CDC14A) and R-spondin 3 (RSPO3) – in eight fold, fibroblast growth factor 18 (FGF18) and cyclin A1 (CCNA1) – in six and four fold, respectively, fibroblast growth factor receptor 3 (FGFR3) and cyclin-dependent kinase inhibitor 1C (CDKN1C) – in three fold. However, expression of mitogen-activated protein kinase 1 (MAPK1) and cyclin-dependent kinase 10 (CDK10) is decreased (in four and five fold, respectively).
Significant induction of gene expression (from four to nine fold) was also observed for many other genes: early growth response 2 (EGR2), interleukin 5 receptor, alpha (IL5RA), interleukin 9 receptor (IL9R), intercellular adhesion molecule 4 (ICAM4), matrix metalloproteinase 15 (MMP15), brain-derived neurotrophic factor (BDNF), gamma-aminobutyric acid (GABA) A receptor, gamma 3 (GABRG3) and cholinergic receptor, nicotinic, alpha polypeptide 3 (CHRNA3). Induction of the expression of IL5RA, BDNF, EGR2 and CDKN1C mRNA was confirmed by polymerase chain reaction using GAPDH mRNA expression as control. These results clearly demonstrated that transcription factor ZXDC participates in the regulation of expression of large group of genes, which control very important cell processes.

Anticancer antibiotic kigamicin D induces of cell apoptosis via a Bax-initiated mitochondria-dependent pathway.

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Kigamicin D is a member of novel anticancer antibiotics which were discovered from the culture broth of Amycolatopsis by their selective killing activity against pancreatic cancer cell line Panc-1 using a new screening strategy that targets the tolerance of cancer cells to nutrient starvation. Pancreatic cancers are known to be among the most aggressive malignancies despite their poor blood supply. We examined the possible molecular mechanisms of cell death caused by kigamicin D using pancreatic cancer cell line KP-3 and PSN-1. Cell lines were cultured in glucose-deprived media with or without kigamicin D (0.1 μg/ml during 1 or 2 hours). Kigamicin D was added to the cell cultures in one hour after pre growing with glucose-deprived media. Results of this investigation shown that kigamicin D induces the accumulation of BCL2-associated X protein (Bax) protein in the mitochondria of KP-3 and PSN-1 cancer cells. Moreover, kigamicin D initiates cytochrome c release from the mitochondria to the cytosol. Effect of kigamicin D was observed in one hour and kept at the same level in two hours. Kigamicin D suppresses the expression of mRNA Bax induced by glucose starvation in PSN-1 cancer cells. Because Clock and activating transcription factor 4 (ATF4) transcription system regulates drug resistance in human cancer cell lines we have also studied effect of glucose-deprivation and kigamicin D on the expression of Clock and ATF4 mRNA in pancreatic cancer cell line PSN-1. Glucose starvation significantly induces expression of Clock mRNA and slightly – ATF4. Kigamicin D was observed to block completely the expression of both Clock and ATF4 induced by glucose starvation in these cancer cells.

We have also shown that human glioma cancer cell line U87 is sensitive to kigamicin D without glucose starvation. Moreover, subline of these cancer cells without IRE-1α signaling are more sensitive to killing by kigamicin D. Blockade of IRE-1α signaling in U87 glioma cells leads to significant increase in Clock and especially death receptor 6 mRNA expression as well as suppress ATF4 and clusterin mRNA expression.

Thus, our results showed an induction of Bax translocation to the mitochondria and cytochrome c release from the mitochondria to the cytosol in two different pancreatic cancer cell lines, demonstrating that this compound induces apoptosis through a Bax-initiated mitochondria-dependent pathway.

Proteomics

Metformin reduces production of reactive oxygen species in renal glomerular podocytes through an AMPK-dependent mechanism.

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Nephropathy is one of the most serious complications in diabetes and a major cause of chronic renal failure worldwide. Recent data suggest that malfunction of glomerular epithelial cells, podocytes, plays an essential role in the development of diabetic nephropathy, although the mechanism of this is unknown. Hyperglycemia increases the production of reactive oxygen species (ROS). NAD(P)H oxidase, producing superoxide anion, is the main source of ROS in diabetic podocytes and their production may lead to oxidative damage. Metformin is one of the major antidiabetic drug with stimulates activity of AMP-dependent kinase (AMPK). We have investigated the effect of metformin on the production of superoxide anion in cultured podocytes and attempted to elucidate underlying mechanisms.

The experiments were performed in normal (NG, 5.6 mM) and high (HG, 30 mM) glucose concentration. Overall ROS production was measured by fluorescence of a DCF probe. Activity of NAD(P)H oxidase was measured by chemiluminescence method. The AMP-dependent kinase (AMPK) activity was determined by immunobloting, measuring the ratio of phosphorylated AMPK to total AMPK. Glucose accumulation was measured using 2-deoxy-[1,2-^{3}H]-glucose.

ROS production increased by about 27% (187±8 vs. 238±9 arbitrary units AU, $P<0.01$) in HG. Metformin (2 mM, 2h) markedly reduced ROS production by 45% in NG and 60% in HG. Metformin decreased NAD(P)H oxidase activity in NG and HG (from 3.16±0.11 to 2.08±0.07 and from 6.86±0.32 to 1.15±0.13 nmol O_2^-/mg protein/min, respectively, $P<0.05$). AMPK activity was increased by metformin in NG and HG (from 0.58±0.07 to. 0.99±0.06, and from 0.53±0.03 to 0.64±0.03; $P<0.05$). We have also shown that most of the antioxidative activity of metformin is determined by its ability to activate AMPK. This is confirmed by the decreased generation of O_2^- by metformin and by no effect of metformin on NAD(P)H oxidase activity when AMPK was inhibited by compound C (100 μM). In fact, our data underscores that AMPK is an important regulator of NAD(P)H oxidase.

These observations provide evidence that, in podocytes, metformin exerts has intracellular antioxidant properties. It decreases ROS production by activating the AMPK, which in turn inhibits the NAD(P)H oxidase activity.

Functional characterization of novel mammalian isoform of adaptor protein ITSN1.

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Investigation of cellular signaling during last decade could be considered as a robust breakthrough in the understanding of the role of signaling molecules such as kinases and small GTPases. Despite this progress, relatively poor knowledge was gained about proteins without catalytic functions that provide frameworks for the signaling. There is a great number of components of signalosomes that are not mentioned as signal transducers or messengers or even are not considered as direct participants. Nevertheless, their role is difficult to overestimate. Adaptor proteins function to bring together components of signaling complexes, to serve framework for the complexes assembly and rearrangements. Our understanding of adaptor proteins becomes more intricate due to existence of multiple splicing events affecting expression, stability, domain structure, regulation of the encoded proteins, etc.

Our research is focused on investigation of adaptor protein intersectin 1 (ITSN1) and plethora of its isoforms in cells of vertebrates. Here we report functional characterization of the novel isoform (ITSN1-22a) of adaptor protein intersectin 1 (ITSN1) with alternative C-terminus. ITSN1 is engaged in clathrin-mediated endocytosis, mitogenic signaling and actin cytoskeleton rearrangements. The most explored role of intersectin deals with internalization events of activated receptor complexes and other cargos. It also participates in cell survival, cell polarity, neurons outgrowth. Expression of ITSN1-22a was observed in all tissues tested, levels of expression were significantly lower in comparison to ITSN1-s. ITSN1-22a is also engaged in clathrin-mediated endocytosis, forms complex in vivo and is codistributed with ITSN1-s isoform. Some differences were observed in isoform localization at the plasma membrane. Alternative C-terminus of ITSN1-22a is engaged in homodimerization via disulphide bonds. Moreover, it provides specific interactions with SH3 domains of amphiphysin 1 and ITSN1. The direct interaction is not needed for engagement of the ITSN1-22a and ITSN1-s in mutual complexes; presumably this interaction is mediated via common protein partners. Both isoforms undergo monoubiqutination; this modification does not depend on serum starvation/stimulation. We have shown that tandem SH3A-22a does not affect dynamin1-amphiphysin1 complex assembly/stability *in vitro* thus, ITSN1-22a and amphiphysin 1 do not compete for dynamin 1. Our results suggest that novel isoform ITSN1-22a complements expands ITSN1 function in mammalian cells linking additional endocytic protein and providing alternative assembly platform consisting of SH3A-22a in comparison to SH3A-E block of ITSN1-s.

Characteristics of ribosomal protein S6 kinase interactions with novel partner - TDRD7

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Ribosomal S6 kinase 1 (S6K1) is an important player in cellular PI3K/mTOR signaling network involved in regulation of cell growth and differentiation. Our recent two hybrid yeast screening, using S6K1 as bait, allowed us to identify tudor domain containing protein 7 (TDRD7) – a protein with unknown function as a novel binding partner of S6K1. TDRD7 is a scaffold protein identified in complexes with proteins which regulate cytoskeleton dynamics, mRNA transport and protein translation apparatus.
To confirm and investigate a role of S6K1-TDRD7 interaction we conducted more detailed studies of these proteins interplay. First, bioinformational analysis of TDRD7 primary structure was carried out. It allowed us to determine several functional domains within TDRD7 and possible S6K1 sites of phosphorylation on this protein. At the next step six different fragments of TDRD7 were cloned, over expressed and purified from bacteria cells. These recombinant proteins were used in a set of pull-down experiments with full-length S6K1. Direct interaction between C-terminal tudor domain of TDRD7 and S6K1 has been shown. This interaction was further confirmed in Far-Western blot on recombinant S6K1 and TDRD7 fragments.
Also, purified domains of TDRD7 were used as antigens for mouse immunizations and generation of monoclonal antibodies (Hybridoma, 2008). The generated antibodies were used for studying S6K1/TDRD7 interaction in mammalian cells in vivo. We have detected the interaction between TDRD7 and S6K1 in reaction of co-immunoprecipitation in HEK293 and some rat tissues extracts.

Adaptor protein Ruk/CIN85 is involved in regulation of EGF/ uPA - dependent signaling in MCF-7 cells

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Cell migration, adhesion and invasiveness play a key role in tumor progression and metastasis. Urokinase-type plasminogen activator (urokinase, uPA), its receptor (uPAR) and inhibitor (PAI-1) are the major regulators of these processes. However, the intracellular mechanisms that mediate uPA/uPAR/PAI action remain incompletely understood. Because cell-surface receptor of uPA lacks transmembrane and cytoplasmic domains, it is assumed that uPA signaling is transduced via growth factor receptors, such as EGFR, associated with uPAR.

Ruk/CIN85 is an adaptor protein implicated to play a role in carcinogenesis by influencing ligand-induced endocytosis of growth factor receptors, cell adhesion, motility and apoptosis. We aimed to explore the dynamics of Akt and ERK1/2 activation by EGF in the presence of uPA using breast adenocarcinoma MCF-7 sublines with different levels of stable Ruk/CIN85 expression. It was shown that in the cells over expressing Ruk/CIN85 both Akt and ERK1/2 were significantly activated already at 1st min after EGF addition and maintained at high levels up to 30-th min after growth factor treatment, as compared to control. After treatment of cells, which over express Ruk/CIN85 with both uPA and EGF together, ERK1/2 activation was much stronger compared to EGF-treatment only.

In order to analyze whether Ruk/CIN85 over expression affects uPA/uPAR/PAI system in MCF-7 cells we developed polyclonal antibodies by immunizing rabbits with PAI-1 protein. As an antigen we used recombinant PAI-1, obtained from lysates of induced *E. coli* BL-21 cells, which were transformed with pT7-PL-PAI-1 vector containing N-terminal His_6-tag sequence. His_6-PAI-1 was purified using Talon Sepharose. High homogeneity of obtained recombinant PAI-1 protein was demonstrated by SDS-PAGE. The specificity of generated antibodies was examined by ELISA and Western blotting.

It was shown that PAI-1 expression was significantly up regulated in MCF-7 sublines with high levels of Ruk/CIN85. In these experiments hypoxia treatment and Ruk/CIN85 over expression had an additive effect on gene expression. Next, MCF-7 cells were cotransfected with a luciferase reporter gene construct containing 806 base pairs of PAI-1 promoter and Ruk/CIN85 expression vector. It was shown that in the presence of Ruk/CIN85 luciferase activity was induced both under normoxia and mild hypoxia. Ruk/CIN85-dependent stimulation of PAI-1 expression was partially blocked by PI3K/Akt pathway inhibitor LY 294002.

In conclusion, our data show that over expression of Ruk/CIN85 affects EGF-induced signaling and expression of uPA/uPAR/PAI system components in MCF-7 breast adenocarcinoma cells.

Metabolomics

Evaluation of the corneal endothelium in patients with diabetes mellitus type I and II

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Aim: To determine corneal physiology and endothelial morphology after proper image analysis technique in type I and II diabetic patients. The HbA1c level and the grade of retinopathy were also recorded and correlated with the endothelial parameters.
Methods: 41 eyes of 21 patients with type I and 59 eyes of 30 patients with type II diabetes mellitus (mean age was 40.97±15.46 and 64.36±10.47 years) were examined and compared to age-matched controls. Endothelial cell density (ECD), mean cell area, coefficient of variation of cell area, central corneal thickness, intraocular pressure, and grade of retinopathy were recorded.
Results: There was a statistically significant decreased endothelial cell density in type I disease (2428±219 cell/mm^2) in comparison with healthy subjects (2495±191 cell/mm^2, P = 0.02). The diabetic corneas were thicker than normal (P = 0.001). The HbA1c level was inversely correlated with the ECD (r = -0.60; P < 0.0001) and correlated with the mean endothelial cell area (r = 0.60, P < 0.0001). Significant correlation was observed between the endothelial morphology and grade of diabetic retinopathy (r = -0.40, ECD; r = 0.38, mean cell area; P = 0.01 for both). In type II diabetes mellitus no significant difference was found in the evaluated values.
Conclusions: The present study disclosed the alteration of the corneal endothelial morphology in type I diabetes mellitus as compared to normal subjects. The results indicated that the type I diabetic corneas are more susceptible to environmental changes than type II corneas.

Expediency of HFE gene mutation screening among patients with idiopathic conditions related to impaired iron metabolism

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It remains controversial whether general screening for HH (hereditary hemochromatosis) mutations should be performed or whether genetic tests should only be done in subjects found to have a disturbed iron metabolism. Individuals with hemochromatosis gene (*HFE*) mutations and iron overload develop hepatocellular and some extra hepatic malignancies at increased rates. Some authors hypothesized that due to the pro-oxidant properties of iron, altered iron metabolism in HFE carriers may promote breast carcinogenesis. Associations have suggested that the *HFE* mutations may also be involved in the development and clinical modification of other non-malignant diseases (cardiovascular diseases, cystic fibrosis, diabetes, arthritis, neurodegenerative disorders, and alcoholic liver disease). The molecular – genetic analysis of C282Y and H63D *HFE* gene mutations have been performed among 155 persons living at Western Ukraine area. Among them 42 children with idiopathic hepatobiliar disturbances, 63 Cystic Fibrosis patients with identified CFTR gene mutations (34 homozygous for F508del), 20 women with incidents of breast cancer in family and 30 persons from control group. For molecular-genetic testing of C282Y and H63D *HFE* gene mutations the PCR based procedures, which required DNA isolation, PCR and RFLP - analysis have been used. 12 heterozygous carriers of C282Y mutation, 35 heterozygous carriers of H63D mutation and one compound heterozygous C282Y/H63D were identified among analyzed group. General frequency of C282Y mutation carriers among studied groups of patients was 8,39%, H63D – 23,2%. Molecular-genetic research showed reliably higher C282Y mutation frequency among patients with hepatobiliar pathology (11,9%) and among CF patients (9,5%) as compared to control group (3,3%) that suggests a relationship between the development of hepatobiliar conditions in CF and *HFE* C282Y mutation. The differences in H63D mutation frequency among studied groups weren't reliable. Among patients with incidents of familial breast cancer 5% individuals were carriers of C282Y and 20% - H63D mutation, that don't differ from general population data. Accordingly to obtained data every 15 – 30 person is heterozygous carrier of C282Y mutation and every forth is heterozygous carrier of H63D mutation that point at necessity of increasing watchfulness concerning HH diagnosis. Molecular – genetic testing of *HFE* gene mutations enable to detect the disease in pre-symptomatic stage and under proper low-cost therapy prevent the complications development, among which hepatocellular carcinoma is the most severe.

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Subcutaneous fat and metabolic risk factors in pre- and postmenopausal women.

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Background: Subcutaneous fat is discussed as a potential risk factor for insulin resistance and cardiovascular disease. We analyzed association between subcutaneous fat and metabolic risk factors with regard to reproductive status in middle-aged women.

Methods: Women aged 45-54 years (n = 602) from general population were evaluated for the presence of cardiovascular risk factors including plasma lipids and markers of insulin resistance, calculated as fasting plasma glycemia (mmol/l) multiplied by fasting insulin (IU/l)/22.5 (HOMA-IR). In addition, subcutaneous fat was measured by ultrasound in all participants. Association between subcutaneous fat and plasma metabolic risk factors (triglycerides, HDL cholesterol, LDL cholesterol, HOMA-IR and C-reactive protein, measured by highly sensitive method) was analyzed, separately for premenopausal (n = 375) and postmenopausal (n = 227) women. The (independent) association between subcutaneous fat and risk factors under study was evaluated by multiple regression analysis (STATA), inculding also age and waist circumference.

Results: In the whole group subcutaneous fat was significantly associated with all metabolic risk factors under study. Nevertheless, after waist circumference was included into the statistical model, no significant association between subcutaneous fat and any risk factor was found. Separate analysis according to the reproductive status revealed significant and independent association between subcutaneous fat and HOMA-IR in premenopausal ($p = 0.014$), but not in postmenopausal ($p = 0.507$) women.

Conclusion: Subcutaneous fat was independently associated with markers of insulin resistance in premenopausal but not in postmenopausal women. This finding may have implications for diagnostic and preventive strategies focused on metabolic syndrome and on the unfavorable fat distribution in women.

The role of aromatic compounds in food on dietetic behaviour of healthy rats

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Maillard reaction products (MRPs) are formed in high amounts in foods during thermal processing. MRPs occur from non-enzymatic glycation, reaction between free amino-groups of proteins and reducing sugars. MRPs render foods aroma, taste and colour. Consumption of diets rich in MRPs was shown to exert deleterious health effects, among others weight gain and diabetogenic effects, both in experimental animals and humans (1). Herein, we addressed the question whether the mechanisms of satiety and behaviour in the adult healthy rats are affected by extractable volatile compounds of bread crusts, or MRPs. Male Wistar rats were randomized into 3 dietary intervention groups (n=8 each): control (CTRL, standard rat chow), bread crust (BC, standard diet supplemented with 25% w/w bread crust), and extracted bread crust (EX, standard diet supplemented with 25% w/w ether-extracted bread crust). During 3 weeks intervention food and water intake, body weight gain and urine volume excretion was recorded. Behavioural tests (openfield, light-dark box, forced swimming and anhedonic test) were performed. Plasma insulin, leptin and adiponectin (rat specific ELISA tests), and urinary excretion of 5-hydroxyindoleacetic acid (5HIAA, HPLC method) was determined. Food intake was slightly higher in the BC and EX groups than in the controls, but CTRL rats gain more weight. Compared to CTRL rats BC and EX groups had higher plasma AGEs levels, reflecting the higher amount of MRPs in their diet ($p<0.001$). Diet enriched with bread crusts was associated with decreased insulin sensitivity (HOMA: $p<0.05$) and increased plasma leptin ($p=0.001$) and adiponectin levels ($p=0.001$). Urinary excretion of 5HIAA was significantly higher in both, EX and BC groups than in the controls. Rats consuming aroma-rich BC diet showed less explorative behaviour and more anxiety. In conclusion, the satiety regulating hormones insulin, leptin, adiponectin and serotonin metabolite 5-HIAA were all most affected by administration of the bread crust diet, whereas no significant effect was observed for the aroma extracted diet compared to the control diet, with exception of adiponectin. These results suggest the effect of both, MRPs and extractable volatile aroma compounds on satiety regulation and behaviour of healthy rats.
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Effects of pituitary adenylate cyclase activating polypeptide in diabetic retinopathy induced by streptozotocin in rats

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Introduction: Pituitary adenylate cyclase activating polypeptide (PACAP) is a neurotrophic and neuroprotective peptide that has been shown to exert protective effects in different neuronal injuries, models of neurodegenerative diseases and cerebral ischemia. About 200 million people are estimated to have diabetes in the world. Diabetic retinopathy, a vascular disorder affecting the microvasculature of the retina is a leading cause of adult blindness and is the most common complication of diabetes. Although neurotrophins, such as PACAP, have been assessed as therapeutic agents for neural complications, their involvement in diabetic retinopathy has not been characterized yet. **Methods**: Diabetes was induced by 70mg/kg streptozotocin in Wistar rats. We administered 3 times intravitreal PACAP (100pmol) injection into the right eye by Hamilton-syringe. We used histological, different kind of immunohistochemical (vertical cryosections and whole mount preparations) and molecular biology (Western blot, RT-PCR) methods. **Results**: In streprozotocin-induced diabetic rats dopaminergic amacrine cells appeared to be degenerated, as revealed by tyrosine hydroxylase (TH) immunoreactivity. Diabetic retinopathy resulted in severe degeneration of dopaminergic amacrine cells in the inner nuclear layer, as shown by the shape of their soma and their connection. The quantification of the dopaminergic amacrine cells showed significant reduction. Neuroprotective effects of PACAP were observed in streptozotocin-induced retinal degeneration. Intraocular PACAP treatment led to a nearly intact appearance of the soma, connections and also cell number. According to RT-PCR and Western blot analyses utilizing TH primary antibody, intensity of immunostaining was increased by PACAP treatment compared to diabetic retinas. **Conclusion**: Intravitreal administration of PACAP protected dopaminergic amacrine cells, demonstrating its therapeutic potential in streptozotocin-induced diabetic retinopathy.