Bulgarian Academy of Sciences

SIXTH WORKSHOP

ON BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS

PROGRAMS AND ABSTRACTS
NOVEMBER 29-30, 2011
SOFIA, BULGARIA

THE SIXTH WORKSHOP

"BIOLOGICAL ACTIVITY OF METALS, SYNTHETIC COMPOUNDS AND NATURAL PRODUCTS"

IS ORGANIZED BY THE INSTITUTE OF EXPERIMENTAL MORPHOLOGY, PATHOLOGY AND ANTHROPOLOGY WITH MUSEUM (IEMPAM)

UNDER THE AUSPICES OF THE BULGARIAN ACADEMY OF SCIENCES

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- ***Institute of Experimental Morphology, Pathology and Anthropology with Museum, BAS***
- ***Organizers are grateful to the firm BIOLAB, specialized in laboratory equipment and scientific consumables, for its kind sponsorship of this Workshop***

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Chairpersons:

Assoc. Prof. Stefka Tepavitcharova, PhD
Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Lora Dyakova
Institute of Neurobiology, Bulgarian Academy of Sciences

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Session B. Metals and Parasitoses

Chairpersons:

Assoc. Prof. Marin Alexandrov, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Delka Salkova, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Ivelin Vladov
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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Delka Salkova, Elvira Arnaudova

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Chairpersons:

Assoc. Prof. Anna Tolekova, MD, PhD
Faculty of Medicine, Trakya Universiyt, Stara Zagora

Assist. Prof. Yordanka Gluhcheva, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Boyka Andonova-Lilova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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Tuesday, 29 November 2011

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Chairpersons:

Assoc. Prof Ivan Goshev, PhD
Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences

Assist. Prof. Albena Alexandrova, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Secretary: Janette Kojumdgian-Ivanova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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Chairpersons:

Assoc. Prof. Margarita Gabrashanska, DVM, PhD
Institute of Experimental Morphology, Pathology and Anthropology with museum, Bulgarian Academy of Sciences

Assoc. Prof. Reni Kalfin, PhD
Institute of Neurobiology, Bulgarian Academy of Sciences

Secretary: Tanya Zhivkova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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Wednesday, 30 November 2011

Session F. Are We in Danger?

Chairpersons:

Assoc. Prof. Mashenka Dimitrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assoc. Prof. George Miloshev, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

Secretary: Veselin Nanev, DVM
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

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Roumiana Todorova

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V. Ormandzhieva, E. Petrova, D. Kadiysky

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Emilia Petrova, Stella Dimitrova, Ralitza Todorova,Yordanka Gluhcheva, Ekaterina Pavlova, Boriana Eremieva, Vera Kolyovska, Denislava Deleva, Mashenka Dimitrova, Dimitar Kadiysky

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Y. Gluhcheva, Ju. Ivanova, I. Ivanov, V Atanasov, N. Antonova, M. Mitewa

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K. Kamenova, Ju. Ivanova, Y. Gluhcheva, S. Arpadjan, M. Mitewa

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Vadim Ermakov, Margarita Gabrashanska
Sergey Tyutikov, Veselin Nanev

Session G. The Treasure of Natural Products

Chairpersons:

Assoc. Prof. Reneta Toshkova, MD, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Assist. Prof. Milena Georgieva, PhD
Institute of Molecular Biology, Bulgarian Academy of Sciences

Secretary: Zina Ivanova
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

14.00 – 14.20

GO1. ARE CANNABINOID AND VANILOID RECEPTORS INVOLVED IN THE ANTIINFLAMATORY ACTION OF N/OFQ(1-13)NH_2 AND [ORN^9]N/OFQ(1-13)NH_2: IN VIVO EFFECTS ON CARRAGEENAN-INDUCED RAT-PAW INFLAMMATION?

Polina Mateeva, Petia Todorova

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Любов Георгиева Христова

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Lora Dyakova, Radostina Alexandrova, Reni Kalfin, Daniela-Cristina Culita, Gabriela Marinescu, Margarita Taushanova, Luminita Patron

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GO4. THE SECRETS OF BILE ACIDS

Lora Dyakova, Radostina Alexandrova, Reni Kalfin, Daniela-Cristina Culita, Gabriela Marinescu, Konstanta Timcheva, Luminita Patron

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Zhivka Ivanova, Andon Toshev, Petia Genova- Kalou

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Радослав Павлов и Ася Кожухарова

16.00 - 16.30 GENERAL DISCUSSIONS AND CLOSING REMARKS

Session A. Nanotechnology and New Materials – Opportunities and Challenges

Chairpersons:

Assoc. Prof. Stefka Tepavitcharova, PhD
Institute of General and Inorganic Chemistry, Bulgarian Academy of Sciences

Assoc. Prof. Radostina Alexandrova, PhD
Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences

Secretary: Lora Dyakova
Institute of Neurobiology, Bulgarian Academy of Sciences

AO1. *IN VITRO* AND *IN VIVO* BIOEFECTS IN THE PRESENCE OF NANOSTRUCTURED BIOPOLYMER (CHITOSAN)

Reneta Toshkova[1], Liliya Yossifova[1], Elena Gardeva[1], Marin Alexandrov[1], Milena Ignatova[2], Nevena Manolova[2], Iliya Rashkov[2]

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Targeted delivery of cytostatics and other drugs into the cancer outbreak is a way of enhancing the therapeutic effect and reduction of the systemic cytotoxicity. Elaboration of new methods and strategies to achieve a specific tumor targeting and destruction of tumor metastasis or delay of their development and overcoming of the drug resistance are major tasks of research in modern cancer therapy.

One such approach is the creation of polymer-based drug delivery systems for local application at the site affected by neoplastic alterations. Novel nanostructured materials based on quaternized chitosan (QCh) and the antitumor antibiotic Doxorubicin (DOX) have been developed by the process electrospinning. They were applied in the present study as nanofibrous mats and implants.

In *in vitro* conditions the antiproliferative activity of nanofibrous mats on human permanent tumor cell lines (HeLa, MCF-7) and on primary culture of *Graffi* tumor cells was studied. The results obtained showed a statistically significant reduction in tumor cell viability, cultured in the presence of two-compound and three-compound nanofibrous mats, which is time and dose dependant of the concentration of quaternized chitosan in the mats. It was found that the cytotoxic effect on tumor cells is through induction of apoptosis, visualized using double staining method with fluorochromes.

It was studied an anti-tumor effect of nanostructured biopolymers implants on the transplantable myeloid tumor in hamsters in *in vivo* experiments. The results showed that QCh

/ coPLA / DOX implants had significant antitumor efficiency combined with low systemic toxicity.

The present study demonstrated that hybrid nanostructured materials containing QCh-nanofibers are an appropriate system for the release of cytostatic drugs and are potentially new therapeutic tool for the local treatment of solid tumors.

Keywords: electrospinning, nanofibers, quaternized chitosan, antiproliferative activity, *Graffi* tumor

Acknowledgement: Financial support from the Bulgarian National Science Fund (Grant DO-02-164/2008) is gratefully acknowledged.

AO2. PLASMON-INDUCED PHOTOTHERMAL EFFECTS OF GOLD NANOPARTICLES ON EXPERIMENTAL MYELOID *GRAFFI* TUMOR IN HAMSTERS

Liliya Yossifova[1], Reneta Toshkova[1], Elena Gardeva[1], Marin Alexandrov[1], Nikolay Nedyalkov Nedyalkov[2], Petar Atanasov[2]

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Neoplastic diseases are of social and economic importance and are the second leading cause for morbidity and mortality in the world. Increasing the number of these conditions is due to a combination of different etiological factors. Therefore, their prevention and treatment are of the priorities of the modern medical science. Multiple negative side effects of traditional methods in the treatment of neoplastic diseases led to the need for new scientific approaches. Recent studies have emphasized on the development of drug delivery systems based on nanosized noble metal particles or metal hybrid structures. A relatively new direction in the treatment of tumor diseases is the co-application of electromagnetic irradiation with nanoparticles and nanostructured objects of noble metals.

The *in vitro* antitumour efficiency of combine nano-photo-thermolysis on a primary culture of the experimental solid tumor of *Graffi* in hamsters was studied. The most pronounced cytolytic effect was observed in the application of plasmonically activated gold nanoparticles (AuNPs) with a size of 40 and 100 nm, 3 pulses and F = 80 mJ/cm^2. Lower but statistically significant inhibitory effect was found in the application of the plasmonically activated AuNPs with a size of 100 nm, 2 pulses, F = 25 mJ/cm^2 and 10 pulses, F = 25 mJ/cm^2, respectively.

In *in vivo* experiments locally combined treatment on hamsters with well-formed subcutaneous tumors (1-1.5 cm in diameter) was applied. It was found that the therapy with nanoparticles (40 nm or 100 nm) and laser irradiation with the density of the laser beam 80mJ/cm^2 effectively suppressed the growth of *Graffi* tumor in hamsters, increased the average survival time of animals and reduced mortality rates of treated animals. Pathohistological studies clearly showed areas of nano-thermolysis of the tumor tissue.

The results obtained showed that application of plasmonically activated gold nanoparticles for the treatment of *Graffi* tumor in hamsters, both *in vitro* and *in vivo* have effective anti-tumor and cytotoxic effect and have a potential to be used for local treatment of small solid tumors.

Keywords: gold nanoparticles, plasmons, photothermal therapy, *Graffi* tumor.

Acknowledgement: The authors acknowledge the financial support from National Science Found under the contract DO 02-293/2008.

AO3. POLY(ALKYLCYANOACRYLATE) NANOSTRUCTURES FOR MEDICAL PURPOSES

Nikolay Marinov, Margarita Simeonova

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Nanotechnology is emerging as a promising area for research in which scientists are making great efforts for its development. One of the direct applications of nanotechnology in medicine as a good example is nanoparticles as drug carriers. Among the suitable nanoparticles used as drug carriers are these based on biodegradable poly (alkylcyanoacrylates) (PACA). PACA nanoparticles with different features can be obtained: nanospheres (matrix-type nanoparticles) and nanocapsules (reservoir-type nanoparticles, either oil- or water-containing) as well as long-circulating and ligand-decorated nanoparticles. Polymerization in heterogeneous media [1] (i.e.,emulsion, dispersion, miniemulsion, microemulsion) is a technique that can be used to obtain polymeric particles, including PACA nanoparticles as colloidal drug carriers for *in vivo* applications. The drugs are loaded to PACA nanoparticles either by incorporation during the polymerization process or by adsorption onto the surface of the preformed particles. PACA nanoparticles actually fulfill all important requirements for the ideal drug delivery systems: easy to obtain and storage in sterile form, good medicinal capacity, low toxicity, excellent biodegradability (the predominant mechanism occurs via the hydrolysis of their side chain ester chains[2], producing alkyl alcohol and poly(cyanoacrylic acid) as metabolites and feasibility of large production. PACA nanoparticles have shown significant results in several pathologies such as cancer, severe infections (viral, bacterial, parasitic) and certain metabolic and autoimmune diseases described in literature [3].

In recent years, the importance of the polymer nanofibers intensively grown, focusing on synthesis methods, properties and applications of nanofibers. These applications include: biomedical applications, nanofiber reinforced composites, membrane and sensor applications, use in electronic devices etc. Currently there are two main methods for obtaining of polymer nanofibers: template-based methods and electrospinning. Recently, a new technique for growth of poly(ethyl-2-cyanoacrylate) (PECA) nanofibers without a template by vapor phase polymerization of the ethyl-2-cyanoacrylate (ECA) monomer on a substrate has been shown [4]. ECA undergoes anionic polymerization initiated by different covalent or ionic bases, including water, applied to the substrate. Vapor phase polymerization of ECA carried out under high relative humidity resulted in different polymer morphologies depending on the type of initiator.

References

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4. Mankidy P, Rajagopalan R, Foley H. Influence of initiators on the growth of poly(ethyl 2-cyanoacrylate) nanofibers. *Polymer* 2008, 49:2235-2242.

AO4. TOXICITY OF METAL NANOPARTICLES AGAINST GRAM-NEGATIVE BACTERIA

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Abstract:
The knowledge about noble metals showed, that different types of silver and gold nanoparticles have antimicrobial activity. Silver nanoparticles are the most used in practice as antimicrobial agent, so understanding of their properties and their effect on microorganisms is main task for investigators. In this study we present the effects of silver and gold nanoparticles upon Gram-negative bacteria *Escherichia coli* and *Pseudomonas putida* (non pathogenic stains). Nanoparticles were produced by the "green" method using citrate as reducing agent. Our experiments showed that silver nanoparticles have strong inhibition effect against tested bacteria in different concentrations and gold nanoparticles didn't show effect in the same concentrations.

AO5. HIGH TEMPERATURE Mg- AND Zn-MODIFIED CALCIUM PHOSPHATES

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Ion modified calcium phosphate-based ceramics and cements have been developed to simulate the composition of the mineral component of bone tissues and to strengthen some specific biologically important behaviors, thus to be prospective materials for bone reconstruction and remodeling. Mg and Zn are preferable among all the substitutes in the biological apatites as they are essential for the organisms. Thus, the biologically active Mg

plays an important role in the formation and initial growth of the bone tissue, while Zn is an important element in the normal growth and development of the skeletal system

Powders of magnesium-modified as well as zinc-modified calcium phosphates (Me-β-TCP and HA) with a $(Ca^{2+}+Mg^{2+}+Zn^{2+}+Na^{+}+K^{+})/P$ ratio of 1.3 – 1.4 and various $Me^{2+}/(Me^{2+}+Ca^{2+})$ ratios (from 0.005 to 0.16) were prepared in biomimetic electrolyte systems at pH 8, mother liquid maturation and further syntering at 600-1000°C. Some differences in zinc and magnesium modifications have been prognosed on the base of thermodynamic modeling of the studied systems and explained by the Mg^{2+} and Zn^{2+} ion chemical behaviour.

The temperature and as well as the degree of Zn^{2+} and Mg^{2+} ions substitutions were found to stabilize the β-TCP structure and this effect was more prononced for zinc. Thus, zinc-modified β-TCP powders consisting of idiomorphic crystals were obtained through sintering of Zn^{2+} ion substituted calcium phosphates precursors at 800-1000°C. The Mg^{2+} ion substitution leads to obtaining magnesium-modified β-TCP with spherical grains.

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AO6. EQUILIBRIUM AND NON-EQUILIBRIUM CRYSTALLIZATION AND CHARACTERIZATION OF THE COMPOUNDS $Gly.MeSO_4.mH_2O$ ($Me = Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}$; m = 0, 3, 5, 6)

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The biological activity of some glycine–metal (II) chloride compounds as $2Gly.ZnCl_2.2H_2O$ [1] and $2Gly.MnCl_2.2H_2O$ [2] attracts the interest to this group compounds as they serve to the organism mutual amino-acid and trace elements, both of them very important for different living processes. Some of these group compounds are interesting also from a physical point of view as they possess ferroelectric (triglycine sulphate, TGS and selenate TGSe, $Gly.AgNO_3$, $nGly.MeX_2.2H_2O$ (Me = Mn, Co; X = Cl, Br; n = 1,2), pyroelectric and optic (TGS and TGSe) or other physical properties. The variations in inorganic ligands lead to changes in the chemical types and in the properties of these compounds but the knowledge is not completed. Any relations concerning synthesis conditions, composition, crystal-chemistry, phase stability, physical behaviour etc. are not available.

Some glycine–metal (II) sulphate compounds, e.g. $Gly.MnSO_4$, $Gly.MgSO_4.5H_2O$, $Gly.FeSO_4.5H_2O$, $Gly.NiSO_4.xH_2O$ (x = 3,5,6), $2Gly.CoSO_4$, $2Gly.ZnSO_4$ and $Gly.ZnSO_4.3H_2O$, have been identified but the data are limited and the results are often contradictory.

The purpose of this study is to elucidate the crystallization processes in the Gly – $MeSO_4$ – H_2O systems, where Me = Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} and to characterize the

crystallizing glycine – metal (II) sulphate compounds by the means of chemical, X ray, IR, Raman spectroscopy and DTA methods.

Equilibrium and non-equilibrium crystallisation of complex compounds was found to occur in the systems Gly – $MeSO_4$ – H_2O (Me = Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}) due to the big variety of chemical species in the solution. Equilibrium crystallization of only one type complex compounds $Gly.MeSO_4.5H_2O$ with a structure derivative from the structure of also crystallizing stable $MeSO_4.xH_2O$, where x=6,7 has been predicted and proved in these systems. The octahedral complexes $[Me^{2+}(H_2O)_6]$ and $[Me^{2+}(H_2O)_4(Gly)_2]$, as well as $[SO_4]$ tetrahedra have been predicted to be predominating and stable in these solutions and proved as main building units of the crystallizing $Gly.MeSO_4.5H_2O$ compounds (triclinic crystal system, space group P^-1). In the case of manganese system with a crystallization of $MnSO_4.H_2O$ (35°C) the only complex compound is the anhydrous $Gly.MnSO_4$.

Earlier announced compounds $Gly.NiSO_4.mH_2O$ (m = 3,6) and $Gly.ZnSO_4.3H_2O$ as well as the new $Gly.CoSO_4.3H_2O$ were explained to be a result of a non-equilibrium crystallization in these solutions with a high viscosity. $Gly.CoSO_4.3H_2O$ was proved to consist of two types of octahedra, $[Co^{2+}(H_2O)_4(Gly)^{(O)}{}_{2/2}]$ and $[Co^{2+}(H_2O)_2(SO_4)^{(O)}{}_2(Gly)^{(O)}{}_{2/2}]$, forming a chain structure (monoclinic crystal system, space group $P2_1/c$).

Vibrational spectra of the $Gly.MeSO_4.5H_2O$ (Me = Mg^{2+}, Co^{2+}, Zn^{2+}) compounds were recorded, analyzed and assigned. The overall character of the spectra is in accord with refined crystal structures.

The thermal behaviour of the $Gly.MeSO_4.5H_2O$ (Me = Mg^{2+}, Co^{2+}, Zn^{2+}) compounds was also studied.

The compounds Gly.MeSO4.5H2O (Me = Co^{2+}, Ni^{2+}, Mn^{2+}) was found to possess paramagnetic properties.

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AO7. ION MODIFIED CALCIUM PHOSPHATES AS PROMISING NEW MATERIALS FOR BONE IMPLANTS

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Bones play an important role in our lives, supporting our bodies and enabling us to perform various motions. Bone disease is a serious health problem that directly impacts on the quality of life of sufferers. It has been predicted that the percentage of persons over 50 years of age affected by bone diseases will double by 2020. Bone and joint degenerative and inflammatory problems, bone fractures, low back pain, osteoporosis, scoliosis ant other musculoskeletal problems need to be solved by using permanent, temporary or biodegradable devices (Kokuko et al., 2003; Guehennec et al., 2004].

The ion-modified calcium phosphates mono- or bi-phase ceramics and cements possess some specific biologically important characteristics. Thus, Mg and Zn are preferable modifiers as they are essential trace elements for the organisms. The biological active Mg plays an important role in the initial formation and growth of the hard bone tissue and the Zn is mportant mediator for more than 200 enzymes (Alexandrova et al., 2002; Algrove, 2009). Mg and Zn modified tricalcium phosphate ceramics were found to exhibit lower solubility than the pure ones (Radin, Ducheyne, 1993, 1994) and hence reduce the resorption rate (Xue et al., 2008).

The aim of this study was to evaluate the effect of nine Mg- and Zn-Mg -modified calcium phosphates (Mg-CP, Zn-CP and Zn-Mg -CP) on viability and proliferation of cultured murine and human cells.

100 mg of each compound was mixed with 0.33 ml distilled water and placed on glass slide (5 cm^2) in Petri dish (10cm in diameter). After incubation for 30 min at room temperature 10 ml D-MEM medium containing 10% FCS and antibiotics (100 U/mL penicillin and 100 μg/mL streptomycin) was added to the petri dish and incubated for 4 h, 8h or 24 h at 37°C. Then the medium (so called TCP-medium) was filtered twice: with a paper filter (FILTRAK) and then a syringe filter (0.2 μm). This TCP medium was used in the biological experiments. Permanent cell lines Lep3 (human embryo cells) and BALB/c 3T3 (mouse fibroblasts) as well as cultures from bone marrow and bone explants from ICR mice were used as experimental models in the investigations. Various cytotoxicity assays (MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique), double staining with acridine orange and propidium iodide, single cell gel electrophoresis (Comet assay) at neutral pH and statistical analysis were performed for the evaluation of cell viability and proliferation.

The results obtained indicate that the tested materials are promising candidates for further in vivo experiments. Additional investigations are underway to clarify better the biocompatibility of the examined TCP materials as well as their osteoconductivity and osteoinductivity.

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AO8. MINERAL CONTENT OF SOFT AND HARD RAT TISSUES SURROUNDING BONE IMPLANTS

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The study evaluated mineral content in the soft and hard local tissue surrounding bone implants in rats. Bone defects were created in femur and tibia of rats. The new materials filled the defects were Zn-modified beta tricalcium phosphate and a mixture of beta tricalcium phosphate and hydroxyapatite. The rats were divided into two groups: group 1- rats with defects filled with Zn-modified beta tricalcium phosphate and group 2 – rats with defects filled with a mixture of beta tricalcium phosphate and hydroxyapatite. The mineral content was estimated 12 weeks post surgery. Hard and soft tissues surrounding implants were studies for the content of several elements (Ca, P, Mg, Fe, Zn, Cu, Co, Cr, Mo and Se) using atomic absorption spectrometry. The results showed that the mineral composition of hard and soft tissue was quantitative similar but a bit qualitative different. It was established higher Zn, Ca, P and Mn level in the soft tissues in rats from gr. 1 where the rest trace elements were in similar levels. The bone mineral composition was similar in rats from both groups. The mineral composition of tissues from tibia and femur were similar in the both groups. Therefore the new materials did not cause any adverse effects in the surrounding muscle and bone tissue as well as by means of histopathology only light foreign body reaction around the implants was found.
The data showed a different response of the trace metal content in the studied tissues in the rats with implants dependent on the applied implants. The similar chemical elemental composition in the muscle and bone tissue indicated good tissue tolerance. Applied new materials appear to be an appropriate material for further use because it has good biocompatibility in bone tissue.

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Session B. Metals and Parasitoses

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BO1. BLOOD MINERAL INDICES IN LAMBS EXPERIMENTALLY INFECTED WITH *HAEMONCHUS CONTORTUS*

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The blood sucking parasite *Haemonchus contortus* causes huge economic losses due to lowered meat and wool production, hides. It is found in the abomasum of the sheep and goats and is responsible for acute outbreaks with mortalities, particularly in young animals. A more recent, lot of studies investigated the effect of parasitic infection on mineral status in hosts.

In this study the aim was to establish serum mineral status in lamb experimentally infected with *Haemonchus contortus*. The levels of Ca, Na, Fe, Zn, Cu and Se were determined in blood of lambs infected and non-infected with *H. contortus*.

Ten lambs of 3-4 months of age were studied. They were allocated into 2 groups on body weight basis – control and infected with *H. contortus*. The second group was infected two times every two days with 1 800 *H. contortus* larvae (L3) per a lamb. The Baermann technique was used to extract the L_3 larval stages of *Haemonchus contortus* intestinal nematodes and counted under a dissecting microscope to determine the larval counts. Blood was obtained on days 58 post infection. Blood was collected using Vacutainer system.

The mean serum phosphorus level of infected lambs failed to show any consistent pattern of changes throughout the observation period. On the other hand, serum calcium was significantly decreased when compared to that of control animals. Mean serum iron of infected lambs was significantly ($P<0.05$) reduced when compared with the non-infected controls. Analysis of serum electrolytes indicated significant ($P<0.05$) decline of serum sodium.

The levels of Zn, Cu and Se were reduced in the plasma of infected lambs compared to non-infected ones.

Analysis of the mineral contents of the blood revealed reduction of iron, calcium and sodium concentrations. Serum phosphorus failed to show a fixed pattern of changes. The reduction of serum iron level in infected lambs could be attributed to the expanded erythropoiesis to compensate for blood loss leading to depression of iron stores. The reduction of antioxidant trace elements may be explained by the antioxidant imbalance developed in the lambs due to the infection with *H. contortus*.

BO2. THE ROLE OF SOME HEAVY METALS IN THE HOST-PARASITE SYSTEM (*M. HIRUDINACEUS*-PIGS)

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There is increasing interest in environmental parasitology and bioconcentration of heavy metals within intestinal parasites of fish and mammals, especially acanthocephalans. Studies of fish parasites suggest that acanthocephalans are the most suitable animals for assessing heavy metal contamination in the aquatic environment.

In the literature there are some studies on the metal accumulation by the archianthocephalan *Macracanthorhynsus hirudinaceus* in naturally infected pigs.

The levels of most elements in the parasites were greater than the levels found in the porcine tissues – the acanthocephalans take up metals from the intestinal lumen of the host. Analysis by electrothermal atomic absorption spectrometry showed that *M. hirudinaceus* contained 85, 85, 56 and 24 times higher lead levels compared with hosts muscle, liver, kidney and intestine respectively. The mean cadmium concentration of the parasite was 32 times higher compared with porcine kidney. Acanthocephalans from mammalian hosts accumulate lead and cadmium and some other elements to levels far in excess of the host tissues. Thus *M. hirudinaceus* might be used as an accumulation indicator for heavy metals in terrestrial biotopes especially as it is a very abundant parasite of domestic and wild pigs throughout the world.

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Session C. Diabetes on Target

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CO1. DIABETES ON TARGET

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The lifestyle changes characteristic to the second half of the 20 century, have evoked diabetes epidemic which drastically impairs the quality of life and is the underling cause of many demises. The number of patients with diabetes mellitus (DM) worldwide is estimated to increase to 300 million by 2025 from 150-220 million in 2010. DM is mainly classified as either insulin-dependent Type 1 or non-insulin-dependent Type 2, according to the definition of WHO [1,2].

Type 1 diabetes (T1D) is an autoimmune disease caused by a combination of various genetic and environmental factors and characterized by destruction of pancreatic β-cells resulting in absolute deficiency of intrinsic insulin secretion. The patients require exogenous insulin injections several times a day. Insulin remains the only treatment available for type 1 diabetes [2,3].

In type 2 diabetes (T2D), characterized by insulin resistance and abnormal insulin secretion, the patients need exercise, diet control and/or several types of hypoglycemics (Sakurai et al., 2010). In the majority of patients with T2D, oral antidiabetic drug (OAD) treatment is the first line treatment after lifestyle measures fail. Two major groups of OAD are used in clinical practice-insulin secretagogues and insulin sensitisers [4].

Several types of insulin preparations and synthetic drugs for T1D and T2D, respectively, have been developed and are in clinical use. However, there are several problems concerning the insulin preparations and synthetic drugs, such as physical and mental pain due to daily insulin injections and defects involving side effects, respectively [1]. Consequently, a

new class of therapeutic agents is anticipated. The aim of the present study is to present some new strategies for diabetes treatment such as metal compounds, neuropeptides, monoclonal antibodies and stem cells.

To clarify better the pathophisiology of diabetes and to improve the current treatment protocols continue to be among the major challenges of medical and biological science. Some of the presented new strategies by themselves or by way of opening up new research frontiers may hold the key to the 'cure' of diabetes that millions of people across the world are hoping for.

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CO2. NEW INSULIN FORMULATIONS AND TREATMENT STRATEGIES

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New strategies for insulin delivery by routes other than intravenous and subcutaneous injection have been investigated since the discovery of insulin in the 1920s. The first successful testing of inhaled insulin occurred in the mid-1990s. The lung has proven to be an organ well capable of absorbing insulin in a reproducible and dose-dependent manner. When Exubera (the first USA FDA-approved inhaled insulin product) was introduced to the market some years ago it was hoped that this would be the first in a series of novel insulin formulations applied by this route. In addition, it was hoped that inhaled insulin would pave the way for other alternative routes of insulin administration, i.e. oral insulin, nasal insulin or transdermal insulin which are only some of the different attempts that have been studied in the last 90 years. The failure of Exubera, i.e. its withdrawal from the market due to insufficient market success, was followed by the cessation of nearly all other attempts to develop inhaled insulin formulations. MannKind Corporation has developed a powder formulation of insulin that allows for a high percentage of the administered insulin to be absorbed via the lung. Their product, AFREZZA (Technosphere insulin), is currently under review by the FDA for use in patients with diabetes. Technosphere insulin appears to overcome some of the barriers that contributed to the market withdrawal of Exubera by the manufacturer. It is very rapid acting, has a relatively short duration of action, and is efficacious in terms of improved glycemic control without contributing to increased weight gain or the incidence of hypoglycemia when compared with other prandial insulin products. Technosphere insulin has demonstrated a favorable safety and tolerability profile in clinical studies to date. The device to administer the insulin is well

designed, small, and easy to use. Technosphere inhaled insulin may provide a useful treatment option for patients resistant to or fearful of initiating prandial insulin injections.

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CO3. STREPTOZOTOCIN - TOXICITY OUTSIDE OF PANCREATIC BETA-CELLS

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Streptozotocin (STZ) is glucosamine-nitrosourea compound, originally identified in the middle of the past century as an antibiotic. Despite its broad spectrum of antibacterial properties, it was found that STZ has strong selectivity and toxicity to pancreatic beta cells. This suggested the drug's use as an animal model of diabetes, and as a medical treatment for cancers of the beta cells. STZ enters the pancreatic beta-cells through GLUT-2 transporter and causes alkylation of DNA, production of reactive oxygen species and non-specific activation of the immune system. This leads to necrosis of the cells. The side effects of STZ are the development of functionating insulinoma, kidney and liver tumors.

For nearly 50 years there are thousand publications from scientists, used STZ for animal models of diabetes. Unfortunately, most of the authors do not share their problems with the mortality of experimental animals during the induction of the diabetes. They only have mentioned some of STZ disadvantages and the subsequent diabetic complications. In addition to well-known action of STZ, in our experiments with Wistar rats, the application of 80 mg/kg STZ caused unexpected effects, including death of animals. Probably the reason for the high mortality of the experimental animals is a general impairment of the body function with a development of acute pancreatitis. This is supported by the observed damages in other organs with GLUT-2 transporters such as liver, kidney and brain. Epithelial cells from pancreatic ducts also have this transport mechanism. Moreover, there is evidence that high glucose levels inhibit fluid and HCO^-_3 pancreatic secretion, as well as enzyme secretion, resulting in both acinar and ductal function impairment. These two statements suggest a rapid damage of the whole pancreas, which is partly confirmed by the pathological findings from STZ - treated rats. It is also interesting that females are less susceptible than males to STZ-induced hyperglycemia, and females exhibit higher resistance to the STZ toxicity.

In conclusion, except its high selectivity to pancreatic beta cells, STZ used in recommended doses for induction of diabetes, has a significant toxic action. It may cause damages to the whole pancreas and other parts of the gastrointestinal tract.

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CO4. SILYMARIN HELPS TO CONTROL BLOOD SUGAR LEVELS IN DIABETES TYPE 2

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Silymarin is a well known hepatoprotector, extracted from seeds of *Silybum marianum* (milk thistle). Silymarin complex consists of four flavonolignan isomers silybin, isosilybin, dehydrosilybin, silychristin, silydianin and a few flavonoids. The most active component of silymarin is flavolingnan silybin (silibinin). Silymarin has strong antioxidant action.

Diabetes type 2 is associated with higher oxidative stress in different organs and tissues. Persistent hyperglycemia may cause high production of free radicals and reactive oxygen species (ROS) which cause DNA damage, protein oxidation and peroxidation of membrane lipids. Silymarin effectively neutralizes superoxides and hence limits lipoperoxidation and cell damage. By limiting lipoperoxidation silymarin maintains lower levels of mean, fasting and daily blood glucose.

In recent years a number of researches show that treatment with silymarin improves the control of blood sugar levels, HbA1c, cholesterol and plasma triglycerides in patients with diabetes type 2. Diabetes mellitus in insulin resistant patients with cirrhosis leads to progressive impairment in insulin secretion together with development of hepatic insulin resistance. Silymarine is effective in reducing insulin resistance in diabetic patients. Silymarin has also neuroprotective effect. Silibinin, a compound of silymarin protects DNA and reduces oxidative stress in brain specific area in mice with diabetes type 2.

Silymarin decreases significantly serum alanin aminotranferase and aspartat aminotransferase and restores liver function in patients with liver diseases and diabetes type 2 along with the control ofblood sugar, HbA1c, cholesterol and plasma triglycerides.

Silymarin prevents developing of nonalcoholic fatty liver disease (NAFLD). NAFLD occurs in up to 75% of patients with diabetes type 2. Silymarin as adjunctive therapy provides effective control of blood sugar levels in diabetes type 2 and delays its complications.

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Session D. In the World of Antioxidants

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DO1. THIOLS - ANTIOXIDANTS OR PROOXIDANTS?

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Thiol administration has been shown to have great potential in a variety of pathological conditions, associated with oxidative stress (1,2). However some investigations showed that certain thiols are able to act as prooxidants producing reactive oxygen species (ROS) at least in vitro (3,4). In the present study the free radical scavenging effects (against superoxide anion (O_2^-) and hydroxyl radical ($^{\cdot}OH$), generated in vitro) of captopril (as well-known sulfhydryl containing substance with indications for possessing an antioxidant effect (2)) were compared with those of cysteamine, mercaptoethanol, dithioeritrol (DTE), and dithiotreitol (DTT).

The O_2^- and $^{\cdot}OH$ were generated in vitro. Deoxyribose (DR) was used as a detector of $^{\cdot}OH$ radicals. The degradation of DR was measured in terms of the formation of thiobarbituric acid reactive substances, which were quantified spectrophotometrically. Superoxide anion radicals were generated photochemically and O_2^--produced nitro-blue tetrazolium (NBT) reduction was measured.

In the present work two distinct $^{\cdot}OH$ generating systems were used – with and without diethylene triamine pentaacetic acid (DTPA) in reaction mixture. The DR test showed that in the absence of the chelator DTPA cysteamine was much more potent inhibitor of the formation of thiobarbituric acid reactive substances (TBARs) than captopril and mercaptoethanol, whereas DTT and DTE in concentrations higher than 0.8 mM enhanced the DR damage. In the presence of DTPA captopril and mercaptoethanol decreased the TBARs formation in presence of H_2O_2 better than cysteamine; the effect of DTT and DTE was weak. Under these conditions the inhibitory effect increased as follows: DTE < DTT < cysteamine < captoprile < mercaptoethanol.

Captopril in concentration of 9.34 mM and cysteamine in concentration of 1.21 mM inhibited the O_2^- -provoked NBT reduction by 50%. Mercaptoethanol up to 10 mM did not manifest inhibitory effect. DTT and DTE enhanced the O_2^- production.

Our results indicate that captopril and mercaptoethanol are potent free radical scavengers, reacting rapidly with $^{\cdot}OH$, whereas cysteamine act preferentially as a chelator of iron and in this way prevent the formation of $^{\cdot}OH$. Both DTT and DTE generate ROS, probably because of presence of trace metals in the reaction mixture, provoking the occurrence of Fenton reaction.

In conclusion, although the presence of a sulfhydryl group suggests antioxidant properties, some thiol-containing compounds can exhibit prooxidant activity under certain conditions.

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DO2. BIOLOGICAL ACTIVITY OF GLUTATHIONE

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Glutathione is a linear tripeptide of L-glutamine, L-cysteine, and glycine. Glutathione is the most abundant non-protein thiol in the cell, often found in the millimolar range (1 to 10 mM, depending on cell type). The liver is the largest Glutathione reservoir in animals. This tripeptide has a gamma linkage between the first two amino acids (instead of the typical alpha linkage), which resists degradation by intracellular peptidases.

Glutathione is consumed in many enzymatic and non-enzymatic reactions, where it serves as a source of reducing equivalents. The sulfhydryl (thiol) group of cysteine serves as a proton donor and is responsible for the biological activity of glutathione. It is an antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. In this process, glutathione is converted to its oxidized form glutathione disulfide (GSSG):

$$2GSH + H_2O_2 \rightarrow GS–SG + 2H_2O$$

The most dangerous among all reactive oxygen species is hydroxyl radical and it arises as a product of the reaction between Fe^{2+} and H_2O_2. This reaction is called Fenton reaction. Fenton rection takes place in the cell when reduced glutathione is depleted.

$$Fe^{2+} + H_2O_2 = Fe^{3+} + HO. + .OH$$

The most notable initiators of lipid peroxidation in living cells are reactive oxygen species, such as $OH\cdot$. Lipid peroxidation is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. In addition, end-products of lipid peroxidation may be mutagenic and carcinogenic. For instance, the end-product malondialdehyde reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts to them, primarily M1G.

Once oxidized, glutathione can be reduced back by glutathione reductase, using NADPH as an electron donor.

$$GS–SG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity. A dynamic balance is maintained between GSH synthesis, it's

recycling from GSSG/oxidized Glutathione, and its utilization. Reduced glutathione is necessary to detoxify peroxides.

There is growing evidence that oxidative stress significantly impairs sperm functions. Due to their high content of polyunsaturated fatty acids spermatozoa are susceptible to damage induced by reactive oxygen species. The glutathione content decreased about 75% during sperm maturation from caput epididymis spermatozoa to ejaculated spermatozoa. The reduction in the glutathione concentration during spermatogenesis especially rendered late spermatids susceptible to mutagenesis by free radicals or other compounds. The high sensitivity of late spermatids to chemically induced DNA damage, dominant-lethal mutations, and oxidative stress was demonstrated in rats and hamsters.

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DO3. BIOLOGICAL ACTIVITY OF NOCICEPTINE AND ITS NEW-SINTETIZED STRUCTURAL ANALOGUES

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The role of nociceptin (N/OFQ) and its receptor (NOP1) in central and peripheral nerve system where they modulate nociception is well known but the data about their toxic and pro/antioxidant status are scanty. That was the reason which determinate the aim of this work – to investigate 1) nociceptine and it's new – synthesized structural analogues in norm and pathology, 2) their effects on cell's pro/antioxidant status, 3) to classify their antioxidant capacity. The structural analogues of nociceptine used in the present work differ in amino acid lysine on position 9, which is substituted respectively with ornitine [Orn^9]N /OFQ (1–13)NH_2, diaminopropanoic acid [Dap^9]N/OFQ(1–13)NH_2, diaminobutanoic acid [Dab^9]N/OFQ(1–13)NH_2 and canavanine [Cav^9]N/OFQ(1–13)NH_2. Lysine is important for materializing the bond between nociceptine and its receptor.

The experiments were conducted in vivo, in vitro and in chemical systems generated reactive oxygen species (ROS). The tested peptides (0.2 mkg/0.5 mkl) were injected intracerebroventricularly, as well as the kainic acid (KA) (0.25 mkg/ in 0,5 mkl). KA induces ROS-provoked neurodegeneration and is widely used as proper method for examination of different substances. In the present study were determinate the lipid peroxidation (LP), total glutathion, and the activities of the following enzymes: superoxide-dismutase, glutathion reductase, glutathion peroxidase, and glucose-6-phosphate dehydrogenase – all of them in presence and absence of KA. Hydroxyl radicals were generated in Fenton system. Superoxide anion radicals were generated photochemically.

The obtained results showed that:

1. In chemical systems, generated superoxide and hydroxide radicals, only [Cav^9]N/OFQ(1–13)NH_2 had significant powerful antioxidant capacity in concentrations of 100mM and 1mM. The inhibitory effects in both ROS generating systems increased as follows: [Dab^9]N/OFQ(1–13)NH_2 < [Dap^9]N/OFQ(1–13)NH_2 < N/OFQ(1–13)NH_2 < [Orn^9]N /OFQ (1–13)NH_2 < [Cav^9]N/OFQ(1–13)NH_2.
2. The in vitro experiments showed that the peptides had different activity. [Dab^9]N/OFQ(1–13)NH_2 and [Dap^9]N/OFQ(1–13)NH_2 tested alone lead to cell's damage (increased LP and decreased GSH); cytoprotective effect was obtained only in presence of N/OFQ and [Orn^9]N /OFQ (1–13)NH_2. In presence of KA, again N/OFQ and [Orn^9]N /OFQ (1–13)NH_2 showed weak beneficial effect in comparison with [Dab^9]N/OFQ(1–13)NH_2 and [Dap^9]N/OFQ(1–13)NH_2 in regard to LP-level. All peptides did not change the other tested parameters.
3. In vivo effects showed that N/OFQ and [Orn^9]N /OFQ (1–13)NH_2 alone did not change the parameters of LP, only [Dab^9]N/OFQ(1–13)NH_2 and [Dap^9]N/OFQ(1–13)NH_2 led to increase, but insignificantly in the levels of LP. The other parameters were unchanged. 1h and 24h after KA injection the levels of LP were increased in presence of all peptides compared with controls, but compared with KA-group after 1h Dab, Dap and Cav-analogues decreased slow LP. 24h after only Cav-analogue decreases LP.

Elucidation of the characteristics of this analogues will be interested and significant not only for their pharmacological properties, but in respect of their pro/antioxidant capacity. The summary of the results might be supplemented to the data of nociceptine and its structural analogues and to serve for base of estimation of their further use in laboratory and clinical practice.

DO4. ANTICANCER AND ANTIOXIDANT CAPACITY OF OCTAPEPTIDE SOMATOSTATIN ANALOGS

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Somatostatin (SST), a cyclic tetradecapeptide secreted from the hypothalamus and peripheral tissues, is a potent inhibitor of endocrine and exocrine tissue secretion. It is an important regulator of cell differentiation and proliferation in many types normal and tumor cells. The action of SST is mediated through a family of transmembrane domain G-protein-coupled receptors. The distribution of SSTRs (somatostatin receptors) ranges widely throughout not only the organs in the human body, but also wide range of tumor cells.

In a previous investigation a set of synthesized 16 SST-analogs have been tested for their anticancer activity. The cytotoxicity of the substances was measured *in vitro* by means of MTS – 3-(4,5-dimethylthiazol-2-yl)-5-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium inner salt – test, using the following cultivated human tumor cell lines: HepG2 (human

hepatocellular carcinoma), HT-29 (human colorectal cell line), HeLa (human cervical cancer cells) and Lep-3 (non-tumor human diploid cell line as a control).

The antioxidant properties were measured according two methods – HORAC (hydroxyl radical averting capacity) and ORAC (oxygen radical antioxidant capacity) using fluorescein-Na_2 as a target molecule. The following peptides were used:

1C = D-Phe-Cys-Phe-D-Trp-Dab-Tle-Cys-Thr-NH_2

2C = D-Phe-Cys-Phe-D-Trp-Dap-Tle-Cys-Thr-NH_2

3C= D-Phe-Cys-Phe-D-Trp-Lys-Tle-Cys-Thr-NH_2

PA1= D-Phe-Cys-Phe-D-Trp-Orn-Tle-Cys-Thr-NH_2

PA3= D-Phe-Cys-Phe-D-Trp-Lys-Thr-Cys-Thr-NH_2

The estimated values ranged within: 460 (PA1) – 590 (1C) μM GAE/g (HORAC) and 49 (2C) – 141 (1C) μM Tr.eq./g (ORAC). It is evident that the expressed activities are high. On the other hand, the tested substances are not polyphenols – typical antioxidants, where HAT (hydrogen atom transfer) mechanism usually takes place. In other words, the regulating activity of SST and its fragments towards tumor cells is related to their ROS-scavenging ability. The mechanism elucidation needs further investigations.

Session E. Searching for New Therapeutic Agents

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EO1. SAFETY PHARMACOLOGY: NEW CHALLENGES FOR TESTING THE BIOLOGICAL ACTIVITY OF NEW DRUGS

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Safety pharmacology is those studies that investigate the potential undesirable pharmacodynamic effects of a chemical substance on major physiological functions in

relationship to exposure in the therapeutic range and above. It is a natural bridge between experimental and clinical pharmacology and toxicology to achieve the main goal of modern pharmacotherapy – to have more effective and safer drugs.

This review will mainly focus on some recent advances in drug discovery and development which speed up with less cost the process of toxicology testing. Some modern approaches to achieve this goal:

1. New achievements in implementing of so called **3Rs principles** for decreased numbers of experimental animals which leads to transition in toxicology toward a more pathway-based *in vitro* and computational approach (covered by FP6/7 project consortium AXLR8).

- ***Replace:*** through successful development of new more informative testing methods such as tissue engineering, cell cultures and stem cell technologies, computer modeling (*in silico*). Further testing of substances applied to the skin in practice will not require the use of experimental animals /cosmetics for example from 2013/.
- ***Reduce:*** re-examining the findings of studies already conducted (e.g. by systematic reviews), by improving animal models, and by use of good experimental design.
- ***Refine:*** this includes better housing, and improvements to procedures which minimize pain and suffering and / or improve animal welfare. This is particularly important for testing of substances with potential psychotropic activity.

2. Alternatives: *In vivo* models may, with advances in methods and technologies, be replaced by in vitro techniques, which do not involve live animals, but still require animals to be bred and killed for scientific purposes. In foreseeable future we can use more transgenic species, isogenic strains, new animal models, or other novel test systems and could include a toxicogenomic evaluation of tissue responses over wide dose ranges.

3. RECENT DEVELOPMENTS (few examples):

- From 2008 **ACUTE TOXICITY** studies are not needed prior to first clinical trials in humans. Instead, information can be obtained from other studies, which are performed at more relevant doses for humans and are already an integral part of drug development.
- New testing methods for better in vitro / in vivo extrapolation were needed to meet increasingly develop **protein based therapies** that specifically target human cells. Bad example is CD28-SuperMAB, a humanized monoclonal antibody develop as a strong agonist for the CD28 receptor of the immune system's T cells for treatment of B cell chronic lymphocytic leukemia (B-CLL) and rheumatoid arthritis. Six volunteers were hospitalized in 2006 suffering from multiple organ dysfunction, despite being administered at a supposed sub-clinical dose of 0.1 mg per kg; some 500 times lower than the dose found safe in animals.
- New tests for **PYROGENIC** contaminants rely on cultured human white blood cells and might replace two existing, more expensive methods - the Limulus assay and testing on rabbits.
- Cultures of human cord blood and mouse bone marrow cells were develop to detect **LOW WHITE BLOOD CELL COUNTS** — a common side effect of cancer drugs.
- **Targeted synthesis** of more effective and safer new drugs with **predictable metabolism** will be discussed which are based on some achievements of pharmacogenomics and are directed to development of personal pharmacotherapy.

EO2. SYNTHESIS OF CHALCONES WITH ANTIBACTERIAL ACTIVITY

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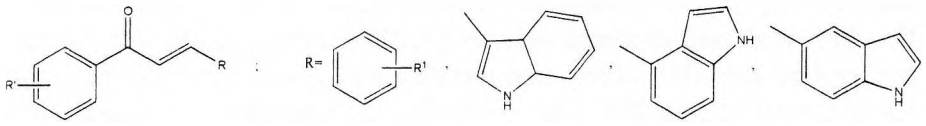

Chalcones (1, 3-diaryl-2-propen-1-ones) are a group of flavonoids widely distributed in plant kingdom. They exhibit a wide range of biological activities such as antioxidant, anti-inflammatory, cytotoxic, anticancer, anti-infective activities, etc. Meanwhile, chalcones do not cause toxic and mutagenic effects on the living cells.[1] For these reasons, chalcones as potencial lead compounds for drug development.
The aim of this research was synthesis of a series of chalcones and their indolyl analogs with antibacterial activity. Their structure was modeled based on the SAR study results for chalcones with proven activity against the Gram-positive bacterium *Staphylococcus aureus*.[2,3]

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EO3. NOVEL 2-STYRYL-8-HYDROXYQUINOLINES (8SQS) DERIVATIVEs WITH ANTI HIV-1 ACTIVITY IN CELL CULTURE

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Earlier it has been reported about anti-HIV-1 integrase activity of styryl-quinolines (Mekouar K. Et al.,1998). In Bulgaria six novel 2-styryl-8-hydroxyquinolines (8SQs) derivatives were synthesized and evaluated for anti-HIV-activity in cell culture. The aim of this work was to target anti-HIV-1 activity of novel substances. Supernatants of chronically infected H9/HTLVIIIB were used as a source of HIV-1. Cytotoxicity tests and microtiter infection assays in MT4 cells were performed (MTT uptake assay). Reverse transcriptase (RT) activity

in supernatants and directly on recombinant RT (Cavidi, Sweden) were checked. Protease as a target was studied by modified screening method using direct spectrophotometric reading of specific substrate utilization by native HIV-1 protease in absence and presence of the substances studied. To check the integrase as a target, mutants were obtained by serial passaging of virus with continuous exposure to increasing concentrations of active compounds followed by sequencing of *in* region. Mitochondrial toxicity was evaluated by RealTime-PCR as mtDNA:nDNA ratio. Two novel 8SQs: 105B and 241, differing in substitutes at C2 in the phenol ring, demonstrated inhibition of HIV replication (105B>95% and 241~70%). 105B showed mitochondrial toxicity accompanied by reducing of both mtDNA and nDNA (mtDNA:nDNA=0,82 compared to 1,01 and 0,97 in MT4 uninfected and HIV-1 infected cells without inhibitor resp.). 241 showed no mitochondrial toxicity. Both derivatives exposed no RT inhibition but weak anti-protease activity (105B – 21% and 241 – 25% resp.). As far as the latter did not explain anti-HIV effect in microtiter assay, we looked for mutations in IN gene in passages 30-32 for 105B and 241. The molecular sequencing found mutations in 105B (N17S, D231I) and in 241 (E10D, D231I), all in *in* region. Evidence demonstrated that 105B and 241 target viral integrase.

EO4. SYNTHESIS AND IN VITRO ANTITUMOUR ACTIVITY OF NOVEL METAL COMPLEXES OF 5-AMINO-1, 10-PHENANTHROLINE AND 1, 10-PHENANTHROLINE

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Aim of the present study is to obtain new coordination compound of alkaline earth metal (Sr) with a rarely used ligand 5-amino-1, 10-phenanthroline (5-NH_2-phen) through a self-assembly process. Via competitive reactions between heavy (Sn and Pb) and alkaline earth (Ca) metals, novel complexes of 1, 10-phenanthroline (phen) and 5-NH_2-phen will be synthesized. For evaluation of the compound's citotoxicity MTS-test will be used.

A metal complex of Sr with 5-NH_2-phen having a composition Sr(5-NH_2-phen)$_4$(NO_3)(OH)(H_2O)$_2$ (**1**) was synthesized via static self-assembly process. This is possible due to weak interactions between organic ligand and metal having s^2-electron configuration. To obtain other three complexes: Sn(phen)(NO_3)(OH)(H_2O) (**2**), Sn(5-NH_2-phen)(OH)(Cl)(H_2O) (**3**) and Pb(5-NH_2-phen)(NO_3)$_2$(H_2O) (**4**) a competitive reactions between heavy metal cations ($Sn^{2+}$and Pb^{2+}), possessing p^2-electron configuration, and Ca^{2+} at the presence of phen or 5-NH_2-phen in a water-alcohol solution under mild conditions were conducted. The new coordination compounds were characterized by elemental analysis, FTIR-spectroscopy and FAB-mass spectrometry. Their cytotoxicity was measured towards human tumour (MDA-MB-231, HT-29, HeLa, HepG2) and non-tumour diploid (Lep-3) cell lines.

As expected all substances showed different activities depending on cell line and amount of the compound tested. The best pronounced cytotoxic effect on all cancer lines showed **1** and **4** at their high amounts as well as **1** at its lower ones ($\leq$ 0.04 mg). Therefore,

strontium complex of 5-amino-o-phenanthroline (**1**) exhibited the widest antitumour activity spectrum, having no toxicity towards non-tumour cells at quantities $\leq 4.10^{-2}$ mg.

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EO5. NEWLY SYNTHESIZED 1, 2, 3-TRIAZOLE RUTHENIUM (II)-BASED COMPOUNDS: *IN VITRO* BIOLOGICAL INVESTIGATIONS AND CHALANGES

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Ruthenium (Ru) is a rare noble metal, unknown to living systems, with a strong complex forming ability with numerous ligands. It allows forming complexes that are useful for a wide variety of applications including industrial, pharmaceutical and medical. Ruthenium, unlike traditional platinum complexes, shows greater resistance to hydrolysis and more selective action on tumors which developed resistance to cisplatin or in which cisplatin is inactive. The most active of these compounds, namely NAMI-A and KP1019, has already completed a phase I clinical trial. 1,4-Disubstituted 1,2,3-triazoles are actively investigated in diverse areas of chemistry as ready-to-make linkers between two functional groups.

The aim of the present study was to evaluate comparatively: 1) The *in vitro* toxicity of four new Ru(II) complexes: two isomeric Ru-dmso compounds, *trans*,*cis*-[$RuCl_2$(dmso-S)$_2$(**PPT**)] (**1**) and *cis*,*cis*-[$RuCl_2$(dmso-S)$_2$(**PPT**)] (**2**), and two half-sandwich Ru-[9]aneS_3 coordination compounds, [Ru([9]aneS_3)(dmso-S)(**PPT**)][CF_3SO_3]$_2$ (**3**) and [Ru([9]aneS_3)Cl(**PPT**)][CF_3SO_3] (**4**). Cytotoxic and cytostatic effect of all tested compounds were compared to the effect of Cis-platine (commercially available anticancer drug); 2) The efficacy against the replication of two *wt* HSV-1 and HSV-2 strains and two resistant to acyclovir mutants with different TK mutations; 3) The anti-influenza activity against A/California/7/2009 (H1N1) and flu B. In all compounds **PPT** behaves as a chelating ligand through the triazole N2 and the pyridyl N. Three human cell lines were used in our experiments – larynx carcinoma cell line (HEp-2), alveolar squamous carcinoma epithelial cells (A-549) and human rhabdomyosarcoma cell line (RD 64) and one canine kidney epithelial cell line - MDCK. The cytotoxic effects of compounds **3 - 5** were evaluated after 48 and 72 h of exposure on all used cell lines. The tested compounds exhibited cytotoxic effects that enabled the

construction of concentration-response curves and calculation of the IC_{50} values. The results show that, in general:

1) The antiproliferative activity of the tested ruthenium compounds is lower than that of cisplatin and basically they are not cytotoxic against the specific cells. Often the activity of **PPT** or of the precursors is higher than that of the half-sandwich derivatives. Notable exception is compound **4** that, on the A-549 cell line shows an IC_{50} value almost one-order of magnitude lower than that of cisplatin. Conversely, **4** does not show any activity on HEp-2 cells, indicating a remarkable selectivity towards the cisplatin-resistant cell line. In contrast to chlorido-derivative **4**, the dmso-derivative **3** exhibits no activity towards both tested cell lines indicating that the leaving group, and as consequence the aquation rate, plays an important role on the activity of such complexes.

2) The most selective against *wt* HSV-1 replication was the compound **4**, and for HSV-2 – compound **2**. The activities of the above compounds were dependent of chemical structure and virus specificities. In conclusion – HSV resistance to ACV can be overcome using appropriate metal complex/s such as **4** and **2**.

3) We investigated the effectiveness of tested above compounds on the plaque formation of infected with flu A and B-MDCK cells. Compound **3** inhibits over 90% plaque formation at the concentration of 100μg/ml no matter the same time as viruses added or after viruses adsorption. It is interesting to know the detail mechanism. Chemical modification of this compound may give a precious information for drug discovery of anti-flu in the future.

EO6. THREE NEWLY SYNTHESIZED ZN (II) COMPLEXES DECREASE VIABILITY AND PROLIFERATION OF CULTURED HUMAN AND ANIMAL TUMOR CELLS

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It is well known that zinc is essential for all life forms and plays a vital role in human nutrition and biochemical functions. This element is an essential co-factor in variety of cellular processes including DNA synthesis, behavioral responses, reproduction, bone transformation, growth, and wound healing. Zinc is the structural component of a wide range of proteins, neuropeptides, hormone receptors and polynucleotides, the metal is required for the biological function of more than 200 enzymes. There is substantial evidence to support an important role of zinc in immune processes. This element is also present in the brain and contributes to its structure and function. Zinc deficiency can promote different disease states including cancer. It has also been found that some zinc compounds possess promising biological activities including anticancer, antimicrobial, antiviral, etc. [1, 2].

The aim of the study presented here was to evaluate the cytotoxic effects of 3 newly synthesized zinc (II) complexes: $[Zn(MorfBig)Cl_3]$ (1), $[Zn(Metf)_2Cl_2.3CH_3OH]$ (2), [Zn(MorphBig)(Metf)Cl]Cl (3), (where MorphBig = morpholine biguanide hydrochloride and Metf = metformin hydrochloride) [3,4].

The following cell lines were used as model systems in our experiments: LSCC-SF-Mc29 (chicken hepatoma induced by the myelocytomatosis virus Mc29) that express v-myc

oncogene and LSR-SF-SR (rat sarcoma induced by Rous sarcoma virus strain Schmidt Ruppin) that carry v-src oncogene; human HeLa (cervical carcinoma cells transformed by human papilomavirus) and MCF-7 (carcinoma of the breast). The investigations were performed by MTT test (which reflects damage to mitochondria), neutral red uptake cytotoxicity assay (indicates damage to lysosomes and Golgi apparatus), crystal violet staining (nuclear staining) and double staining with acridine orange and propidium iodide. The compounds examined were applied at concentrations of 5- 200 μg/ml for 24h, 48h and 72h.

The results obtained revealed that: 1) The examined complexes decreased in a time- and concentration-dependent manner the viability and proliferation of the treated cells;
2) According to their cytotoxic/antiproliferative properties the tested compounds are graded as follows: Zn-1 > Zn-3 > Zn-2 > Morph;
3) The complexes of MorphBig (Zn-1 and Zn-3) express higher cytotoxic/antiproliferative properties as compared to Zn-2 (the complex of Metf);
4) A positive correlation between the data obtained by the above-mentioned methods was observed
Additional experiments are underway to clarify the influence of the investigated Zn (II) complexes on viability and proliferation of nontumor cells.

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ЕО7. ЦИТОТОКСИЧЕН ЕФЕКТ НА ЦИНКОВИ И СРЕБЪРНИ ЙОНИ IN VITRO

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Днес са известни голям брой химични съединения с антитуморно действие, но повечето от тях показват редица нежелани странични ефекти. Това налага необходимостта от търсене на нови, високоефективни антинеопластични агенти с добра биологична поносимост. Специален интерес за учените в това отношение представляват металите и техните съединеия.
Ние изследвахме цитотоксичната активност на четири кристални вещества (модифицирани природни и синтетични зеолити), които имат способност да обменят със средата цинкови или сребърни йони в различни концентрации и на определени интервали от време: природен, модифициран клиноптилолит обменен на Ag+ йони (CPT-Ag); природен, модифициран клиноптилолит обменен на цинкови йони (CPT-Zn);

синтетичен Engelhart титано силикат-4 (ETS-4-ZN) обменен на цинкови йони; синтетичен STS титано-силикат-4 обменен на цинкови йони (STS-4-ZN). Като експериментални модели бяха използвани две човешки туморни (HeLa – карцином на шийката на матката; HepG2 – хепатом) и една нетуморна (3T3 миши фибробласти) клетъчни линии. Проучванията бяха проведени чрез теста за включване на неутрално червено.

При три от изпитваните вещества (ETS-4-ZN, CPT-AG, CPT-ZN) беше наблюдавано статистически достоверно понижаване на клетъчната преживяемост при някои от изпитваните концентрации. Клетките от линия HeLa показаха по-висока чувствителност в сравнение с хепатомните клетки от линия HepG-2.

Получените първоначални резултати за цитотоксичното действие на цинковите и сребърните йони върху култивирани в лабораторни условия човешки ракови клетки представляват интерес и проучванията в тази насока заслужава да бъдат продължени.

EO8. EFFECTS OF METAL (ZN, AU, AG) COMPOUNDS ON VIABILITY AND PROLIFERATION OF CULTURED HUMAN CERVICAL CARCINOMA CELLS

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The aim of our study was to evaluate the influence of three newly synthesized metal compounds (containing Zn, Zn/Au or Zn/Ag) on viability and proliferation of cultured human cervical carcinoma (HeLa) cells. The experiments were performed by MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, single gel electrophoresis (Comet assay) at neutral pH and double staining with acridine orange and propidium iodide. The compounds were applied at concentrations of 1-100 μg/ml for 24h, 48h and 72h. The results obtained revealed that the examined compounds decreased significantly viability and/or proliferation of the treated cells in a time- and concentration-dependent manner. Additional experiments are needed to clarify better their cellular targets and mechanism(s) of action.

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EO9. INHIBITORY EFFECT OF ORALLY ADMINISTERED AMMONIUM VANADATE ON CHEMICALLY INDUCED TRANSPLANTABLE HEPATOMA OF ZAJDELA

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The potential role of vanadium in cancer prevention and therapy and the exact mechanism(s) of its atineoplastic activity are not clarified yet. It has been found in our previous investigations that orally aministered ammonium vanadate (NH_4VO_3) reduces the growth of some experimental tumors in rats such as transplantable sarcoma induced by Rous sarcoma virus strain Schmidt Ruppin and ascites tumor of Guerin. The aim of the present study was to evaluate the influence this compound on the growth of transplantable hepatoma of Zajdela in rats. NH_4VO_3 was dissolved in the drinking water at a concentration of 0.5 ppm and given ad libitum. Four to five week old male Wistar rats were divided into four groups as follows: A: (Control) No implantation of tumor cells, no treatment with NH_4VO_3; B: No implantation of tumor cells, 70 days treatment with NH_4VO_3; Group C: Implantation of tumor cells, no treatment with NH_4VO_3; Group D: Treatment with NH_4VO_3 began 40 days before the implantation of tumor cells and continued until the end of the experiment (70 days).

Ascitic cells obtained from a transplantable hepatoma of Zajdela were implanted subcutaneously (3×10^6 alive cells/animal). All rats were sacrificed 30 days after the implantation of tumor cells. The results obtained revealed that the application of NH_4VO_3 in Group D reduced tumor weight by ~ 80% as compared to Group C. Severe tumour lysis was observed in tumors of the NH_4VO_3-treated animals. No significant differences between the groups were observed in parameters examined (body weight, haematological and biochemical indices).

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Session F. Are We in Danger?

Chairpersons:

Assoc. Prof. Mashenka Dimitrova, PhD
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FO1. PROTEIN DISORDER AND CANCER

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The changes at the DNA level lead to various diseases. Intrinsically disordered protein (IDPs) are involved in various diseases, especially in cancer. Cancer-associated mutations had a preference for ordered regions. The flexibility and plasticity of intrinsically disordered protein regions and the induced-fit changes in the structured regions are both important for binding diversity. Chromosomal translocations, which generate chimeric proteins by fusing segments of two distinct genes, represent the single major genetic aberration leading to cancer. The high level of intrinsic structural disorder enables fusion proteins to evade cellular surveillance mechanisms that eliminate misfolded proteins. The translocation-related human proteins are significantly enriched in disorder (43.3% against 20.7% in all human proteins). The vicinity of the breakpoint is significantly more disordered than the rest of these fusion proteins. The structural disorder is essential to the acquired oncogenic function. IDPs are overrepresented in major disease pathways and are desirable targets for inhibition. The targeting of proteins without defined structures is unclear. Small molecules have been found that bind to the disordered regions of c-Myc, Ab, EWS-Fli1, and various peptides, that are further optimized to increase specificity and affinity. The ability to inhibit the interactions of ID proteins is a potential in chemical biology and drug discovery. Targeted therapy for cancer, without any effects outside of controlling the tumor, is a gold standard for treatment. Ewing's sarcoma contains the potential target EWS-FLI1, as a result of a pathognomonic chromosomal translocation. EWS FLI1 functions by binding to normal cellular protein partners in transcription and splicing, similar to how a virus would corrupt normal cellular machinery for virion production. Interaction between the highly disordered N-terminus of EWS and hsRPB7 was found *in vitro*. The recombinant EWS-FLI1 was a critical reagent for screening a library of small molecules to identify compounds with direct binding.

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FO2. IN VIVO ASSESSMENT OF BREAKS WHICH SOME FOOD ADDITIVES CAUSE TO DNA IN THE CELL

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Damages in the DNA are very dangerous for the cells and hence for the whole organism. They can cause cellular death or malignant transformation. The single- and double-stranded DNA breaks are especially dangerous for the organisms. At a molecular level, these types of damages can lead to chromosomal breakage and recombination and further can induce cangerogenic process as a result of activation of oncogenes or inactivation of tumor-suppressor genes.

In this study we have tested several substances used in food industry and pharmacology. The additives were checked for provoking single- and double-strand DNA breaks. To that end we applied the alkaline and neutral variants of Yeast Comet Assay and standard Comet Assay on higher eukaryotic cells. Additionally, to investigate a possible checkpoint arrest of the cells caused by the tested compounds we performed flow cytometric analysis (FACS).

Our results confirmed the DNA damaging effect of the tested compounds. Furthermore, we established that the Yeast Comet Assay is more sensitive than the variants of standard Comet Assay. Finally, we could not detect significant delay or blocking effect in the cell cycle progressions caused by the tested compounds.

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FO3. EVALUATING THE LEVEL OF POLYMORPHISM IN THE GENOME OF *TARAXACUM OFFICINALE* IN RELATION TO HEAVY METAL POLLUTION

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Heavy metal contamination in the environment leads to genomic rearrangements. The establishment in detail of its impact on organism development is necessary for the purposes of monitoring. The aim of the current study is to examine mutation rates in the genome of plants during heavy metal stress adaptation.

Taraxacum officinale is a good object for this kind of research because of its widespread distribution and high tolerance to broad range of environmental conditions.
As a target for our investigation we chose the intergenic spacer of the ribosomal DNA locus. Coding the genes for ribosomal RNAs the activity of this region has to be one of the firsts reflecting the changes in cellular metabolism. The rDNA intergenic spacer is characterized by high variability in number and length of its repeated sequences, but also possesses some conservation in the regulatory elements. According to the data this region can be considered as evolutionary dynamic. PCR analysis of rDNA intergenic spacer showed different product patterns randomly distributed between probes from rural and polluted areas. After restriction of the PCR products we observed specific level of polymorphism according to the place of samples collection. Interestingly, we assessed lower genetic diversity in probes from contaminated sites than in probes taken from clean sites. These results direct the study to the interesting question about heavy metal pollution and its influence over population's genetic diversity.

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FO4. LITHIUM TOXICITY: BLOOD-BRAIN AND BLOOD-CEREBROSPINAL FLUID BARRIERS

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In this review, we describe the lithium and its toxicity accursed in patients with bipolar disorder receiving lithium therapy. Part of this review focuses on the effect of the metal-induced neurotoxicity on the blood-brain (BBB) and blood-cerebrospinal fluid (CSF) barriers (BCSFB), which are essential to brain chemical stability in the central nervous system (CNS). Normally, lithium is not present in significant amount in body fluid (<0.2 mEq/L). Lithium salts have been used therapeutically for almost 150 years, beginning with its use for the treatment of gout in the 1850s (1). However, the use of lithium became problematic and was discarded due to the serious toxicity associated with the widespread use of lithium in tonics, elixirs, and as a salt substitute. It is not known whether lithium has a physiological role in some organisms, but nutritional studies in mammals have indicated its importance to health, leading to suggestion that it can be classed as an essential trace element with an RDI of 1 mg/day. Observational studies in Japan, reported in 2011, suggested that naturally occurring lithium in drinking water may increase human lifespan (2). Strikingly, no study reported negative effects of lithium. It is the only treatment that has shown efficacy for acute mania and acute depression as well as prevention of recurrent mania and depression (3).

The chemical stability in the brain underlies normal human thinking, learning, and behavior. Psychiatrists define mood disorders as a group of mental disorders in which disturbance of mood is accompanied by either a full or partial manic or depressive syndrome that is not due to any other mental disorder. Bipolar disorder is one of the most common (3–5% worldwide), chronic, recurrent (90%), life-threatening psychiatric diseases (4). The mood disorders entail actual brain damage, which is typically manifested as a loss of gray matter and as reductions in the number and size of neurons and glia (nonneuronal brain cells) in certain regions of the brain. The traditional treatment for both elements of this disorder (mania and depression) has been with lithium, a metallic element found primarily in the alkaline waters of many mineral springs and in certain dry lakes.

The production by the choroid plexuses of the CSF, its circulation and resorption are unique characteristics of the CNS. In conjunction with the BBB, the BCSFB and the flow dynamic of this fluid are the main elements setting the cerebral availability of drugs. The exchanges between the blood and the CSF across the choroidal epithelium are tightly regulated, in the presence of interepithelial tight junctions, by various transports and metabolic processes (5). The BBB and the BCSFB are formed by brain endothelial cells and choroid plexus epithelial cells, respectively. Most blood vessels in plexus choroideus are wide-calibers capillaries (mean luminal diameter 9.16 μm) with thin fenestrated endothelial walls (6, 7).
As the integrity of BBB and blood-CSF barriers, both structurally and functionally, is essential to brain chemical stability, the role of the choroid plexus in metal-induced neurotoxicities has become an important, yet under-investigated research area in neurotoxicology. Our current knowledge on the toxicological aspect of choroid plexus research is still incomplete. Thus, the future investigations need to focus on the role of choroid plexus and brain vessels in early neurodegenerative diseases, and to better understand the BCSFB and BBB as a defense mechanism in overall CNS function.

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FO5. MORPHOLOGICAL CHANGES IN THE AGED RAT BRAIN PROVOKED BY ACUTE LITHIUM INTOXICATION

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Background: Lithium is widely used for both acute and prolonged treatment of bipolar disorders. However, in some cases of improper medication, acute intoxication may occur in patents connected with tremor, ataxia, dyskinesia, seizures and other temporary or persisting neurological symptoms. Morphological studies at autopsy have shown spongiform changes in different parts of the brain. Some of the noticed neurodegenerative alterations however, might be due to the normal brain aging. Animal models can be used for studying the neurotoxic effect of lithium salts. The aim of the present work is to follow up the morphological changes in the aged rat brains provoked by acute lithium intoxication.

Methods: Eighteen-month old Wistar rats were injected i.p. by a single not lethal dose of lithium chloride (250 mg/kg body weight). The treated animals were anesthetized and decapitated. Different parts of the brain – cerebral cortex, cerebellum, thalamus, medulla oblongata at the level of hypoglossal nerve, pons and mesencephalon were studied using the silver-copper staining for neurodegeneration. Healthy animals of the same age were used as controls.

Results: Vacuolization of the brain tissue was observed both in treated and non-treated animals. However, spongiform changes were more profound in lithium intoxicated rats. The number and volume of vacuoles were bigger in that case and they contained cell debris and glial cells. Whereas the non-treated animals had preserved neurons, the lithium treated animals showed lack of normal neuronal bodies. Formation of the zones of spongiosis in the brain of lithium treated rats was more profound in cerebral cortex, mesencephalon and pons. Less intensive vacuolization was registered in the other studied brain parts. Vacuolization in the cerebellum was restricted to the places of missing Purkinje cells.

Conclusion: Age-dependant brain tissue degeneration is strongly accelerated by acute lithium intoxication. The spongiform changes are irregularly distributed throughout different brain parts. The reversibility of the observed neurodegenerative process remains to be studied in the future animal model designs.

FO6. HEMATOLOGICAL AND HEMORHEOLOGICAL CHANGES IN CASE OF SUBACUTE CADMIUM INTOXICATION AND MONENSIN DETOXICATION

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Cadmium (Cd) is a toxic environmental pollutant released both from natural sources (leaching of cadmium rich soils) and anthropogenic activities (mining, smelting, electroplating, manufacturing of batteries and pigments that utilize cadmium) to the aquatic and terrestrial environments [2]. Food, drinking water and inhalation of smoke from tobacco products are the main sources of daily exposure to Cd thus oral administration seems to be the most appropriate in long-term experiment of cadmium as it enters the animal/human body through food and water [3]. The polyether ionophore antibiotic monensin was demonstrated to act as an antidote in case of chronic intoxication. According to Bhavsar et al. [1] monensin treatment of erythrocytes leads to cell membrane scrambling and cell swelling. The purpose of the present study was to evaluate the affect of Cd and monensin treatment on some hematological and rheological parameters. Adult male mice were subjected to subacute treatment with cadmium acetate [Cd m$(CH_3COO)_2x2H_2O$] (group 1), Cd$(CH_3COO)_2x2H_2O$ followed by treatment with low dose monensin (group 2) and Cd$(CH_3COO)_2x2H_2O$ followed by high dose monensin treatment (group 3). Cd $(CH_3COO)_2$ x $2H_2O$ and tetraethylammonium salt of monensic acid were dissolved in distilled water and given daily to the experimental animals. Mice drinking distilled water served as a control group (group 4). Hematological parameters and erythrocyte morphology were evaluated in parallel with whole blood viscosity (WBV). Cd treatment reduced RBC count. The addition of high dose monensin significantly improved erythrocytic indices compared to the control. WBV was significantly elevated in the Cd-treated experimental group in the whole range of shear rates compared to the control group. Addition of low dose monensin reduced WBV. The results suggest that hemorheological parameters such as WBV should be monitored in parallel with the hematological parameters when monensin is applied and heavy metal intoxication is suspected.

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FO7. INFLUENCE OF CADMIUM AND MONENSIN ON IRON HOMEOSTASIS AND LIVER FUNCTION IN MICE, SUBJECTED TO SUBACUTE CADMIUM INTOXICATION

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Cadmium (Cd) is a human cancerogen [1]. It is emitted in the environment in the productions of pigments, alloys, Cd-nickel batteries and fertilizers [2]. Recent studies have demonstated that not only people engaged in the industry could be exposed to Cd-intoxication. It has been estimated that Cd intake in unpolluted areas in Europe varies in interval 10-30 μg per day [3, 4]. The correlation between environmental exposure to Cd and diseases such as anemia, renal dysfunction, hepatotoxicity, cancer, osteotoxicity has been discussed in many studies in recent years [5, 6]. It has been reported that Cd replaces copper (Cu) and iron (Fe) from the cytoplasmic and membrane proteins. The generation of reactive oxygen species (ROS) via Fenton type reactions has been associated with Cd-induced toxicity [6].

Many compounds have been screened as chelators for treatment of Cd-intoxication [5, 6]. Most of them exibit numerous side effects, high affinity for essential metal ions, low absorbability from gastrointestinal tract. Up to now there is no chelating agent, suitable for treatment of human Cd-poisoning [6].

Recently it has been reported that polyether ionophourous antibiotic monensic acid decreases lead (Pb) concentrations in rats intoxicated with Pb(II) acetate and it is much more effective than the dimercaptosuccinic acid (DMSA, traditional chelator for treatment of heavy metal intoxications) [7, 8].

Herein we present experimental evidences that monensic acid. (applied as tetraethylammonium salt) ameliorates Cd-induced liver dysfunction in mice subjected to subacute Cd-intoxication. Possitive effect of antibiotic's salt on Cd-induced iron deficiency is also discussed.

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FO8. RESULTS AND CONSEQUENCES OF TOXIC METALS ON THE COGNITIVE ABILITIES AND BEHAVIOR OF A HUMAN BEING

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The human brain forms and develops over a long period of time compared to other organs, with neuron proliferation and migration continuing in the postnatal period. The blood-brain barrier is not fully developed until the middle of the first year of life. The incidence of neurotoxic or immune reactive conditions such as autism, schizophrenia, Tourette's, ADD, dyslexia, learning disabilities, SLI, etc. have been increasing rapidly in recent years. Some toxic or trace metals compromise normal brain development and neurotransmitter function, leading to long-term deficits in learning and social behavior. High blood lead, mercury, osmium, manganese, aluminum, arsenic, selenium, gallium, lithium, cadmium, bismuth or nickel levels have been found to be associated with many contemporary linguistics conditions such as: attention deficit hyperactivity disorder (ADHD), memory deficits, impulsivity, anger, learning ability, cognitive performance, aggression, inability to inhibit inappropriate responding, juvenile delinquency, and criminality. These metals have also been found to have significant effects on motor-visual ability and performance. In the recent years, chelating is the most effective component of treatment, showing significant improvement in most patients. Chelators such as DMSA are often used or spirulina or chlorella based products. This is supported by selenium, milk thistle, NAC, calcium-D-glucarate, Alpha-ketoglutarate, taurine, methionine, plant based enzymes, GC free diet, omega-3 EFAs, probiotics, vitamins A,C,E, beta carotene, B complex, magnesium, zinc and multiminerals. Also to improving cognitive function scientists use pycogenol, L-theanine for calming effect and CoQ10, L-carnatine, L-carnosine, and DMAE. Regardless of the above ways of treatment the most important measure from the linguistic theory point of view is to provide a theoretical framework for quantitative and precise assessment of language impairments. Better understanding of specific language impairments in different clinical populations is paramount in order to provide individually tailored effective treatment.

FO9. БИОАКУМУЛАЦИЯ НА ОЛОВО В БЪБРЕЦИ НА СЛАДКОВОДНИ РИБИ ОТ ПОРЕЧИЕТО НА Р. АРДА

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Резюме

Екологичните програми, включващи проследяването на биоакумулацията на различни антропогенни замърсители в органи на риби, дават възможност тези водни обитатели да се определят като биомаркер за замърсяване на водоемите.
Настоящото проучване проследява антропогенното въздействие с тежкия метал олово върху водните екосистеми от региона на р. Арда.

Чрез атомно-абсорбционни методи са проучени стойностите на оловото във водата и седимента и е установена биоакумулацията на метала в бъбреците на сладководните риби – червеноперка, уклейка и костур.

Отбелязани са повишени стойности на оловото в тъканните проби на изследваните риби, които надвишават ПДК, в сравнение с нормите в хранителни продукти. Сравнително най-висока концентрация на оловото се наблюдава при костур и уклейка, което може да се отдаде на индивидуалната резистентност на отделните видове риби. Допуска се включването на изследваните риби, като обекти на спортен риболов, но трябва да се изключва тяхното използване като хранителен компонент.

Abstract

The ecological programs that include the monitoring of bioaccumulation of various anthropogenic pollutants in the organs of fishes enable one to define those inhabitants of the water as a biomarker for the pollution of water basins.
The present research traces the anthropogenic impact with the heavy metal lead on the water ecosystems in the region of the Arda River.

Using atom-absorption methods the lead value in water and the sediment were investigated and the metal's bioaccumulation in it and also in the kidneys of freshwater fishes as common rudd, common bleak and European perch was established.

Increased values of lead in the tissue samples of investigated fishes were noted that were greater than the Threshold Limit Value, as compared to the norms in foodstuffs. The comparatively highest concentration of lead was shown in the European perch fish and common bleak fish, which could be due to individual resistance of these species. The investigated fishes can be allowed to be the object of sport fishing, but their use as a nutrition should to be excluded.

Key words: lead, freshwater fish, ecology

FP1. ЗДРАВНИ РИСКОВЕ ОТ ТЪКАННИТЕ ДЕПА НА ТЕЖКИ МЕТАЛИ, КОИТО СЕ НАТРУПВАТ В ОРГАНИЗМА НА ЖИВОТНИТЕ ЗА ХРАНИТЕЛНА КОНСУМАЦИЯ

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In different animal species tissue depots with prolonged storage of heavy metals, which may carry health risks, have been discovered.

Highly toxic metals such as strontium, cadmium, lead, antimony and others are close to the absorption of healthy elements such as calcium, zinc, iron and copper in the body and can be disposed for a long time, mainly in the liver, kidneys, lungs, skin, intestine, placenta (cadmium, lead), bones (cadmium, strontium).

The accumulation of heavy metals and metal protein in the red muscle of birds and fish is significantly higher than that in white muscle.

It has been found that several heavy metals are emitted significantly not only through the kidneys but also through milk.Significant landfills of heavy metals in birds are not only the liver and kidneys, but the gut and the egg yolk.

Heavy metal landfills carry a sanitary risk for bear liver, kidney, eggs, poultry skin, thick fat deposits around the intestine and thick subcutaneous fat used for fattening pigs. Such a risk for fodders is carried particularly by bones, bones from fish gills and skin from poultry.

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FP2. FUNCTIONAL DIFFERENTIATION OF METALS IN THE LOCAL BIOGEOCHEMICAL CYCLES

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The sampling of the basic biogeochemical molybdenum deposits of the Trans-Baikal and Caucasus regions and background areas was realized. Chemical analysis of different

samples is performed by means of AAS, ICP/AES and x-fluorescence with digestion and without preliminary mineralization of objects, including decomposition in a microwave, with use of the standard reference materials: hair CRM 397, plant mixture SBMT-02, soil SRM 2709. Distribution of copper in soils and increased accumulation of copper ore in the soil cover landscapes were discovered. In the laboratory experiment assessed the interaction of copper, molybdenum and tungsten on the accumulation of metals, bluegrass and the Starry and the level of sulfur-containing phytochelatins. A change of the activity of xanthine oxidase of milk, if introduced into the medium of microgram quantities of molybdate and sodium tungstate, copper sulphate was shown. However, the activity of xanthine oxidase of milk from different background regions of Russia and Bulgaria did not differ.

The degree of accumulation of metals by microorganisms from soil agar under aerobic conditions and deficiency of oxygen (in nitrogen) was different.

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Session G. The Treasure of Natural Products

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GO1. ARE CANNABINOID AND VANILOID RECEPTORS INVOLVED IN THE ANTIINFLAMATORY ACTION OF N/OFQ(1-13)NH_2 AND [ORN^9]N/OFQ(1-13)NH_2: IN VIVO EFFECTS ON CARRAGEENAN-INDUCED RAT-PAW INFLAMMATION?

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In the present study, we examined the mechanism of action, as well as the possible interactions of N/OFQ(1-13)NH_2 and its structural analogue [Orn^9]N/OFQ(1-13)NH_2 with cannabionoid CB_1-receptors on acute carrageenan (CG)-induced inflammation in rat paw. The study is also aiming to find out whether the TRPV1-receptors take part in these interactions. Our results showed that the simultaneous treatment of rats with CB_1-receptor agonist HU-210 and the investigated peptides did not change the specific effects of the tested substances. Applied after the blockade of CB_1-receptors, the peptides did not exert their anti-inflammatory effects. Moreover, we determined that, when the TRPV1-receptors were blocked, the antiinflammatory effects of NOP-receptor agonists as a whole remain unchanged.
In conclusion, based on the results obtained, it might be suggested that N/OFQ(1-13)NH_2 and [Orn^9]N/OFQ(1-13)NH_2 influenced the peripheral inflammation by interactions with their own NOP-receptors – located on the primary sensory neurons.

We suppose also that there is a functional link between NOP- and CB_1-receptors. It might be assumed that vacant or activated CB_1-receptors are required for NOP-evoked inhibition of acute peripheral inflammation.

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GO2. РОД *ARTEMISIA* И НЯКОЛКО НЕГОВИ ПРЕДСТАВИТЕЛИ, ХАРАКТЕРНИ ЗА БЪЛГАРСКАТА ФЛОРА - БИОЛОГИЧНО АКТИВНИ ВЕЩЕСТВА, ФАРМАКОЛОГИЧНИ СВОЙСТВА И ПРИЛОЖЕНИЕ

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Няколко класа вторични метаболити характеризират химичното съдържание на растенията от род *Artemisia*. Представени са почти всички класове химични вещества, характерни за живата природа, като особено голям е делът на терепеноидите и флавоноидите. В някои видове са открити восъчни конституенти, полиацетилени и в по-малка степен - азот-съдържащи молекули.

Биологичното и терапевтичното приложение на растенията от род *Artemisia* е резултат от популярна традиция и систематично провеждани химични и фармакологични изследвания. Установени са противоглистни, холеретични, спазмолитични, противовъзпалителни, антибиотични и антимикотични свойства, както и антитуморна и антималарийна активност на отделни вещества или етерично-маслени комплекси на различни видове пелин. Те намират широко приложение и в хранително-вкусовата промишленост като съставки на ликьори, като подправки и овкусители.

Род *Artemisia* е бил обект на множество химични и биологични изследвания тъй като синтезира основно сескитерпенови лактони, кумарини и ацетилени. Много от неговите видове синтезират голям брой еудезманолиди - група сескитерпенови лактони, които показват най-висока антибактериална и противовъзпалителна активност, докато инсектицидните свойства на много видове се дължат на α-туйон, абсинтин, анабсинтин и 1,8 - цинеол. Голямо внимание се отделя на многостранното изследване на *Artemisia annua* L. (едногодишен пелин) заради съдържанието на антималарийният агент артемизинин - кадинанов тип сескитерпенов лактон ендопероксид.

В България видовете *Artemisia chamaemelifolia* Vill., *Artemisia lerchiana* Weber, *Artemisia pedemontana* Balbis и *Artemisia eriantha* Ten. са под закрилата на Закона за биологичното разнообразие. Макар и малко, се намира информация за състава на екстракти при някои от тях, както и за фармакологичните им свойства.

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GO3. THE UNKNOWN FACE OF THE WELL KNOWN NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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Nonsteroidal anti-inflammatory drugs (NSAID) are among the most consumed drug over the world due to their well known analgesic, anti-inflammatory and antipyretic properties. The enzyme cyclooxygenase (COX1 and COX2) has been identified as the common molecular target of all these compounds. COX enzymes use the substrate arachidonic acid to produce prostaglandin (PG) H2, the precursor of all the prostanoids. Prostaglandins are produced in virtually all mammalian tissues and have diverse biologic activities, including vasoconstriction, vasodilatation, stimulation or inhibition of platelet aggregation, and immunomodulation, primary immunosuppression

Many reports have demonstrated that non-steroidal anti-inflammatory drugs suppress malignant transformation and tumor growth, and some NSAIDs are expected to be new anticancer agents. Until now, at least five mechanisms by which COX-2 contributes to tumorigenesis and the malignant phenotype of tumor cells have been identified, including: i) induction of apoptosis; ii) increased angiogenesis; iii) increased invasiveness; iv) modulation of inflammation/immunosuppression, and v) conversion of procarcinogens to carcinogens. For example, increased expression of COX–2 occurs in multiple cells within the tumor microenvironment that can impact on angiogenesis. COX–2 appears to:

a) play a key role in the release and activity of proangiogenic proteins;
b) result in the production of eicosandoid products TXA2, PG12, PGE2, that directly stimulate endothelial cell migration and angiogenesis in vivo, and
c) result in enhanced tumor cell, and possibly, vascular endothelial cell survival by upregulation of the antiapoptotic proteins Bcl–2 and/or activation of PI3K/Act

Multiple mechanisms independent of COX activity have also been proposed such as: activation of protein kinase G inhibition of NK-kappa B activation downregulation of the antiapoptotic protein Bcl-XL, inhibition of PPAR gamma.

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GO4. THE SECRETS OF BILE ACIDS

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Bile acids (BAs) are a group of molecular species of acidic steroids with peculiar chemical and biological characteristics. They are not only important for the absorption of dietary lipids and fat soluble vitamins but are signalling molecules with diverse endocrine and paracrine functions. Primary BAs (such as cholic and chenodeoxycholic) are directly synthesized from cholesterol by hepatocytes, by the addition of hydroxyl groups and the oxidation of its side chain. The secondary bile acids (such as deoxycholic, lithocholic, ursodeoxycholic) are generated in the intestine by bacterial biotransformation of primary BAs. Bile acids are not only important for the absorption of dietary lipids and fat soluble vitamins but are signalling molecules with diverse endocrine and paracrine functions. Their biological effects are mediated through different pathways which comprise the activation of nuclear hormone receptors, of intracellular kinases and of the plasma membrane-bound, G-protein coupled bile acid receptor TGR5/Gpbar-1.General physico-chemical defence based on the detergent action of bile acids protects the body against bacterial endotoxins and other agents (e.g. viruses) that possess lipoprotein or lipid structures.

In recent years steroidal structures have become increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic, supramolecular chemistry and also in nanotechnology. There are well known pharmacological applications of bile acids and their derivatives, including their use in the treatment of liver diseases, in dissolution of cholesterol gallstones, as well as their potential to act as carriers of liver specific drugs and cholesterol level lowering agents. At the same time, there are multiple epidemiologic data and scientific reports suggesting the role of bile acids in pathogenesis of human malignancies, especially those of the gastrointestinal tract. In contrast, other studies have shown the potential antineoplastic effect of bile acids and their derivatives in several cultured cancer cell lines and experimental models in vivo. Furthermore, it has been suggested that bile acid conjugates could be helpful in the development of new approaches for target anticancer therapy. Due to the high efficiency of hepatocytes to take up bile acids, these endogenous compounds or their analogues can be considered as possible carriers for delivering drugs to the liver.

GO5. MONENSIN AGAINST CANCER

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Monensin is a polyether ionophore antibiotic isolated from *Streptomyces cinnamonensis*. It has been used in veterinary medicine as coccidiostatic and antibacterial agent for 40 years [1]. Recently the interest to biological activity of Monensin has been increased by the data concerning its antitumor properties. It has been found that activation of the Na^+/H^+ antiporter and the resulting pH changes (intracellular alkalization) take place in the cell proliferation, differentiation and apoptosis. The compound prevents the maturation of transforming growth factor beta (TGF-beta) [16] that is well known to play a central role in the pathophysiology of several diseases especially tissue fibrosis and malignancies [2,3] Many reports demonstrate the antineoplastic potential of Monensin in cell lines established from some of the most common and aggressive human malignancies (leukemia, glioblastoma multifirme, cancers of the breast, lung, liver, uterine cervix, skin), some of which express MDR1, MRP1 or ABCG2 gene and are multidrug resistant [4-9].

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GO6. ROLE OF METAL IONS IN MAINTAINING THE STRUCTURE AND FUNCTIONS OF VIRUSES

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Metal ions are integral part of some viral proteins and are known to play an important role in their survival and pathogenesis. Several trace metals such as zinc, copper and manganese influence the susceptibility to, the course and the outcome of a variety of viral

infections. To move between different parts of their hosts, most plant viruses exploit the phloem. Plants exposed to subtoxic levels of cadmium ions can resist this viral invasion of their transportation network. Zinc, copper and magnesium are the commonest metals that bind with viral proteins. They participate in maturation of genomic RNA, activation and catalytic mechanisms, reverse transcription, initial integration process and protection of newly synthesized DNA, inhibition of proton translocation, minus- and plus-strand transfer, enhance nucleic acid annealing, activation of transcription, integration of viral DNA into specific sites and act as a chaperone of nucleic acid. Metals are required for nucleocapsid protein-transactivation response RNA interactions. Deficiencies of these metals alter the genome of the viruses and the consequence of this may be the emergence of new infection. It is still not clear why metal ions bind to viral nucleic acids indirectly via a water molecule whereas in tends to bind to proteins directly. Further studies are necessary to reveal how a viral protein selects a specific metal ion from a mixture of ions in the surrounding fluids and why they choose mostly zinc. It is supposed that this selectivity is due to the natural abundance of the metal, its properties as stereochemical and charge or properties of the protein as amino acid residues that form the metal-binding pocket. Recently scientists have developed a method to genetically engineer viruses to bind specific metals.