ROMANIAN JOURNAL OF BIOCHEMISTRY

Volume 51, *Supplementa*, 2014

The Annual International Conference of the SRBMB

&

Workshop "Viral hepatitis from cell culture to clinic"

June 5-6, 2014
Băile Felix, Oradea, Romania

The Faculty of Medicine of the University of Oradea

Abstracts

ROM. J. BIOCHEM., **51,** *Suppl.*, 1–112 (2014)

SPONSORS

Colegiul Farmaciştilor

AMS 2000 Trading Impex SRL

S.C. MUSO S.R.L.

S.C. Phenalex SRL
http://www.phenalex.ro/

SCIENTIFIC COMMITTEE

Institute of Biochemistry of the Romanian Academy

Dr. Stefana Petrescu
Dr. Elena Ganea
Dr. Anca Roseanu
Dr. Norica Nichita

ORGANIZING COMMITTEE

UNIVERSITY OF ORADEA

Faculty of Environmental Protection

Simona Vicas
AnaMaria Morna
Alin Teusdea
Adrian Timar

Faculty of Science

Alina Caraban

Faculty of Medicine and Pharmacy

Mariana Muresan
Otilia Micle
Laura Vicas
Tunde Jurca
Eleonora Marian
Ramona Suciu
Annamaria Pallag
Angela Antonescu
Camelia Mraz
Luciana Dobjanschi
Marieta Lestyan
Ioana Codreanu
Alexandru Jurca

INSTITUTE OF BIOCHEMISTRY OF THE ROMANIAN ACADEMY

Anca Roseanu
Norica Nichita
Paula Florian

ROMANIAN JOURNAL OF BIOCHEMISTRY

VOLUME **51**, *Supplementa* 2014

CONTENTS

PLENARY LECTURES

ORAL PRESENTATIONS

PLENARY LECTURES

ROM. J. BIOCHEM., **51,** *Suppl.*, 9–14 (2014)

[PL1] NEW TECHNOLOGIES FOR PRODUCTION OF ANTIOXIDANT RICH SELENIUM ENRICHED SPECIAL FOODS

József Prokisch, Attila Sztrik, Beáta Babka, Timea Takács, Edina Keresztesi

University of Debrecen, Centre for Agricultural Sciences and Engineering, Böszörményi út 138. H-4032 Debrecen, Hungary;

Selenium is a wellknown antioxidant which behaviour strongly depends its chemical form. A novel technology for producing Se nanospheres in homogeneous in form and size has been developed in our laboratories. The technology developed is a manufacturing process which enables forming of a suspension as well as a powder containing valuable Se spheres having unique characteristics. Material prepared in such a way can be used in the food industry as food or feed additive. The relative simplicity of the technology developed allows for significant decline in prices which can further broaden the range of useful high quality raw materials available. We applied yogurt bacteria for the production of nanospheres. The technique is the first to use lactic acid bacteria and other probiotic bacteria (Species of Lactobacillus and Bifidobacteria, Streptococcus thermophilus) to form the product, Se nanospheres. Our invention enables the production of red elemental Se nanospheres in high purity by using micro-organisms applied in the food industry. These bacteria are commonly used, non-toxic and harmless. The toxicity and bioavailability of selenium nanospheres were tested in plant and animal models. We concluded that this form of selenium has good bioavailability and very low toxicity. Food supplements and diary product were developed by using this new form of selenium and some already available in food stores. The developed selenium enriched yogurt was tested in human study as well. According to the regulations (EU No 432/2012) 6 health claim can be applied for selenium enrich food:

1. Selenium contributes to normal spermatogenesis
2. Selenium contributes to the maintenance of normal hair
3. Selenium contributes to the maintenance of normal nails
4. Selenium helps to protect cells from some types of demages caused by free radicals.
5. Selenium supports the normal function of immune system
6. Selenium is necessary to the production of iodine containing thyroid hormones.

[PL2] THE ANTICANCER ACTIVITY OF MISTLETOE PREPARATIONS RELATED TO THEIR POLYPHENOL PROFILES

Henning M Schramm

Institute Hiscia, Society for Cancer Research, CH-4144 Arlesheim/Switzerland
e-mail: h.schramm@vfk.ch

Although the polyphenols contained in the various mistletoe preparations are known for their specific anticancer activities, their role in the antitumoral properties of these preparations has so far not been reviewed or evaluated.

The flavonoid profiles differ between European mistletoe preparations from deciduous and coniferous trees. Characteristic polyphenols of mistletoe preparations from deciduous trees are: gallic acid, quercetin and chlorogenic acid. Naringenin was not detected here, but is found in conifereous trees, namely in the pine. These polyphenols can be considered as the lead substances in the metabolic antitumoral properties of corresponding mistletoe preparations as their activities – so far found in experimental and clinical studies – correspond to the anticancer mode of action of the polypyhenols contained in them.

What makes the polyphenols so interesting in oncology? Although, in a pharmacological sense, their antitumoral activity seems to be weak in cancer patients, their mode of action nevertheless is distinctive. In in-vitro and in animal studies the polyphenols were found to act differently on cancer cells and on benign cells. The polyphenols may act-prooxidatively in cancer cells whereas in most studies they were found to exert anti-oxidative properties in benign cells. They have anti-estrogenic and aromatase-inhibiting effects on cancer cells, whereas they have an oestrogen-mimetic effect on the bone, thus stabilizing it. They inhibit the activity of fatty acid synthase – a prerequisite for the continous proliferation of certain cancer cells –, whereas they do not provoke weight loss often observed with synthetic fatty acid synthase inhibitors. They inhibit the activation of lipophilic oncogenic substance in the phase I detoxification process, whereas they stimulate the excretion of lipophilic oncogenic substances in phase II of the cell detoxification process.

Further polyphenols may decrease the risk of cancer prognosis factors, namely obesity and leanness. Certain polyphenols reduce the respiratory quotient in obese persons thus inducing a switch in energy fueling from glucose to fatty acids. In lean cancer patients who gain their energy mainly by beta-oxidation of fatty

acids, they stimulate this process in tumour cells where it is reduced or dysfunctional, whereas they stimulate glucose uptake in the muscle. They do not increase beta-oxidation in the whole organism, and thus these substances counteracted weight loss.

In mistletoe the polyphenols do not exert their activities alone but together with other of the plant`s substances and synergy is possible between them. The pro-oxidative action of the polyphenols from deciduous trees, e. g. quercetin or gallic acid, is supported by the high lectin content of these mistletoe preparations. Arginine and leucine have properties that work in synergy with the anti-obese activities of certain polyphenols.

Besides the various ingredients of mistletoe preparations so far studied in relation to their immunological activities, the polyphenols contained in these preparations may play a major role in combating the dysfunctional metabolism in cancer cells, the tumour and the patient.

[PL3] CHALLENGES AND IMPACT OF HIGH-THROUGHPUT TARGETED AND UNTARGETED PLANT/FOOD/MEDICAL METABOLOMICS USING UPLC-MS AND FTIR TECHNOLOGIES

Carmen Socaciu[1,2], Raluca Pop[2,3], Floricuta Ranga[1], Florinela Fetea[1]

[1] Dept. Agrifood Biochemistry, Univ. Agr.Sci. &Vet. Med., Cluj-Napoca, Romania
[2] RTD Centre of Diagnosis and Molecular Therapy, Proplanta Ltd, Cluj-Napoca, Romania
[3] Univ. Medicine & Pharmacy, Cluj-Napoca, Romania

Metabolomics, the "apogee" of the *omics* trilogy, as part of the systems biology (1,2) proved to provide a useful readout of cellular biochemistry and metabolic pathways. Metabolomics provide a downstream of genetic information, amplified relatively to transcriptome and proteome, filling important gaps in systems biology and focusing on interconnected molecular pathways, looking to metabolite networks, easier to understand the fluxomic networks. Using emerging technologies like ultra performance liquid chromatography (UPLC) coupled with mass spectrometry (MS), Fourier Transform Infrared spectroscopy (FTIR) thousands of metabolites can be identified and/or quantified providing a global metabolome, either for a plant/food extract, or for a biological fluid (blood, urine, saliva). Untargeted metabolomics complemented with targeted analysis can provide high level information regarding the key-biomarkers for a specific recognition of a plant or food, or for a specific diagnosis (early or progression-related to a specific disease (3).

New insights and discoveries are related to plant biodiversity, food authenticity and traceability, human and animal cellular metabolism, in vitro tests on normal and tumor cells, cancer diagnosis and prognosis, as well different biological processes. Metabolomics reflect the "personalized" picture for a plant or food product under different influencing factors like environmental stress or processing, or a specific medical fingerprint of a human/animal body fluid, as a noninvasive personalized profile of human or animal samples (by identifying and quantifying specific biomarkers) and offer large information, cheaper to generate, by reliable high-throughput technologies (UPLC/MS or FTIR)(4,5,6). Updated databases of metabolome and metabolic biomarkers are available and increase exponentially every year in all domains (plant/food/nutritional/medical/pharmaceutical/toxicological). The personalized medicine based on specific metabolic fingerprints, related to age, physiology and pathology, is ready to demonstrate beside the genetic background, the nutritional and "lifestyle" influence, providing solutions to modulate metabolic disturbances.

Bioinformatics is an essential tool to make appropriate interpretation of metabolomic data (PCA or cluster or heat map analysis) and can provide added-value information and approaches for modeling therapies. Specific case studies will be discussed to demonstrate the ubiquity of metabolomics involvement in systems biology, across all possible physiological and pathological stages of a living organism.

References

[1] Patti G.J., Yanes O., Siuzdak G., 2012, *Metabolomics: the apogee of the omics trilogy*, Nature Reviews, 13:263-269.
[2] Vaidyanathan S. et all.,(eds), *Metabolome analysis: strategies fos Systems Biology*, Springer, NY, USA, 2005.
[3] Kell D.B., 2007, *Metabolomic biomarkers: search, discovery and validation*, 2007, www.future-drugs.com.

ORAL PRESENTATIONS

[OP1] TAXONOMIC PROFILING OF PROKARYOTIC COMMUNITIES FROM AN EXTREME HYPERSALINE MEROMICTIC LAKE USING A NEXT-GENERATION SEQUENCING APPROACH

Ştefan-Adrian Andrei[1], Michael S. Robeson II[2], Mircea Podar[2, 3], Andreea Baricz[4], Cristian Coman[4], Cosmin I. Sicora[5], Vasile Muntean[6], Horia L. Banciu[1, 6, *1]

[1] *Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babeş-Bolyai University, Cluj-Napoca, Romania*
[2] *Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, USA*
[3] *Department of Microbiology, University of Tennessee, Knoxville, Tennessee, USA*
[4] *National Institute of Research and Development for Biological Sciences (NIRDBS), Institute of Biological Research, Cluj-Napoca, Romania*
[5] *Biological Research Center, Jibou, Romania*
[6] *Department of Molecular Biology and Biotechnology, Babeş-Bolyai University, Cluj-Napoca, Romania.*

Introduction. Halotolerant and halophilic prokaryotes are thriving in saline and hypersaline ecosystems, playing key roles in the overall biogeochemical processes occurring in such extreme habitats. Nevertheless, determining the taxonomic composition of their communities is challenging due to difficulties in detecting and quantifying the low abundance populations.

Aim of research. Presently, high-throughput molecular technologies are employed to profile microbial communities at unprecedented depth and resolution; we applied a next-generation Illumina-based sequencing approach in order to explore changes in diversity and structure of prokaryotic assemblages thriving in an extreme hypersaline stratified lake.

Materials and methods. The prokaryotic community structure of Ursu Lake, the largest athalassic meromictic hypersaline lake in Romania and one of the most important heliothermal salt lakes in Europe, was investigated using pair-end sequencing of the variable V4 region of 16S rRNA genes, on Illumina MiSeq platform and qPCR technique.

* *Corresponding author: horia.banciu@ubbcluj.ro*

Results. The number of DNA sequences obtained per sample varied between 62.000 and 141.000, and the number of operational taxonomic units between 180 and 575. The results showed that *Bacteria* outnumbered the *Archaea* and that alpha diversity increased with depth and salinity. The *Bacteria* were found to be more diverse than *Archaea* and most of the prokaryotic sequences were affiliated within the phyla Actinobacteria, Bacteroidetes, Proteobacteria, Firmicutes, Cyanobacteria, Chlorobi, and Euryarchaeota.

Conclusion. This study is the first attempt to characterize the prokaryotic diversity in an extreme hypersaline meromictic lake from Romania using a second-generation sequencing approach and shows that hypersaline environments have the capacity to harbor a larger extent of prokaryotic diversity than previously estimated.

Acknowledgements. This work was supported by grants of the Romanian National Authority for Scientific Research, CNCS–UEFISCDI, project numbers PN-II-ID-PCE-2011-3-0546 and PN-II-ID-PCE-2011-3-0765.

[OP2] EDEM1 MODULATES PROTEIN DEGRADATION ASSOCIATED TO THE ENDOPLASMIC RETICULUM

Marioara Chiritoiu, Stefana M. Petrescu

Institute of Biochemistry, Molecular Cell Biology Department, Splaiul Independentei 296, 060031, Bucharest 17, Romania

Roughly one third of the cell's proteome is synthesized on ER (endoplasmic reticulum) bound ribosomes and trafficked through the secretory pathway *en route* to either plasma membrane or a specific organelle. ER translocated proteins are subjected to a folding process assisted by the ER resident chaperones and foldases; polypeptides that fail to achieve native conformation are targeted for proteolysis through ER-associated protein degradation pathway (ERAD). EDEM1, a protein functioning in ERAD, was proposed to recognize misfolded proteins exiting folding cycles and target them for degradation. Extensive studies have shown that EDEM1 accelerates the degradation of ERAD substrates and is responsible for delivering these polypeptides to SEL1L, adaptor protein of the retrotranslocation complex. Initially it was believed EDEM1 is able to recognize specific patches exposed on misfolded proteins more exactly demannosylated glycans. Recent studies have shown that EDEM1 interacts with ERAD substrates in a glycan-independent manner and the interaction is mediated by the N-terminal domain of EDEM1.

Previously we showed that tyrosinase and its mutants are degraded through an EDEM1 dependent pathway. We found that EDEM1 requires a specific N-terminal domain to interact with tyrosinase and accelerate its degradation. Conversely we questioned if this might be relevant for other ERAD substrates. In this regard we extended our experiments to other ERAD substrates: alpha 1-antitrypsin and its misfolded mutant NHK, ribophorin 1 (Ri) and Ri332 (truncated mutant) and also the spliced form of β-secretase (BACE-476).

Our results showed that EDEM1 overexpression enhances the degradation of the proteins used in our studies. Also, similar to tyrosinase EDEM1 is able to interact with other ERAD substrates employed here. We conclude that EDEM1 has the same behaviour with other substrates as observed for tyrosinase. Further studies will require the elucidation of molecular determinants supporting the interaction of EDEM1 with the ERAD substrates.

[OP3] MECHANISMS OF CELL DEATH INDUCED BY PHOTODYNAMIC THERAPY WITH INNOVATIVE PORPHYRINS IN MELANOMA, *IN VITRO* STUDY

Baldea Ioana [1], Olteanu Diana [1], Nicoleta Decea [1], Bolfa Pompei [2], Cenariu Mihai [2], Muresan Adriana [1], Rodica Mariana Ion[3], Filip Adriana [1]

[1] Disciplina Fiziologie, UMF, Cluj
[2] USAMV, Cluj, [3]ICECHIM

Background: Melanoma, a cancer that arises from melanocytes is one of the most unresponsive cancers to known therapies. Several studies showed encouraging results of the efficacy of photodynamic therapy (PDT) using different experimental settings *in vitro* and *in vivo* as well as a few clinical reports, suggesting a possible role as an adjuvant therapy in melanoma.

Methods: We assessed the effects of PDT using two porphyrins: tetra-hydroxi-orto-phenyl-porphyrin (THOPP) and tetra-hydoxi-orto-metil-orto-phenyl-porphyrin (THOMOPP) on a melanoma cell line (WM 35). Cells were irradiated with Philips LED lamp with 630 nm wave lengths with irradiation doses of 50 and 75 mJ/cm^2. Cyto and phototoxicity were measured with a photometric cell proliferation assay. Flowcytometry with Annexin V-FITC/ Propidium iodide markers and ELISA measurement of active caspase 3 were used for cell death assessment. We tested the antioxidant activity of superoxide dismutase (SOD) and malondialdehide (MDA), a marker of lipid peroxidation, using a spectrophotometric assay.

Results: Both porphyrins induced a dose related phototoxic, in the absence of significant cytotoxic effect. THOPP was more potent in decreasing cell viability. Based on the viability results we selected THOPP concentrations of 2.5 and 5 μg/ml and respectively 25 and 50 μg/ml for THOMOPP for further experiments. The main mechanism of cell death was apoptosis (annexin-PI staining), however, necrosis was also induced (caspase 3). Antioxidant SOD enzymatic activity was only slightly increased, mainly in the case of THOMOPP($p \leq 0.006$). Only higher doses of PDT increased MDA levels, especially for THOPP($p \leq 0.011$).

Conclusion: The new porphyrins that we used in this study proved to be effective photosensitisers for the PDT in melanoma, in vitro.

[OP4] EFFECT OF QUERCETIN ON OXIDATIVE/NITROSATIVE STRESS IN THE THORACIC AORTA OF ADJUVANT-INDUCED ARTHRITIS RATS

Baltaru D[1], Mureşan A[2], Socaciu C[3], Moldovan R[2], Chiş C.[2*1]

[1] "Constantin Papilian" Military Emergency Hospital, G-ral Traian Mosoiu st., no.22, Cluj district, Zip code 400132, Cluj-Napoca, Romania
[2]Department of Physiology, University of Medicine and Pharmacy "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania, irinnaus@yahoo.com (corresponding author)
[3]Department of Agrifood Chemistry and Biochemistry,, University of Agricultural Sciences and Veterinary Medicine, Calea Manastur 3-5, Cluj, 400372, Cluj-Napoca, Romania

Background: Rheumatoid arthritis (RA) is a systemic inflammatory disease with cardiovascular complications as the leading cause of morbidity. An early manifestation of CVD is endothelial dysfunction which can lead to functional and morphological vascular abnormalities. Oxidative/nitrosative stress and inflammation are both implicated in causing endothelial dysfunction in RA. The excessive production of reactive oxygen and nitrogen species (RONS) are responsible for cartilage and bone destruction and endothelial dysfunction associated with RA. The purpose of this study was to evaluate oxidative/nitrosative stress in the aortic tissue and antioxidant effects of Quercetin (Que) in a rat model of adjuvant-induced arthritis (AIA).

Materials and methods: Arthritis was induced in adult male Wistar rats by injecting of Freund's adjuvant (FA, 0.1 ml) into the left hind footpad. AIA was confirmed 7 days after the injection of FA by clinical examination. Five groups of rats were compared: a non-arthritic control group and four AIA groups treated via an intragastric tube for 21 days, starting the first day after the induction of AIA, with: vehicle (saline solution), non-steroidal anti-inflammatory agents (indomethacin, 2 mg/kg/day or Arcoxia, 10 mg/kg/day) or Que (20 mg/kg/day) respectively. The clinical severity of arthritis was evaluated after 28 days using a macroscopic scoring system and by quantifying the change in the paw volume (as an indicator of oedema) with a plethysmometer. At the end of the experiment, the aortic tissue

* *Contact. author@email.com*

homogenat levels of the free radicals (malondialdehyde, MDA and carbonylated proteins, CP), nitrite plus nitrate (NOx) production, activity of antioxidant enzymes (reduced glutathione, GSH) and inducible nitric oxide synthase (iNOS) protein expresion were measured.

Results: In aortic tissue homogenate, MDA and CP levels, NOx production and iNOS expresion of AIA rats had increased significantly (P<0,005) and GSH activities had decreased significantly (P<0,005) compared to those of the control non-AIA rats. Expression of iNOS, NOx production, lipid peroxidation and protein carbonilation had decreased significantly (P<0,005) in Que-treated AIA-rats compared with non-AIA rats (control groups). However, Que administration significantly increased (P<0,05) GSH activities of the aortic tissue in the AIA rats.

Conclusions: This study suggests that Que treatment can be beneficial in attenuating the oxidative/nitrosative stress associated with RA.

[OP5] **THE ROLE OF ER DEGRADATION-ENHANCING MANOSIDASE-LIKE-2 (EDEM-2) IN *CAENORHABDITIS ELEGANS***

Simona Ghenea, Stefana Petrescu

Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, Bucharest, Romania

The endoplasmic reticulum associated degradation (ERAD) eliminates non-native proteins from ER and is essential for the maintenance of protein homeostasis. Failure in any step of the ERAD process results in accumulation of potentially toxic protein within ER, which could induce ER stress and finally apoptosis. EDEM-2 belongs to the ER α-mannosidase-like family of proteins that accelerate ER disposal and proteasomal degradation of misfolded proteins in cells in culture. Despite of increasing knowledge regarding the molecular mechanism of ERAD, little is known about the role of ERAD at a more global level, in an intact organism.

We investigated the role of the EDEM-2 in an intact organism and here, we show that EDEM-2 is ubiquitously expressed and localize to ER in various cell types. We found that the *C. elegans* EDEM-2 has an evolutionarily conserved function in ERAD, and its expression is modulated by unfolded protein response (UPR). However, we also show that EDEM-2 deficiency has pleiotropic effects under physiological growth conditions. Our results unrevealed a constitutively active role for EDEM-2 in animal development not only in response to inducible ER stress.

[OP6] HEPATITIS B VIRUS ENVELOPE GLYCOPROTEINS AS SUBTRATES FOR ER DEGRADATION-ENHANCING α-MANNOSIDASE-LIKE 3 PROTEIN

Catalin Lazar, Mihaela Uta, Norica Nichita

Institute of Biochemistry of the Romanian Academy, Department of Viral Glycoproteins, Splaiul Independentei 296, Sector 6, Bucharest 77700, Romania

Hepatitis B virus (HBV) belongs to the *Hepadnaviridae* family of enveloped DNA viruses. The HBV nucleocapside is surrounded by a lipid bilayer in which the large (L), middle (M) and small (S) envelope glycoproteins are embedded. It was previously shown that HBV can induce endoplasmic reticulum (ER) stress and activate the IRE1-XBP1 pathway of the unfolded protein response (UPR). As a consequence, the ER degradation-enhancing mannosidase-like proteins (EDEMs) are up-regulated to relieve the ER stress during UPR, by recognizing terminally misfolded glycoproteins and delivering them to the ER-associated degradation (ERAD). A crucial step in ERAD is the trimming of three or four mannose residues from the precursor sugar chain of the misfolded or unfolded glycoprotein. This process is accomplished by the ER alpha-mannosidase I itself or aided by other ER mannosidases. One possible mannosidase is ER degradation-enhancing α-mannosidase-like 3 protein (EDEM3), a soluble homologue of EDEM1 protein.

Previously, we demonstrated that co-expression of the wild-type HBV envelope proteins with EDEM1 resulted in their massive degradation. Surprisingly, the autophagy/lysosomes, rather than the proteasome were involved in disposal of the HBV envelope proteins. In this study we try to demonstrate the involvement of EDEM3 in the biosynthesis and secretion of HBV.

[OP7] MEMBRANE-BOUND AND SOLUBLE FORMS OF RAGE ARE DIFFERENTIALLY PROCESSED IN MELANOMA CELLS

Ioana Popa, Elena Ganea, and Stefana Petrescu

Institute of Biochemistry,
Splaiul Independentei 296, Bucharest 060031, Romania

The receptor for advanced glycation end products (RAGE) is upregulated in cancer cells and plays a role in carcinogenesis, tumor growth and invasion. Multiple receptor forms are generated through alternative splicing or proteolytic cleavage. Several mechanisms of oligomerization and ligand-driven multimerization have been described so far.

In the present study we investigated the expression of the monomeric and oligomeric receptor forms in melanoma cells with different degrees of malignancy. We used SDS-PAGE under reducing and nonreducing conditions, and Western blotting in which we competed for the binding of antibodies with recombinant soluble RAGE. RAGE oligomers of about 200 kDa, formed by disulfide bridges were detected in both primary (e.g. MelJuSo) and metastatic melanoma cells (e.g. SK-Mel28), while the molecular weight of the monomeric receptor was 55-60 kDa. However, in primary melanoma cells other types of interactions are likely to be important for the formation of higher RAGE complexes. We also found a soluble endogenous protein form of RAGE significantly reduced in SK-Mel28 cells compared to MelJuSo cells. Moreover, metastatic cells appeared to have a lower capacity of generating soluble receptor by ectodomain shedding upon overexpression of full length RAGE. Immunofluorescence microscopy showed RAGE had a more clustered, polarized distribution at the plasma membrane in MelJuSo cells, while in SK-Mel28 cells the receptor was localized throughout the cell. Silencing RAGE by siRNA inhibited migration of primary melanoma cells, and had little effect on metastatic cell migration, suggesting different roles and regulation of receptor functions in the two melanoma types.

Acknowledgements. This work was supported by a postdoctoral fellowship to I.P., within the postdoctoral program POSDRU/89/1.5/S/60746 from European Social Fund.

[OP8] ADHERENCE PROPERTIES OF FIBROBLASTS TO DIFFERENT BONE SUBSTITUTE DESIGNED FOR ORTHOPEDIC AND DENTAL APPLICATIONS

Simona Cavalu[1], Dumitrita Rugina[2]

[1]University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania,
[2]USAMV Cluj-Napoca, Romania

Poly(methyl methacrylate) (PMMA) bone cements are extensively used in certain types of total hip or total knee replacements and are of potential utility wherever mechanical attachments of metal to living bone is necessary The main function of the cement is to serve as interfacial phase between the high modulus metallic implant and the bone, thereby assisting to transfer and distribute loads. On the other hand, the development of new zirconia toughened alumina (ZTA) composites is aimed to substitute metallic devices. The aim of this study is to present comparatively the adherence and proliferation properties of human fibroblasts to PMMA bone substitute and alumina/zirconia ceramics. Scanning Electronic Microscopy (SEM) images demonstrated the morphology of fibroblasts after 3, 7 and 24 h incubation in contact with both specimens. The fibroblasts showed a wide variety of shapes: spread multipolar or round, as well as spindle shaped, elongated cells when they are in contact with PMMA. By comparison, the morphology of fibroblasts in contact with alumina/zirconia ceramics, showed a different behavior: in the first stage (3 hours), the cells did not spread out over the surface, avoiding the contact with the ceramic surface. After 24 h the cells forms a shell-like coating covering a large surface of the ceramic material, with numerous filopodia attached to the surface, in contact with each other. The results are also supported by the fluorescence confocal microscopic images, suggesting possible aggregate formation on the surface.

[OP9] CARDIOPROTECTIVE EFFECTS OF LYCIUM BARBARUM EXTRACT FROM OXIDATIVE/NITROSATIVE STRESS UNDER INTERMITTENT HYPOBARIC HYPOXIA EXPOSURE IN RAT

Irina Camelia Chiş[1]*, Doina Baltaru[2], Anca Dumitrovici[3], Andrei Coşeriu[4], Florina Vlad[4], Remus Moldovan[1], Adriana Mureşan[1]

[1]Department of Physiology, University of Medicine and Pharmacy "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania, irinnaus@yahoo.com (corresponding author)
[2]"Constantin Papilian" Military Emergency Hospital, G-ral Traian Mosoiu st., no.22, Cluj district, Zip code 400132, Cluj-Napoca, Romania
[3]Oncologic Institute "I. Chiricuta", Republicii st., no. 34-36, Cluj district, Zip code 400015, Cluj-Napoca, Romania
[4]Student, University of Medicine and Pharmacy, "Iuliu Haţieganu", Cluj-Napoca, Romania

Background: Exposure to high altitude, which is associated with decreased oxygen pressure, could result in oxidative/nitrosative stress, enhanced generation of reactive oxygen and nitrogen species (RONS) in heart tissue. RONS produced in excess cause myocardial injury, cardiomyocyte apoptosis, and cell necrosis. The fruits of Lycium barbarum (LBG), the small red berries, have been used for thousands of years in traditional Chinese medicine for your biological activities including anti-aging, anti-tumor, immune-stimulatory and cytoprotection. Recent studies have demonstrated that extracts from LBG possess cardioprotective activities due to their anti-oxidative effects. The aim of this study is to evaluate the cardioprotective effects of LBG extract in animals exposed to intermittent hypobaric hypoxia (IHH) and therefore exposed to oxidative/nitrosative stress.

Materials and methods: Sixty adult male Wistar rats were randomly assigned into six groups and were exposed to to short-term (2 days) or long-term (4 weeks; 5 days/week) IHH in a hypobaric chamber (5500 m, 8h/day, 380 mmHg, 12% O_2 and 88% N_2) or kept under normobaric normoxia (Nx). Some of the rats were treated with natural antioxidant LBG extract (30 mg/kg body weight) daily, before each IHH exposure; the remaining rats received saline solution. Control rats were kept under Nx and treated in a corresponding manner. The heart tissue homogenate levels of the free radicals (malondialdehyde, MDA and carbonylated proteins, CP), nitrite plus

nitrate (NO_x) production, activity of antioxidant enzymes (superoxide dismutase, SOD and catalase, CAT) and inducible nitric oxide synthase (iNOS) protein expresion were measured one day after the last exposure to IHH.

Results: In heart tissue homogenate, MDA and CP levels, NO_x production and iNOS expresion of IHH-exposed rats had increased significantly (P<0,005) and SOD and CAT activities had decreased significantly (P<0,005) compared to those of the Nx-exposed rats (control groups). Expression of iNOS, NOx production, lipid peroxidation and protein carbonilation had decreased significantly (P<0,005) in LBG-treated IHH-exposed rats compared with IHH-exposed rats (control groups). However, LBG administration significantly increased (P<0,05) SOD and CAT activities of the heart tissue in the IHH-exposed rats.

Conclusions: These results suggest that the activities of LBG on antioxidants and nitric oxide metabolism may be related to its cardioprotective potential on HH-induced cardiac damage.

[OP10] *IN VITRO* STUDY ON THE BINDING PROPERTIES OF NOVEL RUTHENIUM (III) COMPLEXES WITH HUMAN SERUM TRANSFERRIN

Cristina Manuela Dragoi[1], Valentina Uivaroşi[2], Andreea Letiția Arsene[1], Niculina Mitrea[1], Alina Crenguța Nicolae[1]

University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Bucharest, Romania
[1]Department of Biochemistry
[2]Department of Inorganic Chemistry

The transferrin cycle has gained increased interest in recent years and it holds promise as an attractive system for strategies of drug targeting to tumor tissues. Tumor cells exibit a large demand of iron for their growth and therefore express the transferrin receptor at a high rate. As a consequence, transferrin conjugates that retain a good affinity for the transferrin receptor can preferentially interact with cancer cells. This strategy is exploited nowdays for targeting novel anti-cancer drugs.

Following the success of cisplatin, several metal complexes other than platinum have been considered over the years as possible alternatives to cisplatin, particularly it was found that ruthenium (III) compounds possess antitumor and antimetastatic activities. The literature studies showed the affinity of these complexes for crucial biomolecules (like transferrin) and provide evidence for formation of stable adducts between them.

Therefore the present paper presents the transferrin-binding properties of some ruthenium (III) complexes with quinolone antimicrobials, having the general formula $[RuL_2Cl]Cl \cdot nH_2O$ ((1)L:norfloxacin (nf), n=4; (2)L:ciprofloxacin (cp), n=3; (3)L:enrofloxacin (enro), n=5).

In this regard we investigated, *in vitro*, the interactions of these ligands with human transferrin through spectroscopic techniques, with the ultimate goal of preparing adducts with good selectivity for cancer cells. By analyzing the obtained experimental results we can state that all studied complexes interact with human serum transferrin, the molar ratio [complex]/[transferrin] strongly influences the binding.

[OP11] OPTIMIZATION OF SPRAY DRYING OPERATING CONDITIONS OF RED GRAPE EXTRACT USING RESPONSE SURFACE METHODOLOGY

Duduku Krishnaiah[1*], Awang Bono, Collin G. Joseph,
S.M. Aniszzuman, Chia Chee Yen

Chemical Engineering Program, School of Engineering and Information Technology, Universiti Malaysia Sabah, Locked Bag No. 2073, 88999 Kota Kinabalu, Sabah, Malaysia
Tel: +60-88-320000, Fax: +6088-320348

In this study, a conventional solvent extract of red grapes was spray dried using k-carrageenan (2 wt.%) as an additive for antioxidant parameters estimation. The independent variables selected were air inlet temperature (AIT) (110^{o}C-160^{o}C), M_{wall}/M_{core} (1.10–2.60) (volume ratio of additive solution to red grape extract), and extract volume flowrate (EVF) (20 ml/min – 40 ml/min). The spray drying process was optimised by response surface methodology (RSM), using a central composite design for five different responses: moisture content (MC), diameter particle (DP), DPPH scavenging activity, total phenol content (TPC), and total flavonoid content (TFC). The analysis of ANOVA variance showed that quadratic models should be applied for all the responses with high value (>0.9) of coefficient of determination (R^2). The impacts of AIT, M_{wall}/M_{core}, and EVF were found to be significant for all the response. The result showed that the optimal point was obtained at condition of 110^{o}C AIT, 1.83 M_{wall}/M_{corel}, and 28 ml/min EVF with desirability of 0.83.

[*] *e-mail: krishna@ums.edu.my*

[OP12] THE INTRATUMORAL MACROPHAGES AS POTENTIAL TARGET CELL TYPE FOR THE ANTI-TUMOR ACTIVITY OF LIPOSOMAL SIMVASTATIN

Emilia Licarete[1,2], Marius Costel Alupei[1,2], Laura Patras[1,2], Manuela Banciu [1,2]

[1] Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences, Babes-Bolyai University, Cluj-Napoca, Romania
[2] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania

Statins as inhibitors of cholesterol biosynthesis, reduce production of isoprenoids which are involved in the post-translational lipid modifications of small GTP-ase proteins. These regulatory molecules are responsible for modulation of cell proliferation, apoptosis, inflammation, angiogenesis, and oxidative stress. The increasing evidence on statin pleiotropic activities have stimulated extensive research on their anti-cancer therapeutical applicability. However, the majority of the *in vitro* and *in vivo* results demonstrated that statins exert anti-tumor actions at very high doses. Therefore, in the present study we exploit the ability of long-circulating liposomes (LCL) to accumulate simvastatin (SIM) to tumor tissue in B16.F10 melanoma *in vivo*. Thus, the effects of LCL-SIM were compared with those induced by free SIM in B16.F10 melanoma-bearing mice. To assess whether tumor-associated macrophages (TAM) might be a potential cell target for the anti-tumor activity of LCL-SIM we used B16.F10 melanoma bearing mice in which TAM were depleted by treatment with liposomal clodronate. To elucidate the mechanisms responsible for the anti-tumor activity of LCL-SIM tumor production of HIF-1α – a key transcription factor for tumor development was determined. Modulatory effects of LCL-SIM on redox status in tumors were evaluated by measurement of nonenzymatic antioxidants, malondialdehide, and NO levels as well as of superoxide dismutase and catalase activities.

Our data have shown that LCL-SIM inhibited strongly the growth of B16.F10 melanoma tumors (by 85% compared to the growth of controls) via anti-oxidant effects on TAM. Simultaneously, HIF-1α expression was strongly reduced in tumors. Furthermore preservation of TAM production of anti-tumor proteins like TIMP-1, 2 and IL-12 might potentiate the antitumor activity of LCL-SIM in B16.F10 melanoma *in vivo*.

[OP13] TUMOR-ASSOCIATED MACROPHAGES – KEY PLAYERS IN THE INTERACTION TUMOR INFLAMMATION - OXIDATIVE STRESS AS POTENTIAL TARGETS FOR ANTICANCER THERAPIES

Lavinia Luca[a,b] Emilia Licarete [a,b], Laura Patras[a], Manuela Banciu[a,b]

[a] *Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania*
[b] *Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania*

Among the immune cell populations present in tumor tissue, tumor-associated macrophages (TAM) seem the most important in promoting and coordinating tumor growth. Tumor inflammation induced by TAM represents a source of pro-inflammatory/pro-angiogenic cytokines (TNF-α, IL-1, IL-6, VEGF), cellular adhesion molecules, anti-apoptotic proteins, and reactive oxygen species (ROS) which, depending of their level, could modulate the immune system, and affect cell proliferation, stimulating angiogenesis by activation of oxidative stress enzymes responsible for stabilization of some regulatory transcription factors (HIF-1α). Besides protumoral functions of TAM, these cells exert antitumoral functions by direct cytotoxic effects on tumor cells and by production of anti-inflammatory and anti-angiogenic proteins. Therefore, TAM might be a potential target cell type for anti-cancer therapies. In the present study we investigated TAM-mediated processes that can be used for development of anticancer strategies.

To this aim we have tested the proliferation of C26 murine colon carcinoma cells co-cultivated with intraperitoneal murine macrophages *in vitro*. To evaluate the involvement of TAM in the production of three key transcription factors (HIF-1α, AP-1 and NF-kB) we performed western blot analysis. Modulatory effects of TAM on redox status in tumor cells were tested via measurement of nonenzymatic antioxidants, and malondialdehide levels as well as of catalase activity. A screening for angiogenic/inflammatory protein production was also performed.

Our preliminary data have shown that TAM might be a potential target cell type for anticancer therapies that have the ability to inhibit pro-angiogenic, pro-inflammatory, and pro-oxidant functions of TAM, with the preservation of TAM antitumor functions.

[OP14] IDENTIFICATION OF A HIGHLY EFFICIENT SUBSTRATE-TRAPPING MUTANT OF EYE ABSENT PROTEIN TYROSINE PHOSPHATASE

Mentel M., Iancu I., Ionescu A., Munteanu C.V.A.,
Kibedi-Szabo C. Z., Szedlacsek S. E.

Institute of Biochemistry of the Romanian Academy,
Department of Enzymology, Spl. Independentei 296, Bucharest, Romania

Eyes absent (Eya) proteins are known both as transcription factors involved in the Retinal Determination Gene network responsible for eye development in *Drosophila*, and as phosphatases involved in DNA damage repair and regulation of innate immune response. Eya phosphatases are involved in numerous diseases, such as congenital disorders, cancer, hearing loss and kidney defects. However, the physiological targets of Eya phosphatases in cytoplasm are still elusive. Based on the fact that Eya can undergo autodephosphorylation and thus interacts itself, we could evaluate the substrate-trapping capacity of different mutants within the C-terminal conserved domain of Eya phosphatases. Initially, we found that mutation at a key residue in the catalytic domain (other than the catalytic aspartate), conserved among Eya family, abrogates its enzymatic activity and also substantially inhibits Eya autodephosphorylating capacity. Next, we proved by co-immunoprecipitation that this mutant specifically binds wild type and inactive Eya mutants, suggesting that it can be an efficient substrate-trapping mutant. Further on, this substrate trapping-mutant was used to identify and confirm potential Eya substrates. To this purpose we used Aip1/WDR1, a substrate candidate which we previously identified by a microarray assay on more than 6000 tyrosine phosphorylated peptides. Aip1/WDR1 is known to be involved in disassembly of actin filaments in conjunction with ADF/cofilin family proteins. First, we found that WDR1 is phosphorylated by Src kinase, both *in vitro* and *in vivo*, and that the novel Eya trapping mutant interacts specifically with phosphorylated WDR1 protein. Second, we demonstrated that the active form of Eya phosphatase dephosphorylates WDR1 protein *in vitro.* Thus, our results show that i.) the here reported substrate-trapping mutant can be used as a highly efficient tool in identifying novel Eya substrates and 2.) WDR1 is a *bona fide* substrate of Eya and its interaction with WDR1 could be involved in cytoskeletal reorganization.

Acknowledgment. This work was financially supported by POSDRU 89/1.5/S/60746.

[OP15] NEUROBLASTOMA N_2a CELL LINE RESEARCH REGARDING THE ACTIVITY OF ABCB1 TRANSPORTER

Alina Crenguța Nicolae[1], Niculina Mitrea[1], Cristina Manuela Drăgoi[1], Maria Zinaida Constantinescu[1], Vlad Vuță[2], Gabriela Zamfir[2], Andreea Letiția Arsene[1]

[1]UMF „Carol Davila", Faculty of Pharmacy, Biochemistry Department, Bucharest, Romania
[2]Institute for Diagnosis and Animal Health, Virology Department, Bucharest, Romania

ABCB1 transporter, also known as P-glycoprotein (Pgp), is present in various cell types, is a transmembrane pump that belongs to the family of ABC transporters *(ATP-binding cassette)* which plays an important role in the absorption (intestine), distribution (CNS) and elimination (liver, kidneys) of xenobiotics, but also for endogenous products. At the blood-brain barrier (BBB) level Pgp is localized in the apical membrane of brain capillary endothelial cells and transports substrates toward the blood compartment. Therefore, Pgp can limit the penetration into and retention within the brain and thus modulate effectiveness and central nervous system (CNS) toxicity of numerous compounds.

The experimental data led to the hypothesis that, in addition, Pgp is overexpressed in the neuroblastoma cells (like N_2a cell line), where it is involved in the cellular efflux of CNS drugs. Thus, after administration of CNS drugs and their intracellular transport, Pgp pump takes over the compound immediately and removes it in the extracellular space, preventing therefore the cytotoxic effect on cells.

For these reasons, highlighting molecules with inhibitory properties on Pgp pump has become a topic of great interest in CNS pharmacotherapy.

In addition, there is increasing evidence that ABC transporter-mediated drug efflux at the BBB may limit brain drug delivery of several CNS drugs, thereby leading to treatment failure in various brain disorders. In the field of psychiatry, much recent attention had been given to the role of efflux pumps in the pharmacokinetic profile of antidepressant drugs. Emerging evidence suggests that P-gp, in particular, may limit the ability of several antidepressants to cross the BBB, thus resulting in inadequate brain concentrations and therefore contributing to the poor success rate of current antidepressant therapies.

In this paper we investigate the Pgp complex interaction of some structurally diverse central nervous system-active drugs wich were selected based on literature data suggesting differential interactions with Pgp.

[OP16] **NITRIC OXIDE AND ENDOTHELIAL DYSFUNCTION**

Pârvu Alina Elena

Iuliu Haţieganu University of Medicine and Pharmacy

Healthy endothelial cells respond to a number of stimuli by releasing NO, which relaxes the vascular smooth muscle that surrounds them. NO also inhibits platelet aggregation, reduces the endothelial expression of adhesion molecules and thus the adhesion and penetration of leucocytes, prevents the proliferation of vascular smooth muscle cells and limits the formation of oxy-LDL.

Endothelial NO production is intimately regulated by the activity of eNOS. Phosphorylation at key serine residues is the major post-translational modification that is required for eNOS function. Additionally, shear stress, vascular endothelial growth factor (VEGF) and high-density fatty acids can phosphorylate and activate eNOS. In contrast, phosphatases like protein phosphatase-2 dephosphorylates and inactivates eNOS. S-Nitrosylation inhibits eNOS activity by modifying its steric configuration, whereas de-nitrosylation is associated with an increase in eNOS activity.

The release of NO by the endothelial cell can be downregulated and up-regulated. Ageing and certain lifestyle factors, or certain diseases result in a lesser release of NO and an acceleration of the turnover of the apoptotic process in the endothelium. The apoptotic endothelial cells are replaced by regenerated ones, which are dysfunctional, senescent, and incapable of producing the required amounts of NO, facilitating the inflammatory response and leading to the formation of atherosclerotic plaques. The deficiency of NO allows vasoconstrictor mediators to act more efficient leading to vasoconstriction which amplifies the degree of endothelial dysfunction.

NO is also highly reactive with other molecules including superoxide anion (O2-), oxygen (O2) and hemoproteins such as hemoglobin and myoglobin. The intermediate products of these reactions are known as reactive nitrogen species, which promotes many pathophysiologically damaging reactions.

Thus, ECs and NO release may be important predictors of cardiovascular outcomes and independent predictors of future events in patients with cardiovascular risk factors.

[OP17] XANTHOPHYLLS ESTERS – DISTRIBUTION, PROPERTIES AND STABILITY IN FOOD

Adela Pintea

Department of Chemistry and Biochemistry, University of Agricultural Sciences and Veterinary Medicine, Mănăştur 3-5, 400372, Cluj-Napoca, Romania; e-mail: apintea@usamvcluj.ro

Xanthophylls are oxygenated derivatives of carotenes and represent an important group of compounds in the large family of carotenoids which comprises more than 700 representatives. Even if they lack provitamin A activity, xanthophylls have important biological functions in humans, therefore representing valuable nutrients. A diet rich in fresh fruits and vegetables is associated with a reduced risk of chronic and degenerative diseases. The xanthophylls containing hydroxyl groups are often esterified with middle chain saturated fatty acids. Some rich sources of xanthophylls esters are *Tagetes sp.*, *Capsicum sp*., *Lycium barbarum, Hippophae rhamnoides, Diospyros kaki, Physalis sp*..

We present some new aspects regarding the composition of fatty acids esters of xanthophylls in *Hippophae rhamnoides* and *Prunus armeniaca* fruits. Several cultivars of sea buckthorn and apricots cultivated in Romania were analyzed by HPLC/PDA/MS technique.

The biological functions of carotenoids *in vivo* are often related to their antioxidant capacity and to their ability to scavenge the free radicals. A relatively reduced number of studies investigated the antioxidant capacity of xanthophylls esters and the results are contradictory. We obtained pure esters of zeaxanthin and β-cryptoxanthin by semi-synthesis and we determined their antioxidant capacity by different validated methods. Generally, the esterification with saturated fatty acids (myristic, palmitic) did not change the radical scavenging capacity of xanthophylls. Slightly reduced, but not significant, antioxidant capacity was observed for xanthophylls when esterified with unsaturated fatty acids (oleic, linoleic).

Thermal and light stability tests were performed on the above mentioned pure compounds. All the esters with saturated fatty acids showed better thermal stability compared to the free xanthophylls. Similar test are in progress using fruits and food products rich in xanthophylls esters.

Acknowledgements. This work was supported by a grant of the Romanian national Authority for Scientific Research CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0721

[OP18] TUMOR-ASSOCIATED MACROPHAGES ARE INVOLVED IN THE RESISTANCE OF C26 COLON CARCINOMA CELLS TO THE 5-FLUOROURACIL TREATMENT

Laura Patras[1,2], Emilia Licarete[1,2], Lavinia Luca[1,2], Manuela Banciu [1,2]

[1] Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano Sciences, Babes-Bolyai-University, Cluj-Napoca, Romania
[2] Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai-University, Cluj-Napoca, Romania

Despite being the most effective chemotherapeutic agent for colorectal cancer treatment, 5-Fluorouracil (5-FU) has a limited clinical applicability due to a high rate of cancer drug resistance. Constant generation of reactive oxygen species in the tumor microenvironment is one of the main mechanisms promoting tumor progression and drug resistance. Our study aimed to investigate the molecular mechanism driving the resistance to 5-FU and also to elucidate the interplay between tumor-associated macrophages and cancer cells, upon drug administration. For this reason, we assessed the cytotoxic effect of 5-FU on C26 murine colon carcinoma cell line, in the presence or in the absence of murine peritoneal macrophages. Moreover, we measured oxidative stress biomarkers, and the levels of pro- and anti-inflammatory cytokines with the ultimate aim to study the impact of microenvironment generated by active macrophages on the C26 cancer cells.

Our preliminary results show that 5-FU exerted a very strong inhibition of cell proliferation and viability of tumor cells. When C26 cells were co-cultivated with macrophages cytotoxic effects exerted by 5-FU were only moderate. Assessment of the oxidative stress biomarkers suggests that 5-FU counteracts oxidative stress generated by macrophages. Moreover, unveiling the interaction between macrophage-induced oxidative stress and resistance of cancer cells to 5-FU remains a major challenge in order to improve anticancer therapeutic strategies.

[OP19] TESTING OF HYPOGLYCEMIC, LIPID-LOWERING AND ANTIOXIDANT EFFECTS OF *TAMARIX RAMOSISSIMA*

Renata-Maria Popescu, George Dan Mogoşanu, Anca Berbecaru-Iovan, Johny Neamțu, Florica Popescu, Cătălina Pisoschi

University of Medicine and Pharmacy, Department of Pharmacy, 2-4 Petru Rareş, 200349, Craiova, Romania

Background. Pharmaceutical field noted an increase in the consumption of hypoglycemic, lipid-lowering and antioxidant natural products, the management of diabetic disease accounting therapeutic progress. In this context, this study aimed to evaluate *in vitro* total polyphenol and flavonoid content and *in vivo* therapeutic effect of *Tamarix ramosissima (TR)* compared with *Vaccinium myrtillus (VM)* on streptozotocin-induced diabetes in mice.

Material and Methods. Diabetes mellitus was induced with a unique intraperitoneal dose of 180 mg/kg b.w. streptozotocin. The experiment was conducted on adult Swiss Albino mice distributed in four groups: three groups of control animals – Ist group – mice with normal pancreatic function, IInd group – diabetic mice and IVth group – diabetic mice treated with *VM* extract, a plant product recognized for its hypoglycemic effect, and a IIIrd group – diabetic mice treated with *TR* extract of 150 mg/kg b.w. At intervals of seven days, after fasting for 12 hours, we determined glucose, cholesterol and triglyceride levels in plasma, blood being collected from the tail vein. At the end of the experiment the animals were sacrificed and we determined activities of superoxide dismutase, glutathione peroxidase and glutathione reductase and level of lipid peroxides as thiobarbituric acid reactive substances.

Results. After five weeks of treatment, levels of glucose, cholesterol and triglycerides were lower in diabetic mice treated with *TR* extract than those from other diabetic groups. All the antioxidant enzymes had higher activities in mice treated with *TR* extract than those in control groups. Level of lipid peroxides was lower in mice treated with *TR* extract than the groups I,II,IV. The extract of *TR* had higher content of polyphenols and lower content of flavonoids than *VM*.

Conclusion. Our results sustain the efficiency of tested extracts on normalizing metabolic parameters in experimental diabetes mellitus and also their important influence on antioxidant enzymes.

[OP20] **SMART INNOVATIVE CELL ANALYSIS SOLUTION: FLOW AND IMAGING CYTOMETRY**

Riccardo Pasculli

Biotech-Europe, Biotehnologie, Prague, Czech Republic

BioTech-Europe introduces the new methods and technologies in the field of cellular analysis focused on the new advances in cytometry.

Imaging flow cytometry: The Amnis ImageStream is an imaging platform powered by flow – system for basic and clinical research combines the strengths of flow cytometry and fluorescent microscopy in a single system, enhancing recent flow cytometry applications and allowing new and unique applications based on signal localization and cellular morphology.

Imaging Cytometry: IDEA Bio-Medical´s WiScan is a high definition, ultra-fast biological cell-imaging system capable of high resolution imaging at high speeds.The ultimate platform for biological and biomedical research enables modularity and robustness as well as rapid and versatile set-up for a broad range of applications. It has been developed as a multi-user platform, yet it can be readily tailored to perform highly specialized tasks.

Flow Cytometry: The ACEA Bioscience´s New Flow Cytometer NovoCyte brings researchers high performance flow cytometry at a low investment cost. Being equipped with a Unixe optical setup, Novocyte offers a top sensitivity performance as well as an excellent resolution of small particles for study of immunophenotyping, cell cycle, apoptosis, fluorescent protein expression and others.

WORKSHOP

"Viral hepatitis - from cell culture to clinic"

"Identification of class II ADP-ribosylation factors as host factors required for HCV replication"

Dr. Yves Rouille
Molecular & Cellular Virology of Hepatitis C,
Center for Infection & Immunity of Lille (CIIL)
Inserm U1019, CNRS UMR8204, Univ Lille Nord de France
Institute Pasteur, Lille, France

HCV life cycle: is virus entry a potential drug target?

Dr. Dubuisson J.
Molecular & Cellular Virology of Hepatitis C,
Center for Infection & Immunity of Lille (CIIL)
Inserm U1019, CNRS UMR8204, Univ Lille Nord de France,
Institute Pasteur. Lille, France,

Analysis of the Hepatitis C Virus NS2 interactome in infectious virions producing cells

Dr. C. I. Popescu
Institute of Biochemistry, Bucharest. Romania

DISCUSSIONS

POSTERS

[P1] IMPROVED INSULIN SECRETION IN INSULINOMA CELLS BY OVEREXPRESSION OF PROTEINS INVOLVED IN ERAD

Petruţa R. Alexandru, Gabriela Chiritoiu, Mihaela Uta, Simona Ghenea, Florin Pastrama, Ştefana M. Petrescu

Institute of Biochemistry, Molecular Cell Biology Department, Splaiul Independentei 296, 060031, Bucharest, Romania

Insulin is produced by pancreatic beta cells and is a critical regulator of metabolism. Insulin is synthesized as preproinsulin, which is sequentially processed into proinsulin and finally into insulin and stored in secretory granules awaiting release on demand. Insulin synthesis and secretion is regulated by glucose at all levels, including transcription, translation and release. In pancreatic beta-cells, the ubiquitin-proteasome system has been shown to regulate the activation of the VDC channel and K_{ATP} channel, play a role in the regulation of proinsulin transcription, and may also serve to degrade misfolded proteins retrotranslocated from the ER lumen by degradative machinery of the ER-associated degradation system (ERAD), but what role this plays remains unclear. Our purpose was to investigate the role of proteins involved in endoplasmic reticulum-associated degradation (ERAD) in insulin biosynthesis and secretion in the presence of either low or high glucose concentration. For this, we have developed insulinoma cell lines engineered to express the RE resident proteins implicated in ERAD. As controls, was used either parental INS-1 cells or cells expressing the control empty vector. The overexpression of each transfected cDNA was recorded by Western Blotting and confocal fluorescence microscopy. Cell clones were seeded in 6-well plates 2 day before experiments and in the day of experiment the cells were starved in glucose-free medium for 2 h and then stimulated insulin secretion with glucose for 24 hours. After 24h post-stimulation the medium and the cells were collected and were measured by Western blot and Elisa. We found that overexpression of some ERAD proteins in the rat INS-1 pancreatic beta-cells increased the insulin biosynthesis and secretion in a glucose dependent manner. These effects were specific because gene silencing by shRNA reverted the phenotype. We also analyzed the traffic and secretion of the insulin in stable cell lines the by immunofluorescence microscopy. Our results suggest that overexpression of the ERAD proteins specifically increase insulin biosynthesis and secretion in pancreatic beta cells.

Acknowledgements: Alexandru Petruta-Ramona, was *suported by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number SOP HRD/107/1.5/S/82514*.

[P2] PROTEOMICS TECHNIQUES USED IN THE EVALUATION OF PROTEINS PROFILE IN MILK FROM COWS WITH SUBCLINICAL MASTITIS

Andrei Sanda[1], Sorana Matei[1], Adela Pintea[1], Andrea Bunea[1], Leonardo Bianchi[2], Patrice Martin[2]

[1] University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Str. Mănăştur no. 3-5, Cluj-Napoca, Romania, Email: sanda_m_andrei@yahoo.com
[2] French National Institute for Agricultural Research (INRA), Jouy en Josas, Milk, Genome & Health (LGS), Domaine de Vilvert F-78350 Jouy en Josas, France

Milk from cows with mastitis, in the subclinical phases, is characterized by a decrease in fat and lactose content and changes in the concentration of minerals. Total protein concentration did not change significantly, but changes appear in the ratio of casein and soluble proteins.

The purpose of this paper is to evaluate the protein hydrolysis in milk samples from cows with subclinical mastitis compared to those from healthy cows and the correlations that can be established between the severity of infection (SCC - somatic cell counts), the pathogens and intensity of hydrolytic processes. The following objectives were achieved: microbiological examination of milk, SCC determination, the determination of total proteins concentration, proteins profiling by HPLC, electrophoresis (SDS-PAGE and 2D-PAGE), determination of amino acids by GC coupled with MS.

Microbiological examination of mastitis milk samples showed a high number of bacterial species, especially *Staphylococcus intermedius*, *Streptococcus agalactiae* and *Bacillus cereus*. In mastitis milk was a decrease in the concentration of casein simultaneously with the increasing of the concentration of soluble protein with blood origin (bovine serum albumin - BSA and immunoglobulins). These changes occurred in the protein profile are directly related to SCC in milk; total bacterial count and also they depend on the type of pathogen that caused the infection. Pathogens involved in the infection release proteolytic enzymes with different specificity towards caseins (that explains their different hydrolysis) and the body reacts to these infections by a similar immune response (which explains the presence of similar amounts of lactoferrin, BSA and immunoglobulins). The results in terms of the concentration of amino acids showed a significant increase in

mastitic milk samples. For all analyzed samples values was more than 20 times higher compared to the normal milk (as a result of the processes of proteolysis of the caseins).

Acknowledgements: This work was supported by CNCSIS –UEFISCSU, project number 1044/2009 PNII – IDEI 1482/2009 and by Milk, Genome & Health team from French National Institute for Agricultural Research (INRA) Jouy en Josas, France

[P3] NEW SIGNALING MOLECULAR PATHWAYS OF THE ENDOGENOUS INDOLERGIC SYSTEM

Andreea Letiția Arsene, Niculina Mitrea, Cristina Manuela Drăgoi, Alina Crenguța Nicolae

University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Bucharest, Romania, Department of Biochemistry

One of the major concerns of current pharmaceutical research is finding therapeutic resources (drugs) with greater efficiency and reduced side effects , and in this context the pathophysiological and pharmacological potential of bio-indoles (tryptophan, serotonin and melatonin), represents the subject of modern research regarding the organisms integration mechanisms in the wide informational variety of the external environment. These biomolecules are responsible for coordinating and synchronizing the most striking expression of physiological effects of biological rhythms, the body's complex integrative processes in the environment and the social environment, require an order of biochemical systems functionality and determine globally the molecular logic of the living cell.

Indole skeleton is considered by scientists as a "biologically privileged structure" because of its outstanding ability to form biologically active compounds with affinity for different endogenous receptors.

The concept of privileged structure was originally defined as a frame which is selected in order to provide ligands with high affinity for multiple receptors, by appropriate modulation of the auxiliary functional groups .

The current literature mentions a new intracellular signaling mechanism (intranuclear) of endogenous bio- indoles. In this respect, the research conducted by the young research team of the Department of Biochemistry, Faculty of Pharmacy, Bucharest, revealed *in vitro* direct interactions between DNA and endogenous bio-indoles. There were studied the biochemical mechanisms of this interaction, bio-physical-chemical parameters of intercalation, binding constants, denaturation-renaturation processes.

[P4] ASSESSMENT OF THE EXPRESSION OF PTPs POTENTIALLY INVOLVED IN ENDOTHELIAL PROGENITOR CELLS DIFERENTIATION AND ANGIOGENESIS

Rodica Badea, Georgiana Petrareanu, Stefan Szedlacsek

Institute of Biochemistry of the Romanian Academy
Splaiul Independentei 296, 060031, Bucharest 1, Romania

Endothelial progenitor cells (EPCs) represent a minor subpopulation of mononuclear cells found in peripheral blood that are believed to be derived from either hematopoietic stem cells or from endothelium itself. These cells have the potential to proliferate and differentiate into mature endothelial cells. It has been demonstrated that *in vivo*, EPCs are recruited to the sites of injured endothelium or tissues and incorporated into the vessel structure to participate in vascular regeneration

A number of studies on animals revealed the EPCs potential for cell therapy in ischemic diseases as long as they are able to improve the function of ischemic organs either by mediating vasculogenesis and angiogenesis at places with reduced oxygen supply or by stimulating the re-endothelialization of injured blood vessels.

Molecular signaling in angiogenesis, despite significant progress of the last years, is still insufficiently understood. Protein tyrosine phosphatases (PTPs), as key regulators of different signaling pathways, might have important roles in the control the vasculogenic / angiogenic potential of EPCs.

Thus, the goal of the present study is to find a correlation between the EPC differentiation, their vasculogenesis capacity, and the expression pattern of PTPs involved in vasculogenesis/angiogenesis. In order to observe the involvement of PTPs in the vasculogenic capacity of EPCs, we analysed the expression level of four PTPs (two receptor-like and two non-receptor) known to be involved in angiogenesis. We estimated the mRNA and protein expression patterns of studied PTPs over succesive EPCs passages by qPCR and Western Blot, respectively. Next, we compared the mRNA and protein expression levels with those found in mature endothelial cells and we observed notable differences between the PTPs expression in the two cell lines. The various levels of expression may correlate with EPCs differentiation and vasculogenic capacity.

[P5] EICOSANOIDS AND PERIODONTAL REPAIR

Bădulescu Mădălina, Antonescu Angela*

** University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*

"Eicosanoid" is a term that introduce a large family of oxygenated fatty acides containing 20 carbone atomes. This family is made up of three clanes, which includes the prostanoides (prostaglandins and thromboxanes) which are synthesized in reactions that are catalyzed by the "cyclo oxygenase", the leucotrienes and certain mono-, di- and tri- hydroxy acids which are formed via "lipoxigenase" pathways, and the epoxides which are formed by a cytochrome P-450 "epoxygenase" pathway. In other words, one of the most known fatty acid is the arachidonic acid and it can be metabolized in the cell, giving birth eicosanoids. (1)

These compounds have an essential role in cellular signaling processes, acting on receptors. How important could be the eicosanoids in stomatology, considering their chemical structure and their roles? As researchers say, "eicosanoids are very mysterious, but are essential". It is very interesting how eicosanoids are practically hormones, even if they are not the result of a gland secretion. As such they can be considered "super-hormones" because they control the hormonal actions of other hormones. It is well known that nowadays, more and more people suffer from periodontal disease, because of different factors: alimentation, superficial hygiene, heredity etc. (2) Anyway, in some cases, correct brushing, mouth cleaning, and flossing are not sufficient for avoiding the periodontal diseases. If periodontitis is present, therapy begins with a procedure, called "scaling and root planning in which the gingival and root surfaces of the theet are scraped clean using ultrasonic and hand instruments. The theet are then polished and the patient is instructed in oral hygiene to slow the redevelopment of plaque and calculus. The therapy facilitates the disappearance (resolution) of the inflammation and the restoration of a healthy sulcus. The process of resolution is directed by a subgroup of eicosanoids.

References

[1] Biochem. J. (1989) 259, 315-324 (Printed in Great Britain), *The eicosanoids and their biochemical mechanisms of action,* William L. SMITH, Department of Biochemistry, Michigan State University, East Lansing, MI 48824, U.S.A.

[1] M. Levine. 2011. *Topics in Dental Biochemistry,* Springer-Verlag Berlin Heidelberg.

[P6] REVIEW ON CURRENT ANALYTICAL DEVELOPMENTS FOR MUSTARD ANTIOXIDANTS

Georgiana Bîrda[1,2], Jean Negru[2], Diana Negru[2], Romulus Gruia[1]

[1] Transylvania University of Brasov, [2] S.C MUSO S.R.L

Vegetals are an important source of dietary and bioactive substances, used to produce active ingredients for pharmaceuticals. The antioxidants found in medicinal plants can effectively prevent the unwanted action of free radicals, usually resulted in the normal human metabolism. However, the extraction process, as well as the quantitation methods of pure substances, is still a challenge.

Our review focuses on analytical methods used to separate and quantitate phytochemicals with antioxidant action from a very common family of vegetals, the mustard (*Brassica* and *Sinapis*). Further, we sorted the articles that describe analytical method for analyzing phenolic extracts by HPLC, UHPLC, LCMS, etc.

Phytochemicals from *Brassica* and *Sinapis* (mostly in seeds) have antioxidant activity due to mainly phenolic compounds like sinapine and its hydrolysate, sinapic acid, along with other derivatives. Mustard seeds, flour, powder, or oil can be used as raw materials. Due to their affinity for polar solvents, extracts are being prepared in small-chain alcohols (methyl, ethyl and isopropyl alcohol), water, acetone, ethyl acetate, and even by alkaline hydrolysis.

Modern chromatographic techniques are used to separate, identify and quantify phenolic extracts, either as total phenolic content or as individual compounds. Based on reversed-phase chromatography mechanisms, the cited LC methods utilize C8 or C18 stationary phases columns with polar mobile phases in gradient. To create an acidic aqueous mobile phase, modifiers like ortho-phosphoric acid, acetic acid, trifluoroacetic acid or formic acid were added in small amounts (0.1 to 1.2%). In other methods, a buffered pH was used instead. The organic mobile phase was methanol or acetonitrile, in most of the separations. While UV detection was widely used, methods involving fluorescence or mass selective detectors were published, too. The level of detections varied widely by technique and raw material used to prepare the extract.

[P7] HYPOCHLORITE ACTIVATION AT THE HEME IRON CENTER OF HEMOGLOBIN

Cristina Bischin, Radu Silaghi-Dumitrescu

Department of Chemistry, Babes-Bolyai University,
11 Arany Janos street, Cluj-Napoca 400028, Romania

Hypochlorous acid (HClO), generated by myeloperoxidase using chloride and hydrogen peroxide as substrate, is one of the most powerful biological oxidizing and chlorinating agents. A mechanistic link is expected to be between high HClO, which appear in pathological conditions, and higher free Fe level [1]. It has been shown that this molecule can easily penetrate the red blood cells leading to the destruction of one of the most abundant proteins found here, hemoglobin (Hb). There is evidence that an excess of HClO induces similar absorption changes with those observed with an excess of H_2O_2. Some studies revealed that the reaction between HClO and oxy- and met-Hb involved the formation of the strong ferryl intermediate (Fe(IV)=O), followed by heme degradation and protein aggregation. It has been reported that HClO not only binds to heme iron but also destroys free heme iron by the interaction with tetrapyrrole ring.[2, 3]

In the present work, we investigated the reaction between oxi- and met-Hb with HClO. Using stopped-flow technique, for the first time was revealed a reaction intermediate, most probably a Fe(III)-OCl adduct, which rapidly is transformed to ferryl.

Acknowledgements: the work shown here has been supported by the Romanian Ministry for Education and Research (grants PN-II-ID-PCE-2012-4-0488).

[P8] ANALYSIS OF SOME BIOACTIVE COMPOUNDS PRESENT IN EGGS PROVIDED BY LAYING HENS

Andrea Bunea, Florina Copaciu, Simona Pascalau, Claudiu Bunea, Sanda Andrei, Adela Pintea

University of Agricultural Science and Veterinary Medicine, 3-5 Manastur Street, 400372, Cluj-Napoca, Romania, email: andrea_bunea@yahoo.com

Between different diet products which provide important nutrient to the human body, egg plays a specific importance. They contain lipids, aminoacids, fatty acids, minerals and vitamins. Responsible for the eggs yolk pigmentation are the carotenoids, especially xanthophylls. The lipid composition of the eggs depends on the diet, age and also genetic factors plays an important role. Carotenoids represent a widely group of lipid-soluble pigments found in all types of plant. Because animals are not able to synthesize carotenoids, the accumulation in eggs yolk depends on food supplementation. The aim of this study was to determine the carotenoids, vitamin A and vitamin E content in different eggs provided by nine different laying hens species feeded in two ways. The analyses were carried out using high performance liquid chromatography (RP-HPLC-PDA) and the quantification of individual compounds separated was made with standards. The major carotenoids identified were lutein and zeaxanthin. Regarding tocopherol content, HPLC analysis showed as major representatives α-tocopherol and δ-tocopherol.

Acknowledgements. This work was supported by a grant of the Romanian national Authority for Scientific Research CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0721

[P9] APPLICATION OF SPRAY DRYING TECHNOLOGY FOR MICROENCAPSULATION OF ROSEMARY AND OREGANO EXTRACTS

Florina Bunghez, Carmen Socaciu

Dept. Agrifood Biochemistry Facculty of Food Science and Technology, Univ. Agr.Sci. &Vet. Med., Cluj-Napoca, Romania

Spray drying (SD) is an emerging microencapsulating technology used in food industry due to it's good quality products, considering the good ratios of efficiency/ costs equipment. Spray-drying represents a good alternative to conventional drying techniques, since it preserves the stability of many small, secondary metabolites from plants. A key problem is to modulate accurately, during drying process multiple direct and indirect variables for the optimization of the procedure. Actually spray drying is a process of microencapsulation, preparing microspheres made by biocompatible matrix which immobilize molecules from a liquid phase, by atomizing the liquid in a hot gas current, drying bioactive molecules on the matrix, stable at high temperatures.

Among the advantages of this process we can mention the fast processing of a product with default and specific properties (particle shape, size, humidity, flowabilty, degree of dispersibility, solubility) by optimizing the operating parameters, choosing a proper matrix, or standardize the feed solution (emulsion or suspension). A required property of the product imposes a strict screening of the encapsulating materials to be used in addition to an optimization of the operating conditions. Likewise, if the encapsulated compound is of hydrophobic nature, the stability of the feed emulsion before drying should also be considered. Also, water removal by spray-drying is compulsory for engineering practice, used in the food industry to ensure microbiological stability of the products, to avoid the risk of chemical and/or biological degradations/contamination, reduce the storage and transport costs.

In the present paper the SD of rosemary and oregano extract were investigated in order to obtain atomized powders with antibacterial potential, în a NIRO Minor atomizer. The drying process was optimized by different parameters (T_{out}, T_{in}, feed flow rate, matix ratio) in order to assure a good level of total phenolics retention and recovery percentage of spray dried powder. The SD was carried out at 120^{o}C and 145^{o}C, using as matrix maltodextrin in 10% and 15% ratio to extract volume.

We noticed that the matrix had a positive influence on the recovery percentage of spray dried powders, and if the carrier agent (maltodextrin) has a protective effect

on the phenolic compounds in terms of total phenolic content retention. The total concentration of phenolics in powders reached values of 9.88 mg GAE/g powder for rosemary and 13.56 mg GAE/g powder for oregano after drying, with a recovery yield of 40.08% for rosemary and 68.6% for oregano.

The investigation of the physicochemical properties of the powders obtained is under way being considered the particle size, viscozity, atomizing temperature and pressure.

[P10] **ROLE OF EDEM3 IN ERAD ONE STEP AT A TIME**

Cristian Marian Butnaru, Marioara Chiritoiu, Marius Surleac, Andrei J. Petrescu, Stefana M. Petrescu

Institute of Biochemistry, Molecular Cell Biology Departament, Splaiul Independentei, 296, 060031, Bucharest 17, Romania

To maintain homeostasis, the cell developed control mechanisms in key positions to overcome errors in protein synthesis and folding; thus this research is intended to study new aspects about the folding mechanisms and the degradation pathway of proteins destined to the secretory path.

A major part of this control points is located in the endoplasmic reticulum; it is here, where the translocation, post-translational modification, folding and targeting to degradation occurs. For a protein to achieve its native conformation a large number of chaperons and enzymes (OST, GI,GII,CNX, CRT,PDI...) are required to function. If the protein passes the quality control check, it will be packed into vesicles and exported to the Golgi apparatus. If folding fails, the cycle can be retaken several times. Sometimes the polypeptide chain fails to reach its native structure due to sequence defects, post translational modifications and stress factors that disturbs the cellular homeostasis. As response to this, the ER associated protein degradation pathaway (ERAD) is activated. In this process proteins like EDEM 1,2,3, (ER-degradation enhancing alpha-mannosidase-like proteins) OS-9 XTP-3B and others are implicated

The focus of our studies is based on EDEM3 protein and its role in ERAD. According to Hosokwa et all (1), EDEM3 is a soluble member of G47 hydrolase family located in the ER lumen of mammalian cells that accelerates ERAD of misfolded glycoproteins. This mechanism is likely to be different from that of EDEM1 or EDEM2, since EDEM3 greatly stimulates mannose trimming *in vivo*.

Our preliminary results indicate that EDEM3 accelerates protein degradation, whilst displaying also mannosidase activity. To analyse the role of EDEM3 in comparison with teh other members of teh afmily, we determined the half life of endogenous and overexpressed EDEMs in amelanotic melanoma cells.

We have analysed the structure of EDEM3 using bioinformatics and biomolecular modeling. Using proteomics will help to unravel the interactors of EDEM3 and through biomolecular methods to untangle the biological function of this protein.

[P11] BILE ACIDS-THE NEW GUT HORMONES? THE IMPACT OF BARIATRIC SURGERY

Florinela Cătoi[1], Adriana Muresan[1], Alina Parvu[2], Cristina Bidian[1]

[1]Department of Physiology, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca
[2]Department of Pathophysiology, Faculty of Medicine, "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca

Emerging data have raised the question about bile acids as new possible gut hormones with incretinic effect as it seems that they are involved in the regulation of glucose homeostasis. On the other hand, an interplay seems to exist between bile acids and glucagon like peptide 1 (GLP-1) one of the gastrointestinal hormones which holds an incretinic role.

Morbidly obese patients with a body mass index (BMI) over 40 kg/m^2 or obese with a BMI over 35 kg/m^2 and associated diseases are eligible for bariatric/ metabolic surgery. Type 2 diabetes is one of the most common comorbidities of obesity and together they display an impaired incretinic effect. Beyond weight loss, metabolic surgery exerts and important role on type 2 diabetes, i.e favouring the remission/amelioration of the disease through weight independent mechanisms as well.

Surgical methods that involve rerouting of the food due to the rearrangements of the gastrointestinal tract seem to be involved in modelating the weight independent mechanisms which have positive effects on glucose control. More exactly, it seems that they induce an increase in glucagon like peptide 1 (GLP-1) and bile acids levels, which will both promote an early improvement of glycemic control. On the other hand, it seems that sleeve gastrectomy (SG) considered before to be a pure restrictive procedure has an impact on GLP-1 and bile acids levels as well. However, these mechanisms are poorly known and ask for further investigations.

[P12] IDENTIFICATION OF FLAVONOIDS IN SEABUCKTHORN EXTRACT

Alina Cărăban, Sanda Bota, Florin Albu, Alina Cozma

[1] University of Oradea, Faculty of Sciences, Department of Chemistry, Oradea
[2] Agilrom Scientific Bucuresti, Romania

Seabucktkorn is a natural remedy known from ancient times. Its uses are so numerous that it can be rightfully called the word a miracle of natural medicine and the pharmaceutical industry. Seabuckthorn heath benefits include numerous cardiovascular, immunity, cancer, memory, growth, antiinflamatory and skin health. The ripe fruit of seabuckthorn medicinal food is containing many kinds of vitamins, trace elements, vitamins B1, B2, folic acid, C, E, beta carotene and K. It contains carotenoids, flavonoids, terpenes and phenol, at least 20, a mineral cofactors. Seabuckthorn contains more than 60 antioxidants and high ORAC value. Flavonoids has found in all parts of the plant, i.e. leaves (3.8-4%), fruits, juice and seeds. Studies found that juice and dry fruit residue contained 0.2-0.55% flavonoids.

The present work was undertaken to indentified flavonoids in seabuckthorn extract using LC/MS analysis.

According to the polar seabuckthorn flavonoid compounds, it was extracted by ethanol and water through maceration and solid-liquid extraction. Before extraction was used chloroform to get ride of lipo-soluble compounds.

The tests were performed using a column Eclipse XDB-C18 Zorbax, 150 x 4.5 mm and eluented with mobile phase gradient, being composed of A- 2% formic acid in water and B-0,2% formic acid in acetonitrile. The detector was used at DAD, $\lambda = 270$ nm and tripluquad LC/MS for identification of the main ingredient.

There were identified as main components in extract: quercetin, rutin and kaemferol.

[P13] EVALUATION OF YMDGTMSQV EPITOPE GENERATED FROM TYROSINASE GLYCOSYLATION MUTANTS

Gabriela Chiritoiu[1], Camilla Jandus[2], Pedro Romero[2], Stefana M. Petrescu[1]

[1] *Institute of Biochemistry, Romanian Academy*
[2] *Ludwig Institute for Cancer Research, University of Lausanne*

Malignat melanoma is the most agressive type of skin cancer. Immunotherapy is one of the methods used for melanoma threatment. The immune system has the capacity to recognise and reject the tumor, mainly by the action of tumor reactive cytotoxic T lymphocytes (CTL), that bind to specific antigenic peptides exposed by major histocompatibility complex (MHC1) to the cell surface.

An important tumoral antigen is tyrosinase, the key enzyme in melanogenesis, constitutively found in melanocytes and overexpressed in melanoma cells. Tyrosinase is a type 1 transmembrane glycoprotein with 7 potential N-glycosylation sequons. A fraction of wild type tyrosinase and its mutants are not able to reach the correct conformation; consequently they are retained in the ER (Endoplasmic Reticulum) and targeted to proteasomal degradation by ERAD (ER-associated protein degradation). Peptides generated by proteasome activity are loaded on MHC1 complex and presented at cell surface. Tyrosinase generates a specific peptide YMDGTMSQV corresponding to aminoacids 369-377, naturaly found as YMNGTMSQV, where the Asn in position 371 is ocupied by the 7th glycan.

We are interested in depicting the role of tyrosinase glycans in YMDGTMSQV epitope generation. Previous studies in our lab showed that tyrosinase glycan induced different changes in protein conformation. The N terminal glycan induce a partial misfolding, while the glycans from the C terminus induce a higher misfolding grade. Here we show that the tyrosinase behavior is maintained when its mutants are expresed in A375 amelanotic melanoma cell line. The transfectants are able to generate YMDGTMSQV epitope.

[P14] STATISTICAL AND HISTOLOGICAL STUDY OF THE BRONCHOPULMONARY CANCER

Adina Ciursaș[1], M. Pușcașiu[1], Ana – Maria Drăgan[1], Marușca Patricia[2]

University of Oradea, Medicine and Pharmacy Faculty, Department of Morphological Sciences[1], University of Oradea, Medicine and Pharmacy Faculty, Preclinic Department[2]

Bronchopulmonary cancer is the most common cancer in the world today (12.6% of all new cancers,17.8% of cancer deaths).

Patients and Methods: Were taken in study 341 clinically and imaging suspected cases of bronchopulmonary neoplasm in the framework of the emergency Clinical Hospithal Oradea, over a period of 4 years, 2009-2012. All patients were subjected to a bronchoscopy and harvested for biopsies to establish histologic changes and histologic type of bronhopulmonary cancer.

Results: In our group of study almost 80% of the patients were men (78,59%), men/women ratio being 3.7: 1. The majority of patients were aged 51-70 years (67,74%), under 50 years percentage being 10,55% and over 70 years of 21,70%. The average age at diagnosis of bronchopulmonary cancer being 61,88 ± 7.2 years.

The histological lesions: benign 17,01%, represented the dysplasia 7.04%, whereas the malign 75,95%.

Benign lesions were represented in majority by inflammation (16,72%), one case being diagnosed with squamous papilloma.

Dysplasia lesions were seen 24 (5,57%), of which 3 were recorded cases of carcinoma in situ.

Malignant lesions were represented by squamous carcinomas 126 (36,95%). 21,40 (73%) adenocarcinoma, small cell carcinomas 42 (12,31%), large cell carcinoma 12 (3.52%) adenoscuamoase carcinoma 6 (1,74%).

Conclusions: On the basis of this study it was found that statistical data and histological evaluation of bronchopulmonary cancer correspond to those published in the literature.

[P15] ASSESSMENT OF CHANGES IN COAGULATION IN PATIENTS WITH ACUTE LEUKAEMIA

Codreanu Ioana Coralia[1], Iovan Ciprian[1],
Bonta Marinela[1], Ionita Hortensia[2]

[1] *University of Oradea, Faculty of Medicine and Pharmacy*
[2] *"Victor Babes" University of Medicine and Pharmacy, Timisoara*

Acute leukaemia is a heterogeneous group of diseases characterized by the uncontrolled proliferation of myeloid or lymphoid progenitor cells that gradually replace normal haematopoiesis in bone marrow. Genetic changes generated in neoplastic clone generate the multistage production of transformations at molecular level that generate the abnormal proliferation, aberrant differentiation and inhibition of normal haematopoiesis by malignant cells. Disturbances in haemostasis in patients with acute leukaemia are caused by the release of procoagulant substances from leukemic blasts. Changes in haemostasis may include hyper-coagulability states, disseminated intravascular coagulation (DIC) syndrome and reactive secondary fibrinolysis.

Because of the serious consequences caused by the occurrence of disseminated intravascular coagulation (DIC) syndrome, by means of the study herein we sought to highlight the important role of the laboratory diagnosis for DIC since the early stage of its occurrence.

[P16] THE EMERGENCE OF MULTIDRUG RESISTANCE UPON THE SYSTEMIC RESPONSE TO PHARMACOTHERAPY

Maria Zinaida Constantinescu, Niculina Mitrea, Alina Crenguța Nicolae, Cristina Manuela Drăgoi, Andreea Letiția Arsene

UMF „Carol Davila" Bucharest, Faculty of Pharmacy, Biochemistry Department

Multidrug resistance (MDR) is a common phenomenon in which cells become resistant to the effects of various therapeutic agents, structurally unrelated, and with different mechanisms of action. Once installed, the intracellular concentration of active substance is reduced, and the sensitivity of target cells, to the drug, decreases significantly. The development of MDR is a major obstacle in treating tumours with chemotherapy. The ABC transporters (ATP-binding cassette) are a superfamily of 49 transmembrane proteins involved in the active transfer of endogenous substances and xenobiotics. Changes in the normal function of transporters may lead to the development of resistance to structural unrelated drugs (antibiotics, herbicides, chemotherapy) and the occurrence of genetic diseases. The main bio mechanisms through which patients develop MDR are: overexpression of ABC transporters, decreased concentration of the drug due to solute transporters, inhibition of apoptosis, increased processes of DNA repair, inactivation of chemotherapeutic agents by metabolic enzymes. Nowadays, modern scientific research is targeted to identify therapeutic strategies for modulating the activity of these transporters. So far, the administration of drugs that inhibit these transport transmembrane glycoproteins, although effective, require further scientific studies to confirm their clinical utility.

References

[1] Gillet J.P., Gottesman M. M. *Mechanisms of Multidrug Resistance in Cancer*. Methods in Molecular Biology 2010, 596: 47-76.

[2] Vasiliou V., Vasiliou K., Nebert D. *Human ATP-binding cassette (ABC) transporter family*. Human Genomics 2008, 3: 281-290.

[3] Abbasi M., Lavasanifar A., Uludag H., *Recent Attempts at RNAi-Mediated P-Glycoprotein Downregulation for Reversal of Multidrug Resistance in Cancer*, Medicinal Research Reviews, 2013, 33: 33-53.

[P17] HPLC–PDA and HPLC-ESI(+)QTOF-MS FINGERPRINTS OF POLYPHENOLS IN A NUTRACEUTICAL PRODUCT (PROMEN)COMPARATIVELY WITH PLANT INGREDIENTS

Florina Csernatoni[1,2], Carmen Socaciu[1,2], Raluca Maria Pop[2,3], Florina Romanciuc[1], Florina Bunghez[1]

[1]UASMV, Faculty of Agriculture, 3-5 Mănăştur Street, Cluj-Napoca, Romania;
[2]Centre Appl Biotechnology CCD-BIODIATECH, Proplanta Cluj-Napoca, Romania
[3]UMF "Iuliu Hatieganu" Cluj-Napoca, Victor Babes, 8, Cluj-Napoca, Romania

In recent years, an increasing interest towards food supplements (nutraceuticals) isobserved, mainly due to their longterm metabolic activity and prophylactic effect on various diseases.The complex compositions of nutraceuticals based on plant mixtures can provide many biomolecules which can act synergistically as preventive agents, as it is presented here, for prostate-protection. For this reasonquality controlof medicinal plants andnutraceuticalsare becoming of major importance.

HPLC–ESI(+)QTOF-MS analysis represents the best technique to fingerprint, identify and quantify individual molecules from complex mixtures from plant extracts and food supplements.

The aim of this study was to characterize and identify different phenolic derivatives in the plants and yeast powders used as ingredients for an original, nutraceutical formula PROMEN to prevent prostate diseases.

Seven plant sources, namely sea buckthorn (*Hippophae rhamnoides*), nettle (*Urtica dioica*), green tea (*Camellia sinensis*), tomato (*Solanum licopersicum*), fluff with small flowers (*Epilobium parviplorum*), pumpkin (*Cucurbita maxima*) sunflower (*Helianthus annus*) and lyophilized beer yeast (Saccharomyces cerevisiae), used as ingredients of PROMEN, were investigated. Methanolic extracts were preparedusing15% plant concentration and the purified fractions were analyzed using in parallel HPLC-PDA (photodiode array detection) and HPLC–ESI(+)QTOF-MS (detection by mass spectrometry).

By HPLC-PDA analysis coupled with HPLC–ESI(+)QTOF-MSthere were identified 15-25 specific phenolicsin each extract and in Promen, such as Resveratrol, Quercetin, Epigallocatechin, Gallocatechin, Isorhamnetin 3-O-glucoside 7-O-rhamnoside, Quercetin 3-O-galactoside 7-O-rhamnoside, Kaempferol 3,7-O-diglucoside and p-Coumaroylquinic acid,Juglone.The content of polyphenols in Promen was determined by VIS spectrometry and was 76.,61 mg GAE/ 100 ml extract, which was mainly due to the ingredient "fluff with small flowers"having highest content (191,21 mg GAE/100ml extract). The other plants had phenolic

concentrationsranging from 39.38 mg GAE/100 ml sunflower seed extract to 15.38 and 8.8mg GAE/100 ml in tomato and yeasts extracts, respectively.

Combined UV-Vis, HPLC-PDAand LC-MS chromatography are highly recommended as accurate, sensible and reliable tools to investigate theplants and nutraceuticals' fingerprints and to predict the relation between the ingredients' composition and their influence on the quality of final product.

Acknowlegement: "This paper was published under the frame of European SocialFound, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765".

1. Marshall J. R. (2012). Diet and prostate cancer prevention. World J Urol 30:157–165
2. Tang D., L. Hui-Jun , J. Chen, G. Chao-Wei, L. Ping (2008). Rapid and simple method for screening of natural antioxidants from Chinese herb Flos Lonicerae Japonicae by DPPH-HPLC-DAD-TOF/MS. J. Separation Sci. 31(20): 3519–3526.
3. Yanga M., J. Sunb, L. Zhiqiang, C. Guangtong, G. Shuhong, L. Xuan , J. Baohong, Y. Min , D.A. Guo (2009). Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry. J. Chromatogr. 1216(11): 2045–2062.
4. Csernatoni F., C. Socaciu, R.M. Pop, F. Ranga, F. Bunghez, F. Romanciuc(2013). Comparative Fingerprint of Aromatic Herbs and Yeast Alcoholic Extracts used as Ingredients for Promen, a Prostate Preventive Nutraceutical, Bulletin UASVM Food Science and Technology 70(1): 45- 52.

[P18] ASSAY OF COMPONENTS IN THE VOLATILE OILS OBTAINED FROM *SOLIDAGO* SPECIES

Luciana Dobjanschi, Mariana Mureşan, Angela Antonescu, Mihaela Zdrinca, Mraz Camelia, Simona Vicas

Facultatea de Medicina şi Farmacie din Oradea

The goal of this study was to identify and quantify the components of volatile oils extracted from the Solidago species. The species taken in the study were: Solidago virgaurea, Solidago gigantea şi Solidago canadensis. In phitotherapy Solidago virgaurea is well known due to Virgaurea e herba, which is used for it's diuretic [1], saluretic, antiinflamatory, and spasmolythic properties [2].

We studied the upper part of the plant (herba) from the three species of Solidago: S. Virgaurea, S. Gigantea şi S. Canadensis. The analysis of volatile oils from the three species was performed using gas chromatography combined with mass spectrometry.

GC-MS determined the number of components in the volatile oils, their percentage, identity of components, and time of retention [3]. We identified and quantified the main components in the volatile oils obtained from the three species of Solidago. Mircene is characteristic for S. Virgaurea, aristolone is characteristic for S. Gigantea and α- pinene and limonene for S. Canadensis.

References

[1] Tămaş M., Toader S., *Acţiunea diuretică a unor specii de Solidago,* Clujul Medical, 62,(1), 1989, 75-79.

[2] Leuschner J., *Antiinflamatory, spasmolytic and diuretic effects of a comercially available Solidago gigantea extract,* Arzneimittelforschung, 45(2), 165-168, 1995.

[3] Tămaş M., Opreanu R., Roman L., *Identificarea şi determinarea cantitativă a β-asaronei în uleiul volatil şi extracte de Acorus Calamus L.,* Farmacia **44** (5-6), 13-22, 1996.

[P19] **BIOCHEMICAL RELEVANCE OF PLEURAL FLUID**

Ana-Maria Drăgan

University of Oradea, Faculty of Medicine and Pharmacy

Introduction. Pleural effusions (exudates and transudates) are met in respiratory diseases and diseases that affect various organs and apparatuses. The aim of the study was to assess changes in key biochemical tests determined from pleural fluid in patients hospitalized in the Department of Pneumology, between September 2013 and February 2014.

Materials and methods. We have studied 139 patients aged 3-94, of whom 80 males (57.5%) and 59 women (42.4 %). The tests ran on patients were: protein, LDH, glucose, and in the case of 103 patients (74.1%) ADA (adenosine deaminase) was performed too. Investigations were carried out with the Flexor Junior analyzer using BioSystems and Elitech reagents. *Results*: Protein level was >3 mg% in the case of 103 patients (74.1%), pleural effusion being exudate, and the majority of patients were males (59). LDH values obtained ranged between 28-7150U/1; i.e. 52 patients). Glucose values ranged between l–394 mg%, 83 patients were contained in the range 60 - 120mg%, in the case of four patients the level was >120 mg% and in the case 16 patients the level was below 60 mg% (of whom 12 patients presented levels below 10 mg %). ADA values ranged between 119.5 to 2.7 U/l; in the case of 32 patients (31.06%) the values were above the reference value (0 - 33U/1), mostly males (i.e. 21 patients).

Conclusions. Determination and assessment of biochemical tests in the pleural fluid sets the effusion type (i.e. exudate or transudate) and thus we are able to provide information on its etiology. The relevant for therapeutic behavior is complementing the biochemical examination with the cytological and bacteriological examinations. Elevated ADA and proteins levels guide diagnosis to tuberculosis etiology.

[P20] FATTY ACIDS AND CAROTENOIDS IN SOME APRICOT CULTIVARS

Francisc Vasile Dulf[1], Andrea Bunea[1], Elena Andreea Pop[1], V. Al. Oprița[2], Adela Pintea[1]

[1]Department of Chemistry and Biochemistry, University of Agricultural Sciences and Veterinary Medicine, Mănăştur 3-5, 400372, Cluj-Napoca, Romania; e-mail: apintea@usamvcluj.ro
[2]Research Station for Fruit Growing Constanta

Apricots are delicious fruits largely cultivated in Asia and Europe which can be consumed fresh as well as dried or canned. Several biological activities were reported for apricot fruits, such are: antioxidant, antimicrobial, antimutagenic or anti-inflammatory. The complex chemical composition includes simple sugars and polysaccharides, phenolic compounds, lipids, cyanogenic glucosides, carotenoids and volatiles. The objectives of this study were to determine the carotenoid content and profile and the fatty acids composition in the flesh of apricots cultivars – Tudor, Olimp, Harogem and Best of Hungary - cultivated in Romania. Total lipids were extracted and transesterified with acidic methanol an analyzed by GC/MS. Carotenoids were extracted with a mixture of solvents and analyzed by HPLC-PDA on a C30 column using a gradient system

Fatty acids composition of apricots flesh was not yet reported, the former studies being focused on the kernel oil. We identified 17 fatty acids, and a similar profile for all cultivars. Linoleic acid is the major compound (43-46 %), followed by palmitic acid (27-30%) and linolenic acid (13-14 %), while oleic acid was only 1-2 % of total fatty acids. A completely different pattern was found in the kernels, were the major fatty acids were oleic acid (> 55 %) and linoleic acid (32%) and almost no linoleic acid was present (< 0.1 %).

Total carotenoids varied between 1.8 - 4.2 mg/100 g fresh weight. HPLC separation of carotenoids revealed a very similar profile in all cultivars. *All trans* β,β-carotene was the main pigment, accounting for more than 85 % of total carotenoids. Lycopene and γ-carotene were identified in small amounts. Among xanthophylls, lutein, zeaxanthin and β-cryptoxanthin are present almost entirely in esterified form Due to the very specific profile carotenoid of apricots can be used as a tool for the evaluation of authenticity of fruit products.

Acknowledgements. This work was supported by a grant of the Romanian national Authority for Scientific Research CNCS-UEFISCDI, project number PN-II-ID-PCE-2011-3-0721and PNII-RU-PD-2012-3-0245 (contract no. 92/25.06.2013).

[P21] FLOW CYTOMETRYTECHNIQUES (MULTIPLEXED BEADS BASED IMMUNOASSAY) APPLIED FOR THE QUANTIFICATION OF EXTRACELLULAR SIGNALINGFACTORSINVOLVEDIN TUMOR PROGRESSION

Brindusa Dumitriu, Laura Olariu, Emilia Buse

S.C. BIOTEHNOS S.A.

Beads Based Immunoassay (Cytometric Bead Array) is a modern flow cytometry application thatallows the quantification of multiple proteins simultaneously, designed in order to significantly reduce sample requirements and time to resultsin comparison with traditional ELISA and Western blot techniques. This assays provide a method of capturing a soluble analyte or set of analytes with beads of known size and fluorescence, making it possible to detect them using flow cytometry.

The aim of our study was to investigate the release of signaling factors relevant for prostate cancer pathology (the pleiotropic cytokines IL6 and IL8 and the pro-angiogenic factor VEGF) in response to an antitumoral agent of entomological origin. We applythe multiplexed beads based immunoassay technique to quantify theirextracellular level in DU 145 cell culture.IL-8 produced by prostate cancer cells may be responsible for the androgen-independent growth of advanced prostate cancers, as well as tumour metastasis. IL6 is a pleiotropic cytokine implicated in the neoplastic process of a variety of neoplasms, as a mediator of prostate cancer morbidity. An important factor for prostate cancer metastasis is VEGF (vascular endothelial growth factor), responsible for the angiogenic process.

Our result confirm the anti-tumoral role of the entomological preparation. It's activity was proven especially on IL6 inhibition, in a large range of active doses (16 µg /ml - 0,2 µg /ml) and on VEGF (a 59% decrease of extracellular release compared with the cellular control; active doses 10 µg /ml - 0,2 µg /ml).The entomological product has a lower effect on IL8 extracellular release (only a 16% decrease compared with the cellular control; active doses 5µg /ml - 1µg /ml).

The multiplexed beads based immunoassay allowed us a quick feed-back regarding the signaling profile in prostate cancer and certain potential therapeutical approaches.

[P22] INTERRELATION BETWEEN FSP-1 AND TGF-β EXPRESSION IN HEPATITIS C-VIRUS MEDIATED LIVER DISEASE – AN IMMUNOHISTOCHEMICAL STUDY

D. Firu, Camelia Stănciulescu, Anca Predescu, S. Firu,
Ana Marina Andrei, Monica Baniță

University of Medicine and Pharmacy,
2-4 Petru Rareş, 200349, Craiova, Romania

Hepatitis C virus-mediated liver disease (HCV) involves complex mechanisms, fibrosis that follows inflammation being its major complication. Transforming Growth Factor β (TGF-β) is an important mediator of fibrosis but also able to induce phenotypic and functional changes in type 2 EMT (epithelial to mesenchymal transition). Fibroblast-specific protein 1 (FSP-1), a marker of fibroblasts in organs undergoing tissue remodeling, is used to identify fibroblasts that derive from EMT as a response to inflammation.

We evaluated immunohistochemical localization of TGF-β1 and FSP-1 in liver biopsies from HCV-infected patients and correlate them in order to evaluate the relation between inflammation, fibrosis and EMT in disease progression. Fourteen HCV-infected liver biopsy samples were used for histological and immunohistochemical analysis. Histological findings were considered according scoring systems after staining with H&E and Masson. Immunohistochemistry was performed with EnVision+Dual Link System-HRP or avidin-biotin-peroxidase techniques using rabbit anti-FSP-1 and mouse anti-human TGF-β1 as primary antibodies. Liver sections stained revealed various degrees of fibrosis and clusters of inflammatory cells invading portal spaces. The number of TGF-β1-positive cells was directly proportional to the incidence of liver injury: sections with discrete fibrosis revealed positivity only in endothelial cells of sinusoids and occasionally in proinflammatory cells from portal areas. With severe damage of hepatic parenchyma, positive reaction for TGF-β1 expanded to hepatocytes located nearby collagen bundles. In cases of discrete fibrosis, FSP-1 positive cells were observed in cells lining sinusoids. As fibrosis progress, increased number of FSP-1 positive cells was observed mainly in portal areas and along fibrotic septa, most of them appearing to be proinflammatory cells but not fibroblasts.

Even EMT via the activation of TGF-β signaling pathway is a pathogenic mechanism of HCV-induced liver disease, FSP-1 couldn't be used as a marker for fibroblasts that completed EMT. This observation may have implications for therapeutic strategies targeting EMT.

[P23] DEVELOPMENT A NUTRITIONAL SUPPLEMENTS WITH ANTIOXIDANT ACTIVITY

Tunde Jurca[1*], Marian Eleonora[1], Simona Vicas[2], Laura Vicas[1], Mariana Muresan[1], Alin Teusdea[2]

[1]University of Oradea, Medicine and Pharmacy Faculty, Department Pharmacy, Oradea, Romania
[2]University of Oradea, Faculty of Environmental Protection, Oradea, Romania

Various herbal preparations when they are taken in sufficient and correct combinations doses neutralize free radicals before they cause considerable body injury called antioxidant preparations. Based on data from the specialized literature and studies, we have designed a natural product development with a nutritional supplement role.

As standardized active materials we selected: powder from Bilberry fruits (*Vaccinium myrtillus*) powder from Sea buckthorn fruits (*Hippophae rhamnoides*), powder from Sage (*Salvia officinalis*), and powder from flowers Marigold (*Calendula officinalis*).

These vegetale raw materials are derived from indigenous flora. This final product (capsules) oral administrated has real antioxidant properties, with multiple actions in benefit of the human body, so solicited nowadays and a source of vitamins and minerals benefits to the organism.

The antioxidant activity of the final product, we demonstrated by quantitative determination of polyphenols (Folin-Ciocalteu method), flavonoids (colorimetric method), anthocyanins (UV/VIS spectrophotometry method) and metal ions, correlated with the ability to capture free radicals oxide and peroxide involved in infectious and aging processes (DPPH method, FRAP method, ABTS method).

Acknowledgements. This study was supported by a scientific grant from the Ministry of Education of Romania; program (PN-II-IN-CI -2013 -1- 0015), Grant no. 198CI/2013.

[*] *Corresponding author: jurcatunde@yahoo.com*

[P24] FUNCTIONAL LABELING OF HEPATITIS B VIRUS MIDDLE PROTEIN

Paula E. Florian[1] Ramona N. Galantonu[2], Catalin Lazar[2], Anca Roseanu[1], Norica Nichita[2]

[1]Institute of Biochemistry of the Romanian Academy, Department of Viral Glycoproteins, Splaiul Independentei 296, Sector 6, Bucharest 77700, Romania
[2]Institute of Biochemistry of the Romanian Academy, Department of Ligand-Receptor Interactions, Splaiul Independentei 296, Sector 6, Bucharest 77700, Romania

Hepatitis B virus (HBV) is a small human DNA enveloped virus, member of the Hepadnaviridae family, which infects liver cells and can lead to liver cancer. Little is known about hepatocytes HBV receptors and early steps of viral infection. However, great progress has been made regarding HBV replication and genome studies. The HBV DNA is packed in a nucleocapsid, surrounded by viral envelope, which contains three proteins: large (L), medium (M) and small (S). Although all proteins share a common S domain, they have different functions.The S protein is important in subviral particles (SVPs) formation and secretion. The M protein which contains both S domain and a preS2 extension is known to be dispensable for secretion and infection. The L protein which includes M protein and preS1 is important in virion assembley and infectivity. Labeling the virus proteins without impairing the envelope functionality is the challenge of this work. Therefore, the aim of this study is to design and then investigate the infectivity features of HBV fluorescently labeled at M envelope protein. Previous results showed that, under non-reducing conditions, enhanced green fluorescent protein (EGFP)-M fusion viral protein (EGFP.M) revealed a normal intracellular glycosylation and dimerization pattern, but was poorly secreted. We evaluate the incorporation of EGFP.M into the viral envelope and its subsequently secretion by separation of virions from empty envelope particles using isopicnic CsCl gradient centrifugation. HbsAg-specific ELISA and spectrofluorimetry data indicated fluorescence signal in fractions containing SVPs as well as in more dense fractions containing virions. We also employed a second tagging method, making use of a newly developed labeling technique PRobe Incorporation Mediated by Enzyme (PRIME). Coumarin PRIME labeling on M protein will be further investigated. Since M protein is dispensable for infectivity and the tag required for coumarin PRIME is only 13 aminoacids length the new labeling method will not alter the infection properties of the virions and therefore could help resolving the puzzle about the dynamic processes of HBV endocytosis and intracellular motility in living cells.

[P25] ADVANCED MASS SPECTROMETRICAL ANALYSIS OF GLYCATED BSA, AND COMPARATIVE PROTEOMIC SCREENING OF TEMPORAL AND HYPPOCAMPAL OLD BRAIN TISSUE

Cristian Munteanu, Cristian Butnaru, Cristina Jiglaru, Elena Ganea

Institute of Biochemistry, Romanian Academy, Bucharest, Romania

Advanced glycation end-products (AGEs) are the result of non-enzymatic glycosylation (glycation), a post translational, covalent modification of proteins. These compounds can be involved in the development of diabetes, chronic renal failure, neurodegenerative diseases such as Alzheimer's disease, and in some age-related chronic diseases.Mass spectrometry (MS) has been used for the structural analysis of the AGEs-modified proteins in plasma and tissues of patients with diabetes mellitus, cataract, uremia, and other diseases. Matrix-assisted laser desorption/ionization (MALDI)-MS was one of the most popular methods applied for the direct analysis of AGEs-modified proteins such as albumin and IgG. In spite of the advantages of this technique, the broad and poorly resolved peaks sometime obtained, could make difficult the direct analysis of intact AGEs-modified proteins. Various improved MS techniques, recommanded for certain aspects of the research are described in the literature.

In the present work we studied the structural modifications of BSA induced by the glycation with 500mM ribose for 4weeks, analysing the trypsin digested protein by liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS/MS). Complementary fragmentation techniques for the analysis of peptide modifications, like ETD (Electron Transfer Dissociation) and HCD (Higher-energy dissociation) were also applied. Using Sequest search algorithms on the basis of the MS/MS sequence ion data we successfully identified 17 glycated peptides. Identification of specific glycation sites in the glycated BSA has been realized, and compared with similar data obtained by other MS methods, reported in the literature.

We used a part of the above mentioned methods, proposed by us, as well as the BSA results to the analysis of AGE-modified proteins from brain tissue. Comparative proteomic analysis by ESI/MS (LTQ Orbitrap Velos.pro) of hippocampal and temporal regions of an old, normal human brain has been performed and the results corroborated with Western blot analysis of the brain regions using anti-AGEs immune serum. We believe the present work could contribute to demonstrate the important role of protein-linked AGEs in several diseases, being useful in diagnosis and therapeutic control.

[P26] BIOCOMPATIBILITY OF TITANIUM COATED MICROSTRUCTURED SURFACES FOR IMPLANT APPLICATIONS

Madalina Icriverzi, Paula Florian, Livia Sima, Anca Roseanu

Institute of Biochemistry of the Romanian Academy,
296 Splaiul Independentei, 060031 Bucharest, Romania

The aim of this *in vitro* study is to identify the influence of substrate features and topography on cell viability, morphology, and spreading and to evaluate the potential inflammatory capacity of microstructured surfaces coated with titanium. Interbedded elongated shape pattern coated with Titanium (10-45 μm width and 20 μm height) creating an unique micro-architecture were used as substrates. To investigate whether microstructured surfaces could induce an inflammatory effect, an ELISA-type method was used to measure the pro-inflammatory cytokine TNF-α release from THP-1 cells differentiated to surface-adhering macrophages. The results show that THP-1 cells either cultured on glass coverslip or titanium covered surfaces with or without micropattern do not led to a detectable inflammatory effect compared to the cells treated with lipopolysaccharide (LPS) used as a control. Non-radioactive cell proliferation assay performed for 24 and 48 hours revealed no change in viability and attachment of THP-1 cells irrespective of surface topography. Data obtained from immunofluorescent imaging studies show that macrophages spread and display high adherence to titanium coated surfaces and seems to prefer clustering around the elongated motif which increases the contact area for cell attachment.

Our preliminary results conclude that this special three-dimensional layout of the micropatterned surfaces coated with titanium might be suitable for designing and manufacturing medical products for implant applications.

[P27] CHEMICAL AND BIOCHEMICAL PROPERTIES INVOLVED IN THE FORMATION AND DEVELOPMENT OF DENTAL CARIES

Irinyi Izabella-Tunde, Antonescu Angela*

** University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*

Dental decay is due to chemical and biochemical destructive processes of dental hard tissue. These processes can be generated by environmental factors or intrinsic factors, strictly dependent upon the nature of dental substances.

Teeth adherent bacterial biofilms are responsible for the common forms of periodontal disease and dental caries. In people who keep their teeth clean and have no periodontal diseases ,the bacteria in the microbiota is mostly gram positive and resembles that in saliva or adhering to the oral mucosa. Studies have shown that there are many types of bacteria involved in the carious process. Bacteria obtain energy by fermentation, in which shuffling carbon compounds produces ATP without a need of oxygen .The produced NADH is reoxidized by the product of the shuffling which is excreted. If bacteria ferment sugars it will excrete lactate, or ferment amino acids and excrete ammonia, sulfides and chain fatty acids. Fermentation may alternatively reduce an inorganic molecule such as nitrate, produced by salivary glands, to reoxidize their NADH. Saccharolytic bacteria are associated with lactic acid production and dental caries, whereas asaccharolytic bacteria are associated with periodontal disease. Alkaline environment is characterized by calculus precipitated around the teeth.

Bacteria are simple unicellular organisms surrounded by a membrane and a cell wall. The cell walls thickness distinguishes two major classes of bacteria: gram-positive and gram –negative bacteria. The cell wall covers the outer surface of plasma membrane and fimbriae and flagella extrude. Different bacteria utilize each other's products and grow better ,this mechanism wearing the name of symbiosis.

Over the years, various theories have emerged, such as : Chemical-parasitic theory, Theory of proteolysis, Theory of glycogen, The organotropic theory each of them have arguments and drawbacks, but something is sure, dental caries are determined by the interaction of several factors .

[P28] CLONING AND EXPRESSION OF EDEM1 IN MAMMALIAN CELLS

Andrei Juncu

Institute of Biochemistry, Molecular Cell Biology Department, Splaiul Independentei 296, 060031, Bucuresti, Romania

Secretory protiens are synthesized on ER-bound ribosomes and cotranslationally translocated to the endoplasmic reticulum (ER) where the folding process happens. Protein folding is an inefficient process, more than 80% of proteins fail to acquire the native conformation and end up terminally misfolded. Therefore the cells have evolved a quality control mechanisms that can discriminate between folded and terminally misfolded proteins and selectively eliminate these aberrant, potentially toxic proteins. Terminally misfolded proteins are retrotranslocated from the ER to the cytosol and degraded by the ubiquitin-proteasome system through a mechanism known as ER-associated protein degradation -pathway (ERAD). EDEM1 (ER degradation-enhancing α-mannosidase I-like protein 1) is a crucial regulator of ERAD that has been proposed to extract non-native glycoproteins from the calnexin-calreticulin chaperone system. Overexpression of EDEM1 accelerates the extraction of proteins from the CNX binding cycle and their subsequent degradation by the proteasome.

Although a couple of synthetic ERAD substrates and quality control factors as Calnexin, SEL1L, ERdj5, ER mannosidase I and Derlin-2/3 have been reported to associate with EDEM1 in various conditions, its precise role in ERAD is still elusive. We seek to elucidate the mechanism by which EDEM1 interacts with its partners using SPR method. For this, we successfully cloned and optimized the expression of EDEM1 as a fusion protein with His tag in mammalian cells. We are currently optimizing the purification of the fusion protein on Ni-NTA column and subsequently characterize the interactions of this protein.

[P29] COMPARATIVE STUDY OF CHILDHOOD CHRONIC VIRAL B AND C HEPATITIS IN TERMS OF OXIDATIVE STRESS

Ildiko Lenart, R. Spineanu, L. Ritli, Simona Cheregi, C. Sava, Patricia Marusca, Diana Dubau

Universitatea Din Oradea, Facultatea De Medicina

Background. Hepatitis B and C viruses represent major causes of chronic progressive hepatitis in children, being characterized by systemic oxidative stress with excessive pro-oxidative products and incapacity of endogenous antioxidants to counteract their actions.

Objectives. The aims of the study are to assess oxidative and anti-oxidative status in pediatric patients with chronic B and C hepatitis and to compare the two.

Method. The study included 15 pediatric patients with chronic hepatitis B and 15 children with chronic hepatitis C and a control group of 15 healthy children. Viral hepatitis diagnostic was confirmed using hepatocytolysis tests: alanil aminotransferase and aspartate aminotransferase. To confirm viral hepatitis C detection by immunoenzymatic techniques of VHC antibodies and detection of RNA-HVC were performed. For diagnostic of viral hepatitis B levels of Ag HBs, anti HBc IgM antibodies and DNA-HVB were detected. Malondialdehyde was colorimetric dosed through reaction with thiobarbituric acid, and reduced glutathione was assessed by immunoenzymatic method.

Results. Levels of malondialdehyde were significantly elevated in children with chronic viral hepatitis B and C compared with the control group ($p<0.001$). In the patients with chronic viral hepatitis C group levels of malondialdehyde were higher than in the viral hepatitis B group ($p<0.021$). Patients with chronic hepatitis C presented the lowest values of reduced glutathione, 1.8 folds lower compared with the viral hepatitis B group and 2.9 folds smaller than in control group ($p<0.001$). Glutathione values were significantly lower in hepatitis B group compared to control group, also ($p<0.001$).

Conclusions. Comparing malondialdehyde and reduced glutathione levels in children with chronic viral hepatitis B and C versus healthy children group the authors recorded altered values of these parameters characteristic for oxidative stress, especially for the chronic hepatitis C group.

[P30] COMPARATIVE STUDIES REGARDING THE *IN VITRO* ANTIOXIDANT CAPACITY OF ETHANOL AND AQUEOUS EXTRACTS FROM DIFFERENT SPECIES OF *HIBISCUS*

Marieta Lestyan*, Alin Teusdea**, Mariana Muresan*,
Florian Bodog*, Vicas Simona**

** University of Oradea, Faculty of Medicine and Pharmacy, Oradea, Romania*
*** University of Oradea, Faculty of Environmental Protection, Oradea, Romania*

Hibiscus is an evergreen herbaceous plant which belongs from Malveceae family. It contains several hundred species through the world. A high number of research studies showed that flower of *Hibiscus* ssp. has various pharmacological properties [1]. The bioactive compounds from *Hibiscus* flower belongs from flavonol, anthocyans, and phenolic acid classes. The aim of our study was to compare *in vitro* antioxidant capacity of two species of *Hibiscus* (*H. sabdariffa and Hibiscus syriacus)* extracts in order to use it in the future in the field of dermatology and pharmacology. We use two kind of solvent (water and ethanol) and for phytochemical screening, total anthocyanins, total flavonoid and total polyphenols methods were used. The *in vitro* antioxidant capacity of both extracts was evaluate using the following methods: DPPH, FRAP and ABTS. The presence of anthocyanins was only in *H. sabdariffa*, while in *H. orange*, the content of total flavonoid was predominant (160.52 mg QE/g dw *vs*. 60.73 mg QE/g dw in ethanol extracts of *H. syriacus* respectively *H. sabdariffa*). The same result was obtained in the aqueous extract, but the level of flavonoids was less comparative with ethanol extract (90.13 mg QE/g dw *vs.*51.50 mg QE/g dw). The highest capacity of inhibition of DPPH radical was in the case of ethanol extract of *H. sabdariffa* (83.08%), and the lowest capacity was recorded in the case of *H. syriacus* (59.10%). The highest ferric reducing ability was recording also in the case of ethanol extract of *H. sabdariffa* (5.63 mg TE/g dw) comparative with *H. syriacus* (4.80 mg TE/g dw). In the case of annihilation of radical cation ABTS, the highest capacity was recorded in the case of ethanol extract of *H. sabdariffa* (1.06 mg TE/g dw). Between these two species of *Hibiscus* considered by this study, the one with the strongest antioxidant activity was *H. sabdariffa*, probably due to the presence of anthocyanins. Instead, the *H. syriacus* exhibited the highest amount of flavonoids. Knowing the bioactive compounds from plant extracts, these can be used in the desired direction with maximum efficiency.

[P31] BCL-2 – ANTI-OXIDANT AND ANTI-APOPTOTIC PROTEIN – ULTRASTRUCTURAL AND BIOCHEMICAL STUDY

D.S. Marta

Ultrastructural Pathology Department, National Institute of Research-Development in the Pathology Domain and Biomedical Sciences "Victor Babes", Bucharest

Apoptosis is a highly conserved mechanism, by which eukaryotic cells undergo controlled self-destruction. Bcl-2 is the major anti-apoptotic protein, having an important role in the regulation of the process.

Bcl-2 cellular expression was characterized ultrastructurally and assessed quantitatively by a method involving peroxidase labeling of this protein. Ultrastructural investigation of Bcl-2 was performed with standard electron microscopy techniques, using 3,3'diamionobezidyne tetrahydrochloride (DAB) as substrate for peroxidase, and revealed as an electron-dense reaction product of the enzyme. For the quantitative estimation of Bcl-2 we have used 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as peroxidase substrate. ABTS is oxidized in the presence of H_2O_2, and generates a metastable radical, with a characteristic absorption spectrum, having a peak at 415 nm.

We have obtained ultrastructural images revealing the presence of Bcl-2 in the inner mitochondrial membrane, and the nuclear membrane. Quantitatively, the level of Bcl-2 expression in the cells is inversely correlated with the intensity of apoptosis.

References

[1] Marta DS, *Aspecte biochimice şi ultrastructurale in sindromul mielodisplazic,* Editura Universitară, Bucuresti, 2011,

[P32] **XENOBIOTICS IN *LACTUCA SATIVA L.***

Edward Muntean[1], Nicoleta Muntean[2], Marcel Duda[2]

[1] Faculty of Food Sciences and Technology, University of Agricultural Sciences and Veterinary, 3-5 Calea Manastur, Cluj Napoca, 400372 Romania; edimuntean@yahoo.com

[2] Faculty of Agriculture, University of Agricultural Sciences and Veterinary, 3-5 Calea Manastur, Cluj Napoca, 400372 Romania

Lettuce (*Lactuca sativa L.)* is an important leafy vegetable crop, used mainly as a fresh vegetable in salads, but also cooked (Lebeda et al., 2007). Exposing a relatively high surface area of leafs, this plant can be easily contaminated with chemicals from environment, which enter the food chain and can influence the consumer's health (Lee and Shim, 2007). The major aim of this research is to establish the degree of contamination with heavy metals and polycyclic aromatic hydrocarbons (PAH) for lettuce cultivated on three different sites: one with historical pollution, another one located urban area, the third one being a non-polluted site. Four heavy metals were determined (lead, cadmium, copper and zinc) using atomic absorption spectrophotometry, measurements being performed using a Shimadzu AA-6300 double beam atomic absorption spectrophotometer with both flame and graphite furnace, after microwave-assisted digestion. 15 from the 16 Environmental Protection Agency priority PAHs were assessed (naphthalene, acenaphtene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene), using high performance liquid chromatography (HPLC). HPLC analyses were achieved using an Agilent 1100 system equipped with an Envirosep PP column, with acetonitrile:water as mobile phase (45:55 v/v). The recorded concentrations for heavy metals showed maximum values for the studied elements for samples originating the historical pollution site (1.41 μg/ kg Pb, 0.09 μg/ kg Cd, 2.96 mg/ kg Cu and 5.09 mg/ kg Zn), while the PAH's concentrations were highest in samples from urban area, where pollution is caused mainly by automobiles (8 μg/ kg total PAH's), the average PAHs' content ranging from 0.08 μg/ kg for benzo(g,h,i)perylene to 3.27 μg/ kg for naphtalene. The obtained results revealed a moderate contamination of the studied food products with the studied xenobiotics, the main contributors being soil pollution and traffic.

[P33] HEAVY METALS CONTAMINATION OF SOME COMMON CROP PLANTS

Nicoleta Muntean[1], Edward Muntean[2], Marcel Duda[1]

[1] Faculty of Agriculture, University of Agricultural Sciences and Veterinary, 3-5 Calea Manastur, Cluj Napoca, 400372 Romania
[2] Faculty of Food Sciences and Technology, University of Agricultural Sciences and Veterinary, 3-5 Calea Manastur, Cluj Napoca, 400372 Romania; edimuntean@yahoo.com

Researches on human exposure to environmental contaminants have seen a huge development in recent years, in the meantime with the increasing consumers' interest in organic products and increasing awareness of the population regarding the harmful effects of pollution on health (Jarup, 2003). Such exposure may pose a potential risk to human health, especially for cases of consumption for plant crops cultivated in urban and peri-urban polluted areas. In this framework, our research focused on the study of contamination with heavy metals of four important plant crops: *Triticum aestivum* – winter wheat (Arieşan variety), *Zea mays* – maize (Turda 200 hybrid), *Phaseolus vulgaris* – beans (Ardeleana variety) and *Solanum tuberosum* – potatoes (Sante variety). These were cultivated on three different sites: one with historical pollution (in Copsa Mica area), another one located urban area (Cluj Napoca), the third one being a non-polluted site. Lead, cadmium, copper and zinc were determined using atomic absorption spectrophotometry, measurements being performed using a Shimadzu AA-6300 double beam atomic absorption spectrophotometer with both flame and graphite furnace, after microwave-assisted digestion. Comparative analysis of heavy metal contamination of the studied plant matrices revealed that the major phytoconcentration of these elements was achieved by potatoes cultivated in Copsa Mica area, these accumulating 1.94 μg lead / kg and 0.06 μg cadmium / kg; potatoes from urban area cultivated in Cluj Napoca accumulate maximum amounts of zinc (3.89 mg/ kg), while the same area is the origin of the highest concentrations of copper for wheat (2.92 mg/ kg) and beans (2.62 mg/ kg). While the concentrations of heavy metals in the studied matrices are correlated with that of heavy metals in soil, some plants show a higher phytoconcentration ability, being therefore recommended to avoid using them on systematic bases in human consumption, given the risk associated with heavy metal accumulation on human body (Pendergrass and Butcher, 2006).

[P34] MASS SPECTROMETRY EVALUTATION OF A T CELL PRESENTED EPITOPE OF TYROSINASE

Cristian V.A. Munteanu, Gabriela Chiritoiu, Stefana M. Petrescu

Institute of Biochemistry of the Romanian Academy (IBAR)

Melanoma cells present antigenic peptides to T cells for the adaptive immune system activation. These peptides are the results of the intracellular limited proteolysis of specific proteins. Until now, mass spectrometry has been widely used for characterization of the proteome of different cancer cell lines, but linking this information to the antigenic peptidome of these cells still remains a key-aspect in understanding how cells can control T cell responses. Tyrosinase is a membrane glycoprotein found in melanosomes that can generate epitopes for T cell activation following its rapid degradation in proteasome. One of the epitope (YMDGTMSQV) is generated by Peptide -N-Glycosidase F (PNG-ase), following the removal of the 7^{th} glycan structure subsequent deamidation of the asparagine residue to aspartic acid and the degradation of the resulting polypeptide in the proteasome. [1] We aimed to characterize this epitope by eluting the peptide from the surface of melanoma cells stable expressing tyrosinase and analysis of the eluted material using nanoflow liquid-chromatography coupled with tandem mass spectrometry. We have successfully identified this peptide in the elutions from melanoma cells surface. For validation of the identification we used a standard peptide to asses specific parameters like retention time, ionization and MS/MS fragmentation. XCalibur will be further used for quantification and characterization of this peptide in multiple biological samples to correlate T-cell epitope presentation with different intracellular events like protein N-glycosylation or degradation. This will further aid in establishing a mechanism regarding protein epitope generation and presentation in melanoma cells.

References

[1] Skipper, J.C., et al., *An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins.* J Exp Med, 1996. 183(2): p. 527-34.

[P35] THE CORRELATION BETWEEN SERUM LEPTIN AND CYTOKINES IN OBESE AND NON-OBESE PATIENTS WITH HTA SUBMITTED TO HEMODIALYSIS

M. Muresan[1], S. I. Vicas[2], A. Antonescu[1], Alin Teusdea[2], I. Ratiu[3], L. Dobjanschi[1], O. Micle[1]

[1] Department Preclinical Disciplines, University of Oradea, Medicine and Pharmacy Faculty, Oradea, Romania
[2] Faculty of Environmental Protection, University of Oradea, Oradea, Romania
[3] Department Internal Medicine, University of Oradea, Medicine and Pharmacy Faculty, Oradea, Romania

Background. An important contribution to the morbidity and mortality has inflammation in patients submitted to chronic hemodialysis. Adipose tissue secretes inflammatory cytokines such as leptin, IL-6, tumor necrotic factor alpha. Inflammation plays a more important role in the pathogenesis of cardiovascular disease. The aim of the study was to investigate the serum level of leptin, IL-6, IL-8 TNF-α, in obese and non-obese patients with HTA submitted to hemodialysis.

Methods. We enrolled 37 patients that are divided into two groups: 19 obese patients with HTA and 18 with non-obese HTA submitted to dialysis. 30 normal healthy volunteers served as control group. For measuring the concentration of IL-6, IL-8, TNF-alpha we used an automated immunechemiluminescence assay (Immulite 1000 instrument, Siemens, Germany). Serum leptin level was determined using the DRG Leptin ELISA Kit—a solid phase enzyme-linked immunosorbent assay based on the sandwich principle on automated ELISA Adaltis instrument (Italy).

Results. The concentrations of leptin, IL-6, IL-8 and TNF-α in obese patients with HTA submitted to dialysis were increased significantly when compared with the control group. The serum levels of **leptin** in obese dialysis patients with HTA were significantly higher than in non-obese patients with HTA submitted to hemodialysis and control group (46.877ng/ml, 18.271 ng/ml, respectively 4.838 ng/ml). The same results were obtained in the case of levels of **IL6**, 15.779 pg/ml for obese dialysis patients with HTA, 8.778 pg/ml for non-obese HTA dialysis patients and control with 2.829 pg/ml. The levels of serum IL=8 was significantly higher in the case of obese patients with HTA submitted to dialysis, comparative

with the non-HTA obese dialysis patients (53.526 pg/ml, respectively 30.056 pg/ml). Instead, the level of **TNF α** was significantly higher in both patients with HTA (obese and non-obese) submitted to hemodialysis compared with control group (25.174, 23.750 respectively 6.817 pg/ml) but non-significantly between these two groups of patients.

Conclusion. Our preliminary findings suggest that leptin and cytokine levels were higher in obese patients with HTA compared with healthy group. Further studies must be done to understand the role of leptin in predicting survival and cardiovascular events in hemodialysis patients.

[P36] INTERACTION OF LIPID LANGMUIR MONOLAYERS WITH PROPRANOLOL

Roxana-Diana Pasca[1], Aurora Mocanu[1], Gheorghe Tomoaia[2], Santa Szabolcz[1], Csaba Racz[1], Ossi Horovitz[1], Maria Tomoaia-Cotisel[1]

[1]Babes-Bolyai University of Cluj-Napoca, Research Centre of Excellency in Physical Chemistry, Arany Janos Str., no. 11, 400028, Cluj-Napoca, Romania; mcotisel@gmail.com
[2]Iuliu Hatieganu University of Medicine and Pharmacy, Orthopedic Department, Mosoiu Str., no. 47, 400132, Cluj-Napoca, Romania;

The influence of propranolol, a well-known antihypertensive drug, on a monolayer containing different ratios of dimyristoyl phosphatidyl choline (DMPC) and cholesterol (CHO) is investigated by surface pressure versus area per molecule measurements using Langmuir technique at the air-water interface. From these isotherms, the interaction of propranolol with these lipid systems is determined. Firstly, the isotherms are expanded, depending on the increased propranolol content. Secondaly, the collapsed pressure of the lipid-propranolol systems is rather high, even higher than that one corresponding to the DMPC monolayer, indicating a high stability of the mixed monolayers. Thirdly, the lipid-propranolol monolayers are transferred from the air-water interface, using Langmuir-Blodgett technique, on solid supports (e.g. mica and glass) and further visualized by atomic force microscopy (AFM). The AFM images show a significant modification of mixed lipid-propranolol monolayers morphology as compared with the pure lipid layers features. These data indicate that propranolol stabilizes the lipid membranes.

References

[1] I. Viera, M. A. Gómez, J. Ellena, A. J. Costa-Filho, E. R. Migliaro, L. Domínguez, M. H. Torre, *Synthesis, structural characterization and ex vivo biological properties of a new complex [Cu(propranolol)$_2$]$\cdot$2H$_2$O, a potential beta-blocker,* Polyhedron, 28 (2009) 3647–3653.

[2] H. Sugawara, K. Tobise, S. Onodera, *Absence of antioxidant effects of nifedipine and diltiazem on myocardial membrane lipid peroxidation in contrast with those of nisoldipine and propranolol*, Biochemical Pharmacology, 47 (5) (1994) 887-892.

[3] A. Mocanu, R.-D. Paşca, O. Horovitz, M. Tomoaia-Cotişel, *Behavior of Mixed DMPC-Cholesterol Monolayers at the Air-Water Interface,* Studia Universitatis Babeş-Bolyai- Chemia, 2 (II) (2010) 303-312.

[P37] EDEM1 WT AND EDEM1 IDR INTERACTORS IN ERAD PATHWAY IDENTIFIED BY MASS SPECTOMETRY

Florin Pastramă, Cristian V. A. Munteanu, Marioara B. Chirițoiu, Gabriela N. Chirițoiu, Petruța R. Alexandru, Ştefana-Maria Petrescu, Andrei-José Petrescu

Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031, Bucharest, Romania

Whilst properly folded proteins are allowed to leave the ER and are further trafficked through the secretory pathway, incorrectly folded copies are recognized and send to degradation directly from the Endoplasmic Reticulum (ER) by a mechanism commonly known as the endoplasmic reticulum-associated degradation (ERAD) pathway. Perturbations along this pathway may result in severe diseases including diabethis, Parkinson's disease or cystic fibrosis - known to be due to the accumulation of various incorrectly folded glycoproteins into the ER.

One of the main player in ERAD is considered to be EDEM1 (**E**R **D**egradation **E**nhancer alpha-**M**annosidase like 1). Even if our knowlege on its functions is incomplete, it is commonly accepted that EDEM1 plays a central role in the first steps of ERAD involving the recognition of misfolded glycoproteins.

The aim of this study is to identify EDEM1 interactors of ER. In this research, we used INS-1 pancreatic β, HEK 293T and A375 cells to identify EDEM1 co-immunoprecipitated proteins by mass spectrometry (MS). Experimental data have shown that EDEM1 interacts with proteins involved in several processes of the ER including ERAD. We constructed a mutant lacking the intrinsically disordered domain that we recently found to be involved in tyrosinase recognition within the ER. We present here a comparative study of specific interactors of wild type and mutant EDEM1.

We compare here using mass spectrometry the sets of interactors of wild type and mutant EDEM1 involved in the recognition and dislocation of misfolded proteins from the ER into the cytosol These results will form the basis of further investigations intended to confirming the putative interactors of these two protiens by complementary methods.

Acknowledgements. This paper is suported by the Sectorial Operational Programme Human Resources Development (SOP HRD), financed from the European Social Fund and by the Romanian Government under the contract number SOP HRD/107/1.5/S/82514.

[P38] OXYGEN CONCENTRATION REGULATES THE SECRETION OF EXTRACELLULAR PROTEINS

Florentina Pena, Alina Stroescu, Andrei Juncu

Oxygen controls various cellular functions such as metabolism or gene expression. The endoplasmic reticulum (ER) is the cellular compartment responsible with folding of secretory proteins. Here N-glycans are attached and disulfide bonds are introduced to stabilize proteins conformation. Very recently it has been shown that these two processes have different requirement for oxygen. While N-linked glycosylation is an oxygen-independent process, the formation of disulfide bonds depends on oxygen if they are formed post-translationaly (1). We cultured different cell lines under various oxygen concentrations and found that anoxia induces activation of the unfolded protein response, a cellular process that leads to up-regulation of ER-resident proteins. We confirm that disulfide bonds can be introduced into two model proteins (tyrosinase and immunoglubulin M) in the absence of oxygen, though a fraction of IgM molecules were found in high molecular weight complexes under hypoxic conditions and sent to degradation. PDI-like proteins are responsible with disulfide-bond formation and their redox activity is determined by the redox state of cysteine residues from the CXXC motif (the active-site). Using the AMS (4-acetamido-4'-maleimidylstilbene-2,2'-disulfonic acid) modification assay, we analysed the redox state of a series of ER-resident proteins (ERp57, P5, ERp72, Erdj5, Ero1) and found that hypoxia induces moderate alterations in the ratios between oxidized and reduced forms of these proteins. We also aim to investigate how the interacting partners and substrates of PDI family members are modified under hypoxic condtions. In conclusion, oxygen modulates the function of the endoplasmic reticulum, thus controlling the amount of proteins from the secretory pathway that reach their final destination.

References

[1] Koritzinsky, M., Levitin, F., van den Beucken, T., Rumantir, R.A., Harding, N.J., Chu, K.C., Boutros, P.C., Braakman, I., and Wouters, B.G. 2013. *Two phases of disulfide bond formation have differing requirements for oxygen.* J Cell Biol 203:615-627.

[P39] COMPARATIVE PROTEOMICS TO INVESTIGATE PLASMA MEMBRANE PROTEINS WITH A POTENTIAL ROLE IN HBV INFECTION

Catalina Petrareanu[1,2], Alina Macovei[2], Izabela. Sokolowska[1], Alisa Woods[1], Catalin Lazar[2], Costel Darie[1], Norica Branza-Nichita[3]

[1]Department of Analytical Chemistry and Enviromental Engineering, Faculty of Applied Chemistry and Materials Science, POLITEHNICA University of Bucharest, Calea Grivitei, 132, Sector 1
[2]Department of Glycoproteins, Institute of Biochemistry of the Romanian Academy, Splaiul Independentei, 296, Sector 6, Bucharest, RO
[3]Biochemistry & Proteomics Group, Department of Chemistry & Biomolecular Science, Clarkson University, 8 Clarkson Avenue, Potsdam, New York, US

HepaRG cells are the only proliferating human hepatoma cell line permissive for Hepatitis B virus (HBV) infection after a differentiation process, in the presence of dimethyl sulfoxide. During treatment with DMSO, HepaRG cells are differentiated in hepatocyte- and biliary-like cells. Nowdays, more than 350 million people are known to carry the virus around the world. The viral particle is composed of a nucleocapsid containg the partially double-stranded DNA genome surrounded by the viral envelope. Viral infection begins with receptor recognition at the host cell plasma membrane, followed by highly specific cell-virus interactions. The early steps of HBV entry in target cells are largely unknown, because of the poor infectivity in vitro and the absence of a robust tissue-culture model to support virus infection.

Here we have employed the HepaRG cell line to identify host-cell proteins with a potential function in the HBV infection. Plasma membrane was purified from non-differentiated and differentiated cells by subcellular fractionation and subjected to SDS-PAGE. The proteins were further extracted from gels and analyzed by nanoliquid chromatography-tandem mass spectrometry. The results have shown that the protein expression pattern was significantly changed after the differentiation process. In the next experiments, we focused on the up-regulated proteins with a potential role in viral infection. Further analysis by Western blotting and confocal microscopy, confirmed an increased expression of Cyclophilin A, Cathepsin D, Annexin 1, PDI and PDI A4, during DMSO treatment. Moreover,

these proteins were differentially expressed in those two distinct cells types, suggesting a different function in these cells.

Future studies will focus on functional assays on selected proteins to investigate whether these proteins play an active role in HBV infection.

[P40] CELLULAR AND STRUCTURAL FACTORS WHICH REGULATE THE EXPRESSION AND INTRACELLULAR PROCESSING OF MELANOMA ANTIGEN TYROSINASE RELATED PROTEIN

Popa I., Alexandru P., Sima L., Milaca., Chiritoiu M., Negroiu G.

Institute of Biochemistry, Romanian Academy

Malignant melanoma (MM) is a type of neoplasm resulted from the transformation and proliferation of melanocytes, the cells responsible for the production of melanin pigment. The unusual resistance of melanoma cells to radio- and/or chemo therapy compared to other types of neoplasms makes melanoma a still unbeatable disease. Tyrosinase Related Protein-2 (TRP-2) is a member of TRP-family which includes Tyrosinase (Tyr) and Tyrosinase Related Protein-1 (TRP-1). TRPs are all melanosomal proteins, regulatory enzymes of melanin biosynthesis and melanoma antigens. Unlike other TRPs. TRP-2 has been acknowledged as participant in a pathway which enables tumor cells to be unresponsive and resistant to environmental and therapeutic stress and an anti apoptotic molecule in development.

The aim of this study was to identify which factors related to cellular processes or TRP-2 structure are involved in modulation of TRP-2 fate in melanoma cells and subsequently of the specific pathway mediated by this antigen.

Results and Conclusions: Our study identified that in conditions of severe depletion of nutrients TRP-2 expression was distinctly up-regulated in cell lines with different pathophysiological characteristics. Cell treatments with specific pharmacologic agents, immunofluorescence and co-immunoprecipitationstudies demonstrated that TRP-2 is a member of lipids rafts membrane microdomains. The knock down of gene expression for particular protein constituents of TRP-2 containing membranes had significant impact on intracellular processing and subcellular distribution of TRP-2.

The selective roles of transmembrane and cytosolic structural subdomains of TRP-2 polypeptide in TRP-2 maturation along the secretory pathways were also demonstrated.

[P41] *IN VITRO* CITOTOXICITY OF SIMVASTATIN ON B16.F10 MURINE MELANOMA CELLS UNDER HYPOXIC CONDITIONS

Valentin-Florian Rauca[a], Emilia Licarete [a, b], Manuela Banciu [a, b]

[a] *Department of Molecular Biology and Biotechnology, Faculty of Biology and Geology, Babes-Bolyai University, Cluj-Napoca, Romania*
[b] *Molecular Biology Centre, Institute for Interdisciplinary Research in Bio-Nano-Sciences, Babes-Bolyai University, Cluj-Napoca, Romania*

Metabolic cooperation between tumors beyond a size of 2 mm^3 and healthy tissue surrounding the tumor is insufficient for maintenance of tumor cell viability. Consequently, the core of tumor becomes hypoxic and necrotic. To overcome these toxic effects, hypoxia generated in tumor will activate a transcription factor- **h**ypoxia **i**nducible **f**actor-1 (HIF-1α). HIF-1α will further stimulate expression of proteins involved in angiogenesis, inflammation, cell proliferation and survival. Moreover, aggressive tumor cell lines overexpress HIF-1α under normoxic conditions. Increased non-hypoxic production of HIF-1α plays an important role in cell invasion and proliferation. Our previous *in vitro* studies have demonstrated that under normoxic conditions, simvastatin exerted high cytotoxicity on B16.F10 murine melanoma cells that belong to a cell line with very high metastatic potential. These antitumor effects were mainly based on its suppressive actions on HIF-1α expression and redox status of B16.F10 cells. Based on these findings, in this study we investigated whether the beneficial effects of simvastatin noted under normoxia might be preserved under hypoxic conditions. Therefore we tested the effects of different simvastatin concentrations on B16.F10 melanoma cells cultivated with 200 μM $CoCl_2$ as hypoxic agent. *In vitro* effects of simvastatin were investigated with regard to B16.F10 murine melanoma cell proliferation and viability. Furthermore, we assessed the simvastatin actions on the expression of HIF-1α, but also on the levels of NF-kB- a transcription factor with important role in the regulation of HIF-1α under hypoxic conditions. Our preliminary results have shown that simvastatin exerted strong cytotoxic effects on B16.F10 cells under hypoxic conditions at concentrations much lower (10 times lower) than those necessary to exert similar effects on this cell line under normoxic conditions. Moreover, high inhibition of HIF-1α production in melanoma cells was also noted after simvastatin administration.

[P42] SERUM OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY ALTERATIONS DURING 4NQO INDUCED CARCINOGENESIS

Ioana Scrobota, Adriana Nicoleta Pirte

Department of Dental Medicine, Faculty of Medicine and Pharmacy, University of Oradea, Romania

Although the exact mechanisms of carcinogenesis are still unclear it is established that oxidative stress is implicated in induction, promotion and progression of cancer by playing a crucial role in ADN alteration. The aim of the present study is to investigate the modifications of some oxidative stress biomarkers during 4NQO induced carcinogenesis in Wistar albino rats. Two groups of rats received by topical application on the oral mucosa 4NQO in polyethylene glycol and the vehicle alone respectively for 12 weeks. After 16 weeks animals were anaesthetized and blood was collected from the retroorbital sinus. Carbonyl groups (PC) (Reznick method) as markers of oxidative stress and hydrogen donors (HD) (Janaszewska method), indicators of antioxidant capacity were assayed. Carcinogen administration resulted in increased PC values, not statistically significant maybe because of the body antioxidant system reaction. In supporting this supposition comes the observation that the levels of HD were significantly (p=0,001) elevated in the 4NQO induced carcinogenesis group probably in the effort of the organism to established the equilibrium in the oxidants-antioxidants balance. These results may be helpful in giving additional date regarding the implication of oxidative stress in carcinogenesis and the possibility of using antioxidants in chemoprevention.

[P43] **ENAMEL HYPOPLASIA**

Restea Patricia-Andrada, Antonescu Angela

Tooth enamel represents the more highly tissue, and it is an acellular tissue. The enamel is made by ameloblasts , cells of ectodermal origin ,unrelated to osteoclasts which are of mesodermal origin. Enamel is the only calcified tissue. Tooth enamel forms a tight, impenetrable seal around the dentin , much like a skin.

A tooth enamel defect is enamel hypoplasia (HE) . HE is defined as defective or incomplete formation of the organic enamel matrix. The formation of organic enamel matrix are in the embryonic stage of the tooth..The enamel hypoplasia is caused during tooth formation, by infections, illness or malnutritions. Most cases of HE occur before the age of four.

The signs of enamel hypoplasia are seen when they erupt in the mouth. In extreme cases, the tooth enamel is missing entirely.

There are two types of enamel hypoplasia: hereditary type and environmental type. The hereditary type represents an ectodermal disturbance. Disturbance occurred during the embryonic development of the enamel. The environmental type is caused by the environmental factors that causes damage to the enamel cells. The environmental factors which produce enamel hypoplasia are seven. Fortunately, HE can be managed.

References

[1] *Oral Pathology – Clinical pathologic correlations,* Joseph A. Regezi, James J.Sciubba, Richard C.K. Jordan, Sixth Edition, Elsevier, 2012.

[P44] HEMOGLOBIN AND HEMERYTHRIN BASED BLOOD SUBSTITUTES

Vlad Toma[1,2], Anca Farcaş[1,2], Denisa Hathazi[1,2], Eva-Fischer Fodor[3], Florina Scurtu[1], Ioana Roman[2], Bogdan Sevastre[4], Radu Silaghi-Dumitrescu[1]

[1]*Department of Chemistry and Chemical Engineering, Babes,-Bolyai University, 11 Arany Janos Street, Cluj-Napoca 400028*
[2]*Institute of Biological Research, 48 Republicii St, Cluj-Napoca, 400015*
[3]*Ion Chiricuta Cancer Institute – Comprehensive Cancer Center, 34-36 Republicii St, Cluj-Napoca 400015*
[4]*University of Agricultural Science and Veterinary Medicine, 3-5 Manastur St, Cluj-Napoca 400372, Romania*

Most of the recent attempts to produce protein-based artificial carriers implies a series of chemical modified hemoglobin (1-3), although a recent line of research using another oxygen transporter – hemerythrin - instead of hemoglobin is also described (4). Here, we present preliminary results on the work in progress that implies both the *in vitro* and *in vivo* experiments. Tests on human lymphocytes shows that derivatized proteins are not cytotoxic; also, the immunological, hematological and biochemical tests on animals shows no significant difference between the treatment and control groups since the values variation falls within acceptable ranges.

References

[1] Deac, F., Todea, A., and Silaghi-Dumitrescu, R. (2009) *Glutaraldehyde derivatization of hemoglobin: a potential blood substitute.* in Metal Elements in Environment, Medicine and Biology Tome IX (Silaghi-Dumitrescu, R., and Garban, G. eds.), Cluj University Press, Cluj-Napoca, Romania. pp 165-173

[2] Deac, F., Iacob, B., Fischer-Fodor, E., Damian, G., and Silaghi-Dumitrescu, R. (2011) *Derivatization of hemoglobin with periodate-generated reticulation agents: evaluation of oxidative reactivity for potential blood substitutes.* J Biochem (Tokyo) **149**, 75-82

[3] Scurtu, F., Zolog, O., Iacob, B., and Silaghi-Dumitrescu, R. (2014) *Hemoglobin-albumin cross-linking with disuccinimidyl suberate (DSS) and/or glutaraldehyde for blood substitutes.* Artif Cells Blood Substit Immobil Biotechnol **42**, 13-17

Acknowledgements. Financial support from the Romanian Ministry for Education and Research (grant PCE 488/2012) is gratefully acknowledged.

[P45] FGF2 MODULATION OF MESENCHYMAL STEM CELLS OSTEOGENIC FATE

Livia E. Sima, Mari Chiritoiu, Gabi Chiritoiu

Institute of Biochemistry, Romanian Academy,
Spl. Independentei 296, Bucharest, 060031, Romania

Mesenchymal stem cells (MSCs) represent a heterogenic population of progenitors that are able to undergo differentiation towards various cell lineages such as osteoblasts, chondrocytes and adipocytes. The bias to a specific cell fate is dependent on the stimuli received by the cells. *In vitro*, osteogenic induction is successfully obtained using a cocktail of three differentiation factors: dexamethasone, ascorbic acid, and beta-glycerophospate. During the first stages of implantation, the coverage of biomaterials with progenitor cells with increase potency to generate bone cells is of key importance. Usage of growth factors to enhance the production of bone for *in vitro* experiments or for tissue regeneration purposes is well documented. FGF2 was already described as an efficient proliferation enhancer when added in the expansion media of MSCs. Although FGF2 was shown to maintain osteogenicity and increase osteogenic and chondrogenic differentiation *in vitro*, little is known about the potential molecular mechanisms involved. We have used a combinatorial approach to study the role of FGF2 addition to the osteogenic induction medium (OIM) on MSCs that were pre-treated with 1ng/mL FGF2 for two weeks. The effect of FGF2 on MSCs proliferation and osteogenic differentiation was characterized by MTS assay and Alizarin Red mineralization staining. We have screened for treatment conditions that would be beneficial for the efficient generation of bone tissue. Using flow and image cytometry, we followed MSCs phenotype during treatment with FGF2. MAPKs (pERK1/2, pp38, pJNK) and beta-catenin expression was quantified during cell differentiation in the presence or absence of FGF2. The impact of signaling pathways modulation on Runx2 transcription factor-driven osteogenic commitment was investigated at single-cell level. These studies are a preamble to the design of polymeric scaffolds embedding FGF2 and other growth factors to improve osseointegration and angiogenesis during bone healing by controlled release of these molecules.

Acknowledgements. The study was supported by the National Partnership Grant FABIO3D, 153/2012.

[P46] ASSESSMENT OF SNAIL-1, E-CADHERIN AND VIMENTIN EXPRESSION IN GINGIVAL FIBROMATOSIS

Camelia Stănciulescu, D. Firu, Ana Marina Andrei, Monica Baniță, Cătălina Pisoschi

University of Medicine and Pharmacy, 2-4 Petru Rareş, 200349, Craiova, Romania

Background. Gingival fibromatosis (GF) is a rare condition of gingival overgrowth which develops as a localized or generalized enlargement of gingiva and could be non-syndromic or part of a syndrome due to chromosomal abnormality. Even some molecular and cellular changes are revealed, the pathogenesis of GF is still unknown. Because no increase in fibroblast expression of proliferation markers was noted is not clear whether cell proliferation contributes to GF. Epithelial to mesenchymal transition (EMT) was hypothesized as an event responsible for phenotypic heterogeneity of fibroblasts. Snail-1 is a zinc finger transcription factor expressed in cells that undergone complete EMT. The main mechanism by which Snail induces EMT is downregulation of epithelial markers and upregulation of those mesenchymal. In this study we assessed expression of Snail-1, E-cadherin and vimentin in GF by immunohistochemistry.

Material and Methods. Gingival tissues from 7 patients clinically diagnosed with GF and from 2 healthy donors were processed for paraffin embedding. 3 μm sections were used for Masson staining and immunehistochemistry with biotin-avidin-peroxidase technique using as primary antibodies: rabbit polyclonal anti-Snail and two mouse monoclonal anti-E-cadherin and vimentin.

Results. Histological staining revealed a thickened hyperkeratotic epithelium, abundance of collagen bundles in lamina propria. Cells from the epithelial ridges and mesenchymal cells from their proximity showed an intense positive reaction for Snail-1. The antibody for vimentin labeled a lot of cells in the lamina propria, an increased number of positive cells being in the superficial and less in the deep chorion. We also noted cells with cytoplasmic positivity present in the basal epithelial layer. We observed a diminished E-cadherin expression in the epithelium surrounding the chorionic papillae.

Conclusion. Resuming these data we presume that EMT could be a pathogenic mechanism of gingival fibromatosis.

Acknowledgements. Part of this work was financed in the 563 Ideas PN II project, contract no.1137/2009.

[P47] COPPER BIOSORPTION IN AN AQUEOUS SOLUTION BY BREWER'S YEAST

Andreea Stanila, Carmen Socaciu

University of Agricultural Sciences and Veterinary Medicine, 400372, No.3-5 Manastur str, Cluj-Napoca, Romania

Heavy metal pollution in waste water is one of the most important environmental problems today. The yeast biomass has been successfully used as biosorbent for removal of Ag, Au, Cd, Co, Cr, Cu, Ni, Pb, and Zn from aqueous solution. Yeasts of genera Saccharomyces are efficient biosorbents for heavy metal ions.

As biosorbent was used non-living brewer's yeast type Saccharomices cerevisae at 0,5% and 1% yeast dose. Copper solution of 1mg/L was prepared using CuSO4·5H2O of analytical reagent grade. The experiments were conducted at three pH level (3,5; 5; 6) in order to study the effect on biosorption. Sampes were withdrawn at 15, 30, 60 and 120 minute intervals during the biosorption experiments and analyzed for Cu(II) using AAS method.

The experiments were conducted with yeast and Cu(II) aqueos solution of initial concentration of 1mg/L, mixed at pH range from 3,5 to 6 and shaken at a constant speed of 120 rpm in a shaking at 200C for 15-120 minute. The samples were centrifuge dat 2500 rpm for 15 minute and the supernantant were analyzed for copper quantification.

There were increases in biosorption uptake equilibrum adsorption capacity (qe) with increasing pH level from 59,94 µg/g at 15 minute and pH=6 to 79,6 µg/g at 120 minute and same pH value for a 0,5g/100ml yeast dosage. For 1g/100ml yeast dosage there were also increases in adsorption capacity with the increasing of pH level, but these were lowers , between 60,49 µg/g at 15 minute to 69,16 at 120 minute. As the pH is lowered the overall surface charge on the cells will be positive, which will inhibit the approach of positively charged metal ions.

Acknowledgement. This work was supported by a grant of the Romanian Ministry of Education, CNCSIS – UEFISCDI, PN-II-RU-PD-2012-3 - 0055", nr. 47/30.04.2013

[P48] IDENTIFYING INTERACTORS OF HUMAN TOPOISOMERASE IIα AND IIβ THROUGH COMBINED BIOINFORMATICS AND MASS SPECTROMETRY

Marius D. Surleac[1], Cristian Munteanu[1], Ram Ganapathi[2], Mahrukh Ganapathi[2], Andrei J. Petrescu[1]

[1]*Institute of Biochemistry of the Romanian Academy, Splaiul Independentei 296, 060031, Bucharest 17, Romania*

[2]*Levine Cancer Institute, Cancer Pharmacology, North Carolina, Charlotte, USA*

Topoisomerase II enzymes are involved in regulation of essential cellular processes such as altering the topology of chromosomal DNA by breaking and re-joining double-stranded DNA. Topoisomerases can relax or unwind negatively or positively supercoiled DNA. Topoisomerase IIα enzyme is mostly found in fast dividing cells (essentially during the S and G2+M cell cycle phases) while topoisomerase IIβ enzyme is expressed in all tissues and its expression is not cell cycle dependent. There is an increased repertoire of interactors for both isoforms. Some proteins are involved in specific interactions with each topoisomerase, while other proteins interact with both.

Here we use qualitative and quantitative Mass Spectrometry (MS) based proteomics to assess the interactome of TopoIIα/β and compare it with documented TopoIIα/β interactions as resulting from BIOGRID, STRING and literature data mining. The work aims to refine the TopoII□/□ interactome mapping and assess correlations between the levels of expression for each topoisomerase in various conditions and how TopoIIα/β overexpression influence the cellular phenotype and the level of the expression of interacting proteins.

[P49] PHYSICOCHEMICAL PROPERTIES, BIOCOMPATIBILITY AND INTRACELLULAR FATE OF POLY (LACTIC-CO-GLYCOLIC) ACID (PLGA) AND PLGA-CHITOSAN NANOPARTICLES

C. M. Sabliov[1], P.E. Florian[2], C. E. Astete[1], M. Trif[2]

[1]Louisiana State University Agricultural Center, Baton Rouge, LA, USA
[2] Institute of Biochemistry, Romanian Academy, Bucharest, Romania

Entrapment and delivery of bioactives of various functionalities with polymeric nanoparticles (NPs) is anticipated to significantly impact food, health, and agriculture. Long-term biodistribution studies are needed to determine accumulation/clearing from the body of the NPs and their toxicity. In response to this need, NPs interaction with mammalian cells was assessed by cytotoxicity measurements, fluorescence microscopy investigations and flow cytometry quantitations. The PLGA and PLGA-Chi NPs were covalently linked to fluoresceinisothiocyanate (FITC) to aid in nanoparticle tracking. The particle size of PLGA NPs measured 112±9nm in diameter with a ζ potential of 2.1±0.8 mV, and PLGA-Chitosan (PLGA-Chi) NPs were 134±8nm in size with a zeta potential of 4.9±2.0 mV. All NPs tested did not induce cell toxicity, up to a concentration of 2500μg/ml in Madin-Darby bovine kidney cells (MDBK) and human intestinal epithelial cells (Colo 205). In MDBK cells all NPs were internalized as early as 30 minutes, a process more evident in the case of PLGA-Chi. PLGA NPs seemed to have a peak of accumulation at 3h, while PLGA-Chi did not show an intracellular accumulation even after 24h. In Colo205 cells 85% of the cells internalized the PLGA, compared to 65% in the case of PLGA-Chi after 24 hrs. It appeared that the presence of chitosan inhibited or delayed NPs internalization. ER co-localization experiments in MDBK cells revealed that PLGA NPs partially overlay with ER marker, while PLGA-Chi did not, despite their perinuclear localization. The NPs did not seem to target ER in Colo 205 cells and the agglomeration of NPs detected at plasma membrane suggested that the endocytosis was slow but highly specific in these cells. Our data showed that the charge of the particles plays an important role in particle cell interaction and intracellular trafficking. The results of the work will provide a platform for a broad understanding of the fate polymeric nanoparticles designed for future applications.

Acknowledgements. EPA-NSF-USDA (EPA-G2010-STAR-N2 Food Matrices), Subaward number 61369.

[P50] THE EFFECT OF TWO PROSTAMIDE F2α ANALOGUES TREATMENT ON OCULAR TISSUE PATHOLOGY

Denisa Ioana Udeanu[1], Iuliana Ionașcu[2], Cristina Ciofrângeanu[1], Cristina Manuela Dragoi[3], Andreea Letiția Arsene[3], Alina Crenguța Nicolae[3], Ana Corina Ioniță[1]

[1]*Department of Clinical Laboratory and Food Safety, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", Bucharest*
[2]*Department of Ophthalmology, Faculty of Veterinary Medicine, University of Agronomical Sciences and Veterinary Medicine, Bucharest*
[3]*Department of Biochemistry, Faculty of Pharmacy, University of Medicine and Pharmacy "Carol Davila", Bucharest*

Nowadays, many researches are dealing with the precursors of arahidonic acid, arahidonoylethanolamide (anandamide), linoleic acid, docosahexaenoic acid and cholesterol involved in chronic diseases caused by oxidative stress. Thus, prostaglandins derivatives, isoprostanes, homologous derivatives of arahidonic acid, neuroprostanes and oxisterols represent potential future biomarkers of the *in vivo* oxidative stress. Prostamides (prostaglandine-ethanolamides) are neutral lipid mediators with various biological role like: ocular anti-hypertensive effect, stimulating hair growth, the modulating role on adipocytes differentiation and regulator effect on immune system cells.

Prostamides are expressed in different tissues including ocular cells and macrophages. In normal physiological conditions, the aqueous humour contains small concentrations of hydrogen peroxide and superoxide anion. The photochemical reactions and increased concentration of oxidants expose the ocular tissues to an increased level of oxidative stress. A low level of prostamides correlated with oxidative stress induced molecular modifications with toxic potential involved in the generation of glaucoma and neurodegenerative diseases.

Many researches deal with the cytoprotective and antioxidant effect of prostamides on cells of trabecular meshwork and ciliary muscle, but less is known about the effect on immune system cells like macrophages also present in the eye tissues. The prostamide analogues were obtained by stereocontrolled synthesis within the Department of Synthesis of Natural Products from the National Institute for Chemical and Pharmaceutical Research and Development, Bucharest. The ocular antihypertensive effect was determined on animals diagnosed with glaucoma at the

Department of Ophthalmology within Faculty of Veterinary Medicine, University of Agronomical Sciences and Veterinary Medicine, Bucharest.

The murine macrophage cell line RAW264.7 (ATCC) was grown in Dulbeco Minimum Essential Medium with Earle's salts (DMEM) supplemented with 10% fetal bovine serum (FBS). The response of two synthesised prostamides treatment on cell viability, lactate-dehydrogenase, superoxide-dismutase and glutationperoxidase activity were determined on RAW264.7 macrophage murine cell line.

[P51] MECHANISM OF HEPATITIS B VIRUS PROTEINS DEGRADATION

Mihaela Uta, Catalin Lazar, Stefana Petrescu, Norica Nichita

Institute Of Biochemistry Of The Romanian Academy

More than 350 milion people worldwide are chronically infected with hepatitis B virus (HBV) and at high risk to develop hepatocellular carcinoma (HCC), the third leading cause of death from cancer. The effectiveness of current therapies (interferon and nucleoside analogues) is limited due to the recurrence of viremia after treatment interruption and development of mutants during the prolonged treatment with nucleos (t) ide analogs. Antiviral therapies have no direct effect on the covalently closed circular DNA (cccDNA) minichromosome, which serves as a transcriptional template for the viral mRNA synthesis. The accumulation of cccDNA in the host cell nucleus leads to persistence and chronicity, making this replication form a strong, yet difficult target for therapy. Based on data published previously showing that the envelope proteins may regulate cccDNA amplification, we hypothesized that their significant degradation during the Unfolded protein Response (UPR), triggered by HBV infection, may be a key component of this regulatory mechanism. To investigate this hypothesis, we monitored the interactions between components of the endoplasmic reticulum associated degradation (ERAD) and the HBV envelope proteins. Expression of the ERAD regulators, EDEM1-3 was modulated in cell lines which support different stages of the HBV life-cycle. A HepG2.2.15 cell line permissive for viral replication assembly and secretion, constitutively expressing tetracycline repressor (TetR) was first obtained and characterized. Lentiviral constructs expressing shRNAs targeting EDEM1-3, as well as a scrambled sequence, were cloned and further used to transduce the HepG2.2.15 TetR cell line. The newly established cell lines were treated with tetracycline to induce expression of the corresponding shRNA and the EDEM1-3 down-regulation was evaluated by reverse transcription-PCR, using sequence-specific probes. The results have shown that synthesis of the EDEM1, EDEM2 and EDEM3 mRNA was inhibited by 70, 50 and 70%, respectively, in the cell lines bearing the corresponding shRNA. Interestingly, EDEM1 and EDEM3 knock-down resulted in accumulation of secreted HBV subviral particles (SVPs) suggesting that an excess of folding-competent envelope proteins are available for assembly in these cells. In contrast, EDEM2 down-regulation had no effect on SVPs production. Further investigation of the relationship between cccDNA amplification and the envelope proteins levels in these cells is in progress.

[P52] CHANGES IN PLASMA ANTIOXIDANT ACTIVITY OF WISTAR RATS AFTER INGESTION OF FOUR DIFFERENT FOOD SUPPLEMENTS

Vicas Simona[1], Teusdea Alin[1], Timar Adrian[1], Czirjak Tibor Zsolt[1], Laslo Vasile[1], Bisboaca Simona[2]

[1] University of Oradea, Faculty of Environmental Protection
[2] S.C. Phenalex SRL

Food supplements are concentrated sources of nutrients or bioactive compounds, such as polyphenols, flavonoids, phenolic acids etc. with benefit effects on human health.

The aim of research study was to investigat *in vitro* and *in vivo* antioxidant capacity of four different food supplements: Antioxivita, Propolis, Mistletoe and Viprovi, all produced by Phenalex SRL, Romania.

The bioactive compounds, such as polyphenols and flavonoids, were investigated in food supplements. Regarding to the total polyphenols, the highest value was recorded for Antioxivita, followed by Viprovi, Mistletoe and Propolis, (212.51±2.11, 80.22±0.48, 36.84±0.42 respectively 31.24±0.64 mg GAC/ml). The highest level of total flavonoid was recorded also, in the case of Antioxivita (57.49±0.49 mg QE/ml), followed by Mistletoe, Viprovi and Propolis (31.14±2.93, 28.11±0.19 respectively 26.85±0.59 mg QE/ml). For the evaluation of *in vitro* antioxidant capacity of food supplements, TEAC (Trolox Equivalent Antioxidant capacity) method was used. The antioxidant capacity of food supplements revealed that Antioxivita (584.47±3.37 TE/ml) perform the highest scavenging ability of long-life radical anion $ABTS^{+}\cdot$, followed by Viprovi (481.03±1.94 TE/ml), Propolis (135.87±33.72 TE/ml) and Mistletoe (25.69±1.94 TE/ml).

Each of the three variables values were subjected to one-way of analysis of variance, ANOVA ($P = 0.05$), in order to detect the statistical significance differences between food supplements. In each case there were significant differences between the variables performances of food supplements.

The effect of foods supplements on antioxidant capacity of plasma in Wistar rats was investigated, using TEAC method. Wistar rats were divided in 5 groups, each with seven animals. The groups were named: Control (CTRL), Propolis, Antioxivita, Mistletoe and Viprovi. After ingestion of extract as drinking, for 15 days, the animals were sacrificed and the plasma antioxidant capacity was investigated. Our results shown that after ingestion of Viprovi, Propolis and Mistletoe, the antioxidant capacity of plasma increased with 7.82% ($P > 0.05$), 19.35% ($P < 0.05$) and 18.37%

($P < 0.05$), respectively, comparative with Control. In the case of Antioxivita the plasma antioxidant capacity decreased with 4.72% ($P > 0.05$) comparative with the Control. The statistical significances were calculated with the one-way analysis of variance, ANOVA, after outliers' removal. This test stated that there are significant differences ($P = 0.02$) between the food supplements. Multiple comparisons were performed with Dunnett post-hoc test ($P = 0.05$); the statistical significance results were previously displayed next to the relative ratio (%) compared with the Control.

Acknowledgement. This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI – UEFISCDI, project number PN-II-IN-CI- 2012-1-0295.

[P53] INDUCIBLE *TRANS*-ENCAPSIDATION SYSTEM FOR HEPATITIS C VIRUS ALLOWS TEMPORAL SEPARATION BETWEEN THE DIFFERENT STAGES IN THE VIRAL LIFE CYCLE

Ovidiu Vlaicu[1], Leontina Banica[2], Dan Otelea[2], Andrei-Jose Petrescu[1], Costin-Ioan Popescu[1]

[1]Institute of Biochemistry of the Romanian Academy, Bucharest, Romania
[2] National Institute of Infectious Disease „Matei Bals", Bucharest, Romania

Hepatitis C Virus (HCV) infects 130 million people worldwide and it represents the major cause of liver transplantion in the western world due to cirrhosis. The current therapy consisting of interferon alpha and ribavirin has been recently upgraded by the FDA approval of direct acting antivirals against the viral protease and polymerase. Despite major advance in HCV therapy, the drug resistance still represents an issue. Thus, new antiviral therapies with milder side effects and higher barrier to resistance are still an unmet need. In order to better understand the virus – host interaction in the different stages of the viral life cycle and to facilitate the identification of stage specific inhibitors, we designed an inducible *trans*-encapsidation system where the capsid protein is expressed *in trans*. We obtained a Huh7 cell line derived clone which expresses HCV core in a TetON system. The capacity of this cell line to package core free HCV subgenomic replicons into virus like particles (HCV-VLPs) was determined by qRT-PCR and FFU assay. The specificity of HCV-VLP entry was determined by neutralizing antibodies inhibition assay. Huh7 TetCore cells were able to package VLPs which were able to infect Huh7 naive cells. The replication and secretion/ assembly steps in the viral life cycle were temporaly separated in this system and quantified by a reporter gene inserted in the packaged subgenomic replicons. This system will be a valuable tool in studing pre- and post-assambly events in HCV life cycle and for the identification of stage specific antivirals.

[P54] GLUCIDIC TYPE MACROMOLECULES ON THE ANTIOXIDANT ACTIVITY OF ISOFLAVONE GENISTEIN

Mariana Voicescu[1**], Florentina Gatea[2], Aurelia Meghea[3]

[1]*Romanian Academy, Institute of Physical Chemistry "Ilie Murgulescu", Splaiul Independentei 202, 060021 Bucharest, Romania*
[2]*National Institute of R&D for Biological Sciences, Splaiul Independentei 296, 060031, Bucharest, Romania*
[3]*University POLITEHNICA of Bucharest, Faculty of Applied Chemistry and Materials Sciences, Polizu 1, 78126 Bucharest, Romania*

Genistein is an 4', 5, 7-trihydroxy isoflavone and phytoestrogen with a wide spectrum of physiological and pharmacological functions, especailly as inhibitor of cell proliferation [1]. The work aims to simulate *in vitro* the effects caused by oxidation of genistein using the chemiluminescent system luminol-hydrogen peroxide, in phosphate buffer, pH 7.4. The contribution of glucidic type macromolecules, Dextran 40, Dextran 70, α -, β- and γ-Cyclodextrins, to the antioxidant activity of genistein, in the chemiluminescent system luminol-hydrogen peroxide, has been investigated. ABTS [(2,2`-azino-bis(3-ethylbenzothiazoline-6-sulfonic) acid diammonium salt] free radical scavenging assay for i*n vitro* antioxidant activity of genistein, has been also used. The stable ABTS radical scavenging activity of the genistein has been expressed as Trolox equivalent antioxidant capacity. The results are discussed with relevance to the oxidative stress process.

References

[1] Q. Bian, J. Liu, J. Tian, Z. Hu, *Binding of genistein to human serum albumin demonstrated using tryptophan fluorescence quenching*, Int. J. Biol. Macromol., 34, 275-279, 2004.

Acknowledgements: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNCS – UEFISCDI, project number PN-II-RU-TE-2012-3-0055.

[**] *Corresponding author: E-mail: voicescu@icf.ro (M. Voicescu).*

[P55] MOLECULAR MARKERS PERFORMED BY IMMUNOHISTOCHEMISTRY IN BREAST CANCER

Dana Carmen Zaha

Preclinical Department, Faculty of Medicine and Pharmacy, Oradea University, 1 December Square, No.10, Oradea, Romania, Tel. +040 0723604496E-mail: danazaha@rdslink.ro

Immunohistochemistry is playing an increasing role in the modern pathology of breast disease, as well as in other benign or malignant tumours. A growing list of available antibodies, improved antigen retrieval techniques have all contributed to the broader utility of IHC for solving important problems in breast pathology. Myoepithelial markers are the most useful in helping to distinguish benign lesions from the malign ones. The common immunohistochemical breast cancer prognostic and therapeutic markers used include: ER, HER2, Ki-67, PR, p53. In addition, markers of angiogenesis such as VEGF and apoptosis (Bcl-2)are important.

In this paper we will provide a presentation about established immuno-histochemical markers, and discuss challenges in integrating novel molecular markers into clinical practice using the available data from the literature.

[P56] **NEXT-GENERATION BREAST CANCER TESTS**

Dana Carmen Zaha, Tirb Alina

Preclinical Department, Faculty of Medicine and Pharmacy, Oradea University, 1 December Square, No.10, Oradea, Romania, Tel. +040 0723604496 E-mail: danazaha@rdslink.ro

Early detection is one of the keys to successful treatment in cancers. If breast cancer is diagnosed early, the tumour can be surgically removed and with the appropriate treatment, most patients can fully recover. In addition to classical tests or methods, there arenext-generation genomic breast-cancer: the Blue Print molecular diagnostics assay and the Mamma Print gene signature that determine molecular subtypes and risk of recurrence, the Target Print, an ER/PR/HER2 expression assay and the Oncotype DX.

A key attribute of the MammaPrint is the ability to identify the likelihood of distant recurrence in the first five years following diagnosis. It delivers a powerful means of individualized risk assessment by correctly stratifying patients into low risk and high risk.

The BluePrint molecular diagnostics assay is the most widely available test providing molecular subtyping of individual breast cancers; it classifies breast cancer into one of four molecular subtypes: Luminal A, Luminal B, HER2-type, and Basal-type. BluePrint also provides information about neoadjuvant chemosensitivity more accurately than an IHC/FISH assessment does.Molecular subtyping provides a more precise prognosis and valuable guidance about the best treatment for early-stage breast cancer.

The Oncotype DX Breast Cancer Assays help guide treatment decision making in patients with ductal carcinoma in situ or invasive carcinoma; can predict the potential benefit of chemotherapy and likelihood of distant breast cancer recurrence in women with node negative or node positive, ER-positive, HER2-negative invasive breast cancer.

These assays are designed to support treatment planning and can lead to a more personalized treatment approach.

Plenary Lecture:

[PL4] MOLECULAR TOOLS FOR THE IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF MUTATIONS IN NEUROGENETIC DISEASES

Octavian Henegariu

M.D./Ph.D., Associate Research Scientist; Department of Neurosurgery; Yale University School of Medicine; 1 Gilbert Street TAC S310; New Haven, CT 06510

Using modern molecular technologies, our laboratory is identifying and functionally characterizing novel mutations in genes implicated in either neurogenetic malformations, such as microcephaly, or in a variety of intracranial tumors, such as meningiomas and gliomas. We use blood collected from patients and family members to prepare DNA for exome or whole genome sequencing. In case of tumors, we use tissue sections to isolate DNA, RNA (for gene expression) or proteins (for Western blot) or simply to stain with various antibodies, in order to validate some of the genetic findings. We routinely perform whole genome genotyping, gene expression profiling and whole genome DNA methylation (usually Illumina platform). Our statisticians are analyzing and integrating the information from various platforms to search for mutations and, in case of tumors, for copy number variation, gene amplification, splice defects, gene methylation, etc. We use animal models and in vivo assays to validate the respective genes and their mutations. Drosophila melanogaster (fruit fly) is inexpensive to grow and maintain and fly lines with targeted deletion of every desired fly gene already exist. For example, one of our candidate microcephaly gene, when knocked down resulted in more brain reduction in fly compared to known genes, such as WDR62 or ASPM. In a tumor model, combining EGFR activation with PTEN mutation resulted in massive abnormal growth of the fly brain, when compared to normal controls. We are also using zebrafish (Danio rerio) to apply the recently described CRISPR/Cas9 technology to target novel candidate genes that are thought to induce microcephaly, or vascular changes such as aneurisms. In addition, genes of interest are cloned in expression vectors using the Gateway technology and the various found mutations are introduces via site-directed mutagenesis. The wild-type and mutant candidate genes are transfected into cell lines to investigate the role of the mutation(s) in cell division, migration, invasion or in signaling pathways.

[P57] **LIVER FIBROSIS: FROM EXPERIMENTAL MODELS TO PROTECTIVE STRATEGIES**

Simona Clichici, Diana Olteanu, Marina Dudea, Andras - Laszlo Nagy, Adriana Filip, Petru A. Mircea

Department of Physiology, UMF "Iuliu Hatieganu", Clinicilor st., no.1, Cluj district, Zip code 400023, Cluj-Napoca, Romania,

Liver fibrosis, a common condition occurring during the evolution of almost all chronic liver diseases, is the consequence of hepatocyte injury that leads to the activation of Kupffer cells and hepatic stellate cells (HSC). Reactive oxygen species (ROS) and some cytokines, especially transforming growth factor β1 (TGF-β1), are among the most potent activators of HSC. There are a lot of experimental models to induce liver fibrosis and we performed two of them: CCl_4-induced fibrosis and bile duct ligation (BDL). In those experimental models different protective strategies were assessed for their potential to inhibit the initiation and the progression of liver fibrosis: Silymarin (Si), an herbal product, Chitosan (CS) a polysaccharide obtained from chitin, Rosuvastatin (Ro). To quantify liver injury and protective effects in early and late phases of fibrosis we assessed: hepato-cytolysis (aminotransferases and LDH), oxidative stress, fibrosis (histological score, hyaluronic acid), TGF-β1, markers of HSC activation (α – SMA expression by western blot) and activation of Kupffer cells by immunohistochemistry. We also assessed nuclear factor kB (NF-kB and pNF-kB) and metalloproteinase (MMP) by western blot and cytocheratin 19 and proliferating cell nuclear antigen (PCNA) by immunohistochemistry.

Our data showed the protective effects of Silymarin, in different doses, in early and late phases of liver fibrosis, the protective effects of chitosan in early stages after BDL and the contradictory effects of Ro in early stages of cholestasis.

[P58] THE CYTOTOXIC EFFECT OF AN EGFRTYROSINE KINASE INHIBITOR ON LOW-PASSAGE HUMAN BRAIN TUMOUR CULTURES IN VIVO

Oana Stefana Purcaru[1], Ada Maria Georgescu[1], Oana Neamtu[1], Daniela Elise Tache[1], Ligia Gabriela Tataranu[2], Vasile Ciubotaru[2] and Anica Dricu[1]

[1]*UMF Craiova, Biochemistry Unit.*
[2]*Bagdasar-Arseni Emergency Hospital Bucharest, Neurosurgery Department*

Using cancer cell lines as tumour model has been controversial; some cell lines provided to be very successful, whereas others were completely irrelevant. Many scientists believe that failure of cell line models to mirror clinical setting is the prolonged cell culture, which may induce genetic changes. Drug failures in the clinic may also be due to the fact that preclinical models do not represent the heterogeneity that is observed in human tumours.Compared to established cell lines, low passage cell lines were reported to better preserve features of cancer. At low passage, cancer cell lines are a mixture of several cell populations and should better mimic the tumour heterogeneity *in vivo*. For this reason, they are supposed to have better value as tumor models. In this study, we used a low passage primary brain tumour cell line derived from glioblastomatumours, to analyze the effect of AG556 (aEGFR inhibitor) alone or in combination with Temozolmide (TMZ) (a common drug for brain cancer). Both AG556 (1, 5 and 10μM) and TMZ (1 and5μM) treatment, induced significant cytotoxic effect on glioblastoma cells in a dose- and time-dependent manner. Dual treatment with AG556 and TMZ resulted in synergistic cytotoxicity at a frequency of 93%, when compared to single treatment.

[P59] AN *IN OVO* STUDY OF ZINC EFFECT ON CADMIUM-INDUCED CHANGES IN THE ACTIVITY OF CHOSEN LYSOSOMAL HYDROLASES

Dżugan M.[1], Hęclik J.[1], Lis M. W.[2], Droba M.[1]

[1]Department of Chemistry and Food Toxicology, Faculty of Biology and Agriculture, University of Rzeszów, 2 Ćwiklińskiej St., 35-601 Rzeszów, Poland
[2]Department of Poultry and Fur Animal Breeding and Animal Hygiene, University of Agriculture in Cracow, 21 Mickiewicz Avenue, Cracow, Poland

The aim of the study was to examine if cytotoxic effect of cadmium can be reduced by simultaneous zinc administration using *in ovo* model. Because cadmium at cellular level can provoke lysosomal membrane destabilization chosen lysosomal hydrolases were used as biomarkers. Chicken eggs (n= 40 eggs per group, on 4th day of incubation) were injected by 50 μL of 0.7% NaCl solution containing cadmium ions (0 or 50 nmol per egg) and/or zinc ions (100 or 500 nmol per egg). The Cd and Zn distributions in tissues (liver and kidneys) of 1-day old chicks were examined by ICP-OES method. The activities of lysosomal hydrolases: N-acetyl-β-D-glucosaminidase (NAG) and β-D-mannosidase (β-MAN), and arylsulfatase (ARYL) in chicks tissues were tested spectrophotometrically.

The hatchability in experimental group treated *in ovo* by combination of 50 nmol Cd and 500 nmol Zn per egg reached 61% and was comparable to the control group (61.9%), while the hatchability of remaining groups was from 30 to 50% lower ($P \leq 0.05$). Cd accumulation in liver and kidney of chicks exposed to this metal increased significantly in both tissue to 0.07 μg/g, whereas in the presence of higher zinc dose was reduced to 0.04 and 0.05 μg/g, respectively. Moreover the increase in activity of lysosomal hydrolases induced by treatment of separately Cd and Zn was abolished by simultaneous administration of both ions ($P>0.05$). The most pronounced increase for NAG, as the most active studied enzyme, was observed by 5, 18 and 23% in blood plasma, liver and kidney, respectively.

The cadmium disturbing effect on lysosomal enzymes activity was demonstrated. This negative action was completely recovered in the presence of high zinc dose (in 10-fold higher molar concentration). It was concluded, that adequate zinc supplementation can protect organisms against cadmium cytotoxicity.

Acknowledgement: This work was supported by the research project NN 304 291 140.

[P60] COMPARISON OF ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF FRUITS AND LEAVES OF BLACKBERRY (*RUBUS PLICATUS*) AND RASPBERRY (*RUBUS IDAEUS*)

Dorota Grabek-Lejko[1], Katarzyna Wojtowicz[1]

[1]*University of Rzeszow, Faculty of Biology and Agriculture, Department of Biotechnology and Microbiology, Zelwerowicza 4, 35-601, Rzeszow, Poland.*

Two plants – *Rubusplicatus* and *Rubusidaeus* were screened for antibacterial activity against *Staphylococcus aureus* strains, methicillin susceptible (MSSA) and methicillin resistant (MRSA), two from world collection culture and two isolated from patients. All tested extracts from fruits and leaves exhibited antibacterial activity, both against MRSA and MSSA strains. Stronger antibacterial activity had leaves extracts than fruits extracts. The strongest bacterial inhibition was observed against strains from collection culture, lower against strains directly isolated from patients' wounds. Leaf extract from *R. idaeus*exhibited stronger antibacterial activities than extracts from *R. plicatus* leaves. Antioxidant activities and phenolic content were also tested. Generally leaves of two tested plants had about 4-fold higher concentration of phenolics than fruit extracts. Leaves and fruits extracts from *R. plicatus* exhibited 2.3-fold higher concentration of phenolic compounds than *R. idaeus*. Also antioxidant activities of *R.plicatus* extracts were higher than in *R. idaeus* extracts. The present investigation expresses that plants have great potential as antimicrobial compounds against microorganisms, especially against methicillin resistant strains. Moreover these plants are a good source of antioxidants, especially leaves and fruits of *R. plicatus*, which can be used as a good exogenous sources of antioxidants in our diet.

References:

Deighton, N., Brennan, r., Finn, Ch., Davies, H.V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. *Journal of the Science Food and Agriculture,* 80, 1307-1313.

Otter, J.A., French, G.L. 2010. Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Europe. The *Lancet Infectious Diseases*, 10(4), 227–239.

Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis,G.R. 2007. Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and Cornelian cherries. *Food Chemistry,* 102(3), 777-783.

[P61] ANALYSIS OF THE PROPERTIES OF CIDERS PRODUCED BY NATURAL METHOD

Kluz M.[1], Grabek – Lejko D.[1], Dżugan M.[2],Sybirnyj W.[2] , Pasternakiwicz A.[2], Wesołowska M.[3], Hanus P.[3]

[1]Department of Biotechnology and Microbiology,[2]Department of Chemistry and Food Toxicology,
[3] Students Scientific Group of Food Technologist "FERMENT",University of Rzeszow, Faculty of Biology and Agriculture, M. Ćwiklińskiej Str., 35-601 Rzeszow, Poland

Abstract

The aim of this work was to asses antioxidant activity, level of sugar and alcohol and to assess the consumer ciders produced naturally. For the production of apple cider fermented grape used for wine yeast strains of noble breed *Sauternes* and *Sherry*. Measurement of sugar content was determined using the refractometric method. Determination of ethanol is made using a chemical method of reducing effects of alcohol in relation to potassium dichromate in the highly acidic environment. Excess of no reduced dichromate was determined by indirect titration iodimetry.

Evaluation of antioxidant activity using by fluorescence method was made using a PHOTOCHEM. The measurement was carried out using reagents prepared by the producer. The PHOTOCHEM gives the value of antioxidant per nmol of ascorbic acid.

The samples showed a similar antioxidant content in ciders, and differences between ciders with yeasts *Sherry* and *Sauternes* were minor and amounted to 0.128 - 0.143 nmol ascorbic acid. The level of alcohol in the cider test ranged from 3.38 to 4.66 %. When assessing the consumer received higher ratings ciders fermented with the participation of yeast *Saccharomyces cerevisiae* sp. *sherry* than *sp. sauternes*.Cider apples have high levels of phenolics - antioxidants linked to protection against stroke, heart disease and cancer. In addition, cider include of many minerals, vitamins and has a refreshing and alkalizing properties, which also confirmed at the conducted by our researchteam.

[P62] USABLE PARAMETERS OF BAKER'S YEAST CULTIVATED IN THE PRESENCE OF METAL IONS IN THE GROWTH MEDIUM

Anna Pasternakiewicz
Department of Food Chemistry and Toxicology, Faculty of Biology and Agriculture, University of Rzeszow, Cwiklinskiej 2 str Rzeszow, Poland

Baker's yeast belong to the species *Saccharomyces cerevisiae*, but not always are derived from a pure culture of one race. This may be a mixture of three or four races. Bread produced on yeast has a higher nutritional value than baked on sourdough because yeast contain easily digested protein, a few percent of fat, and most of all vitamins: B1, B2, B6, B12, PP, vitamin D and pantothenic acid, which decompose only partially in baking process.

Important technological traits of baker's yeast are: biomass yield, the protein content, and also fermentation activity and enzymatic activity. Many factors have an impact on these characteristics, among others the chemical composition of the culture medium and the metabolic state of cells, connected with the presence of toxic substances.

The aim of this study was to determine the usable parameters of yeast hybrid YT411x5p cultivated for 18 h in Biostat B fermentor, on the synthetic Rose medium (pH 4.5) supplemented with metal ions. After completion of the yeast production the biomass yield, protein content, fermentation activity and β-fructofuranosidase activity were determined.

Ionic composition of the culture medium has an effect on the technological value of the yeast. The biomass yield, β -fructofuranosidase activity and fermentation activity, only for yeast cultured on a substrate containing magnesium and cobalt ions was lower than in with the control, in all other cases the increase of tested parameters was observed. In all attempts it was found a relatively low crude protein content - 45.35% d.m. in the control sample and 46.38% d.m. for the sample with the addition of magnesium and cobalt, in other cases the amounts of protein was less than 30%.

References:
1. Blackwell K.J. Tobin J.M., Avery S.V. 1995. Metal cation uptake by yeast: a review. Appl. Microbiol. Biotechnol. 16: 633-688.
2. Brady D., Duncan J.R. 1994. Cation loss durind accumulation of heavy metal cayionsby *Saccharomyces cerevisiae*. Biotechnol. Lett. 16 (5): 543-548.

[P63] DEVELOPMENT OF AN ENZYMO-CHEMICAL METHOD FOR METHANOL DETERMINATION IN THE PRESENCE OF ETHANOL IN ALCOHOLIC BEVERAGES

Władimir A. Sibirnyj[1], Dorota Grabek-Lejko[1], Mykhailo V. Gonchar[2,3]

[1]*Department of Biotechnology and Microbiology, University of Rzeszow, Zelwerowicza, 4, Rzeszow 35-601, Poland;*
[2]*Institute of Applied Biotechnology and Basic Sciences, Sokolowska Str. 26, 36-100 Kolbuszowa, Poland;*
[3]*Department of Analytical Biotechnology, Institute of Cell Biology, NAS of Ukraine, Drahomanov Street, 14/16, Lviv 79005, Ukraine.*

Alcoholic beverages may contain the minimum allowable impurities of methanol; however, there are cases of poisoning including fatal when consuming beverages, acquired in the trade network. Therefore, it is important to define the content of methanol in alcoholic beverages. The aim of this study is to develop a new enzymo-chemical method for determining methanol in alcoholic beverages.

In the proposed approach, methanol is analyzed by monitoring formaldehyde (FA), produced in alcohol oxidase-catalyzed reaction, followed by formation of colored product in reaction of FA with Purpald with previous masking of FA in reaction with MBTH. To avoid an interference of excessive amounts of MBTH on methanol assay, the optimization of MBTH content was performed. It has been shown that decreasing MBTH concentration in the reaction mixture results in a better sensitivity of the assay, as well as in a wider linearity of the calibration curve. It was shown that MBTH concentration of 0.02 mg/ml is the optimal.

Using the developed method, methanol content was determined in a variety of strong alcoholic beverages produced in Poland, Ukraine, Scotland, and the USA: three cognacs, three whiskeys and 5 samples of vodka. It was shown that methanol concentration in the tested cognac samples ranges from 20.6 to 24.0 mM, in whiskeys samples - from 19.0 to 23.4 mM, and in tested samples of vodkas - from 22.6 to 26.0 mM. These results indicate that the content of methanol in the tested strong drinks (cognac, whiskey) and rectified (vodka) is in similar range.
To evaluate the practical significance of the developed enzymo-chemical method, gas chromatography measurements as a reference method will be performed.

Abbreviations: MBTH - 3-Methyl-2-benzothiazolinone hydrazone hydrochloride hydrate; Purpald (AHMT) - 4-Amino-3-hydrazino-5-mercapto-1,2,4-triazole.

Acknowledgement: This work was supported by the Cross-border Cooperation Programme Poland–Belarus–Ukraine 2007-2013 (the project IPBU.03.01.00-18-452/11-00 "Scientific integration of the Polish-Ukrainian borderland area in the field of monitoring and detoxification of harmful substances in environment") and by the Polish Ministry of Science and Higher Education funds for science in 2013-2015 for an international co-financed project (decision number 3020/PBU/0755/11/13/2014/2 - "Scientific integration of the Polish-Ukrainian borderland area").

AUTHOR INDEX