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VI NATIONAL CONGRESS OF PHARMACOLOGY

POSTER PRESENTATIONS

COORDINATION COMPOUNDS OF CU(II), CO(II), NI(II), ZN(II) AND LA(III) CONTAINING BILE ACIDS ANIONS AS LIGANDS AND THEIR INFLUENCE ON TUMOUR CELL VIABILITY AND PROLIFERATION

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In recent years steroidal structures have become increasingly important in a number of fields such as pharmacology, medicinal chemistry, biomimetic, and also in nanotechnology. There are well known pharmacological applications of bile acids and their derivatives, including use in dissolution of cholesterol gallstones, in the treatment of liver diseases, as well as their potential to act as carriers of liver specific drugs and cholesterol level lowering agents. Furthermore, it has been suggested that bile acid conjugates could be helpful in the development of new approaches for targeted anticancer therapy. The main goal of this work was to study the coordination compounds of Co(II), Cu(II), Zn(II) Ni(II) and La(III) with the anions of cholic (dedyrocholic, lithocholic, deoxycholic, hiodeoxycholic, ursodeoxycholic) acids. In order to understand the coordination manner, all the complexes have been characterized by elemental analysis, IR, UV/VIS and EPR spectroscopy, as well as magnetic measurements. The influence on cell viability and proliferation was examined by MTT test, neutral red uptake cytotoxicity assay, trypan blue dye exclusion technique, colony-forming method and autoradiography on cultured human tumor cell lines 8 MG BA (glioblastoma multiforme), MCF-7 (breast adenocarcinoma), HepG2 (hepatoma), Hep-2 (carcinoma of the larynx), HeLa (cervical carcinoma) and K562 (erythroleukemia). The ability of the compounds to induce DNA damages (single stranded DNA breaks) was studied by alkaline variant of single cell gel electrophoresis (Comet assay). The results obtained revealed that the compounds investigated reduce cell viability and proliferation in a time- and concentration- dependent manner. Cell specific response was also observed. Applied independently, the ligands were found to be less effective as compared to the corresponding metal complexes.

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VIRUS-TRANSFORMED TUMOUR CELLS AS EXPERIMENTAL MODELS FOR THE SCREENING OF NEW AGENTS WITH ANTINEOPLASTIC PROPERTIES

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It is widely accepted that tumour cell lines have played an important part in our understanding of cancer and have been used extensively in the discovery and characterization of new chemotherapeutic drugs. Two permanent cell lines established from transplantable tumours in chicken (hepatoma induced by the myelocytomatosis virus Mc29 – LSCC-SF-Mc29) and rat (sarcoma induced by Rous sarcoma virus strain Schmidt-Ruppin – LSR-SF-SR) have been applied in our investigations to evaluate the putative cytotoxic and antiproliferative properties of 32 newly synthesized transition metal complexes with various ligands (aminoacids, Mannich bases). The investigations were performed using MTT test, neutral red uptake cytotoxicity assay and colony-forming method. The results obtained revealed that LSCC-SF(Mc29) and LSR-SF-SR tumour cells could be considered as suitable test-systems for the initial screening of new potential antineoplastic agents because of the following main reasons: i) as compared to the other cell lines included in the experiments (obtained from human malignant neoplasms of the brain, breast, liver, larynx) LSR-SF-SR and especially LSCC-SF-Mc29 were found to be the most sensitive to the cytotoxic and antiproliferative properties of the compounds investigated; ii) primary cultures from various tissues of healthy chickens or rats could be easily prepared to serve as "controls" for comparative investigations with chicken hepatoma and rat sarcoma cells, respectively; iii) according to the literature available up to now the effect of synthetic substances and natural products on viability and proliferation of virus-transformed cells is not fully clarified; iv) the viral oncogenes v-myc (LSCC-SF-Mc29) and v-src (LSR-SF-SR) are integrated in genome DNA of chicken hepatoma or rat sarcoma cells, respectively. The cellular analogues of these oncogenes are proved to be involved in pathogenesis of a wide variety of human malignant neoplasms.

Acknowledgements: This study was supported by Grant DOO-2-39/2009 between the Bulgarian Ministry of Education and Science and the Romanian Ministry of Education and Research.

THE EFFECT OF VASODILATIVE DRUGS ON *IN VITRO* VISCOELASTIC CHARACTERISTICS OF AGING RAT AORTA

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The arterial wall is stiffening with age, which influences its biomechanical behaviour [1]. <u>*The aim*</u> was to study the effects of vasodilative drugs on the viscoelastic characteristics (*VEC*) of aging rat aorta. <u>*Methods and materials*</u>: Three groups of different age male Wistar rats were used: mature (4 months), middle-aged (10 months), and old (18 months). *In vitro* thoracic aorta cylindrical preparations were immersed in and perfused with modified Tyrode solution and subjected to forced oscillations of the intraluminal pressure (p) [1]. The frequency of pressure excitation (f_{exc}) was swept up and down in the range 3–30 Hz at several mean-pressure levels with the response volume oscillations being recorded. Resonance curves were plotted for each pressure level and the following *VEC* were estimated: natural frequency (f_0), dynamic modulus of elasticity (E') and coefficient of viscosity (□). Pressure-dependence of each *VEC* was drawn. The experimental protocol was repeated in the presence of vasodilators with different mechanisms of action: Sodium nitroprusside and Isoptin hydrochloride at concentrations of 10, 30 and 50 □M, which have been applied for 15 min with the Tyrode medium. <u>*The results*</u> revealed different changes in the curves $f_0(p)$, $E'(p)$ and □(p) depending on drug category, drug concentration and direction of f_{exc} alteration. The highest concentration (50 □M) of both drugs produced the most significant increase of distensibility (E' decrease) of old aorta, despite the natural frequency was lowered. E' decrease is determined by the decrease of f_0. However extreme diminishing of f_0 is unacceptable because the impairment of arterial wall integrity. <u>*Conclusion*</u>: It is concluded that the drug-induced vasodilation could improve in a concentration-dependent manner the distensibility of the aged aorta wall.

<u>*References*</u>: [1] Antonov P., Antonova M., Nikolova N., Antonova N., Vlaskovska M., Kasakov L. Age dependent changes of arterial wall viscoelasticity. Clin. Hemorheol. Microcirc. 2008, 39: 63-68; [2] Antonova M. A device for biomechanical investigations of the viscoelastic characteristics of vital and artificial arterial segments. Clin. Hemorheol. Microcirc. 2004, 30: 477-480.

<u>*Keywords*</u>: Aging rat aorta, vasodilative drugs, resonance curve, distensibility, modulus of elasticity, viscoelasticity

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IN VITRO STUDIES OF THE VISCOELASTICITY OF THORACIC AORTA IN LIPOFUNDIN TREATED RATS

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It had been shown that intravenous infusion of lipid emulsion Lipofundin can rapidly induce in rat morphological changes of the aortic intima resembling the early pathological alterations in the atherosclerotic process in human [1]. <u>*The aim*</u> was to study the viscoelasticity of atherosclerosis-like stiffened aorta. <u>*Methods and materials*</u>: Arteriosclerotic changes in the aorta of 10-month-old male Wistar rats (n=5) were induced by 10-day intravenous administrations of 20% Lipofundin MCT/LCT (1 ml/100 g b.w.). Age-matched Wistar rats (n=7) were used as the controls. *In vitro* thoracic aorta cylindrical preparations were immersed in and perfused with modified Tyrode solution and subjected to forced oscillations of the intraluminal pressure (p) [2]. The frequency of pressure excitation (f_{exc}) was swept up and down in the range 3–30 Hz at several mean-pressure levels with the response volume oscillations being recorded. Resonance curves were plotted for each pressure level. The viscoelastic characteristics estimated were: natural frequency (f_0), dynamic modulus of elasticity (E') and coefficient of viscosity (□). Pressure-dependence of each VEC was drawn. <u>*Results*</u>: The results showed that $f_0(p)$ diminished linearly with lower values than in the controls. $E'(p)$ was exponential with values at p>50 mm Hg higher than the values in the controls. An extreme increase was observed in E' at p>100 mm Hg (6 times at p=125 mm Hg). □-values increased linearly with p being higher in comparison to control rat aorta, demonstrating raised intrinsic friction in the vessel wall. <u>*Conclusion*</u>: It is concluded that in arteriosclerotic aorta distensibility dramatically decreased, especially at higher intraluminal pressures. Present data quantified the stiffening of the vessel wall, which is observed by the more conventional pharmacological approaches.

<u>*References*</u>: [1] Saladino CF, Klein RA, Jonas EA. Induction of early atherosclerosis in rats using parenterally administered lipid emulsions. Artery. 1987, 14: 304-315; [2] Antonova M. A device for biomechanical investigations of the viscoelastic characteristics of vital and artificial arterial segments. Clin. Hemorheol. Microcirc. 2004, 30: 477-480.

<u>*Keywords*</u>: Arteriosclerotic rat aorta, resonance curve, modulus of elasticity, viscoelastic characteristics

<u>*Acknowledgement*</u>: The authors would like to thank Mrs. N. Nikolova for the excellent technical assistance.

THE EFFECT OF VASODILATIVE DRUGS ON THE *IN VITRO* VISCOELASTICITY OF LIPOFUNDIN INDUCED ATHEROSCLEROSIS-LIKE ALTERATIONS IN RAT AORTA

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It is widely known that arteriosclerosis impairs the elasticity of arterial wall, which could change the biomechanical characteristics. *The aim* of this investigation was to study the effect of vasodilative drugs on viscoelastic characteristics (*VEC*) of atherosclerosis-like stiffened aorta. *Methods and materials*: Arteriosclerotic changes in the aorta of 10-month-old male Wistar rats (n=5) were induced by 10-day intravenous administrations of 20% Lipofundin MCT/LCT (1 ml/100 g b.w.). *In vitro* thoracic aorta cylindrical preparations were immersed in and perfused with modified Tyrode solution and subjected to forced oscillations of the intraluminal pressure (p) [1]. The frequency of pressure excitation (f_{exc}) was swept up and down in the range 3-30 Hz at several mean-pressure levels with the response volume oscillations being recorded. Resonance curves were plotted for each pressure level. Natural frequency (f_0), dynamic modulus of elasticity (E') and coefficient of viscosity (□) were estimated from resonance curves and the pressure-dependences for each of *VEC* were drawn. The experimental protocol was repeated in the presence of vasodilators with different mechanisms of action: Sodium nitroprusside (*SN*P) and Isoptin hydrochloride (*Isoptin*) at concentrations of 10, 30 and 50 □M, which have been applied for 15 min with the Tyrode medium. *Results*: The results showed that $f_0(p)$ diminished linearly, which was different for each drug or concentration. $E'(p)$ was exponential with values at *SNP* 50 □M significantly lower than those in untreated preparations. *Isoptin* had no significant effect on E'. □(p) of both drugs were significantly lower at concentrations of 50 □M. *Conclusion*: It is concluded that vasodilators *SNP* and *Isoptin* at higher concentrations increased the distensibility and decreased the inner friction of atherosclerosis-like stiffened aorta. The improvement of the distensibility has been observed in the physiological range of intraluminal pressures being more significant at higher pressure, at which the vessel wall is mostly endangered.

References: [1] Antonova M. A device for biomechanical investigations of the viscoelastic characteristics of vital and artificial arterial segments. Clin. Hemorheol. Microcirc. 2004, 30: 477-480.

Keywords: Arteriosclerotic rat aorta, vasodilative drugs, resonance curve, modulus of elasticity, viscoelasticity

Acknowledgement: The authors would like to thank Mrs. N. Nikolova for the excellent technical assistance.

OLFACTORY BULBECTOMY IMPAIRS ACTIVE AND PASSIVE AVOIDANCE LEARNING IN RATS

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Olfactory bulbectomy is a widely used animal model of depression. Bilateral removal of the olfactory bulbs in rats results in a constellation of behavioral, neurochemical, neuroendocrine, and neuroimmune alterations, many of which are reported in patients with major depressive disorder. Two methods to assess learning and memory abilities were used –active avoidance test (shuttle box) and passive avoidance test (step through). The experimental male Wistar rats were divided into two groups: olfactory bulbectomized (OBX) rats and sham opeated controls. Fifteen days after olfactory bulbectomy, OBX rats showed significant impairment of learning and memory - related behaviors, as measured by active and passive avoidance tasks. The primary measure of learning and memory in active avoidance task is an increase in avoidance responses. In OBX rats the number of avoidances significantly decreased on learning tests (1st and 2nd training days) and on the retention test (24th h after the 2nd training day).
In the passive avoidance task OBX rats showed diminished tendency to avoid the foot-shock, as expressed by a decrease of the latent time on the retention tests (3h and 24 h after the acquisition trial). None of the OBX rats reached the learning criterion, compared to the sham-operated controls. These findings suggest that the olfactory bulbs play an important role in the learning and memory processes. In conclusion, olfactory bulbectomy significantly impaired learning and memory of rats. The neurodegenerative changes in many brain areas and the impairment on neurotransmitter systems, induced by the lesion probably account for the memory deficits exhibited by the OBX rat.

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HOW MIF-1 AND TYR-MIF-1 AFFECT ENDOGENOUS NITRIC OXIDE AFTER THREE ACUTE MODELS OF STRESS?

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The question of the role of nitric oxide (NO) in physiological functions has been studied intensely in recent years. It is now clear that NO system affects the secretion of stress hormones and fulfils the main criteria of a stress-limiting system. NO is synthesized by the enzyme nitric oxide synthase (NOS), which had widespread distribution in the brain. Also, NO is involved in NO-molecular ways, which affect through auto regulation different signalling molecules – like opioids, endocanabiotics and others.
One of the mechanisms known to play a part in the response of an organism to stress is activation of the endogenous opioid system. Endogenous opioid peptides take part in various functions as hormones or neuromodulators. Tyr- MIF-1 neuropeptides/neuromodulators are able to inhibit the expression of some forms of stress.
Literature data showed that periaqueductal gray (PAG) is a major module in the circuitry mediating stress-induced analgesia. Stimulation of opioid receptors within the PAG activates descending inhibitory pathways and suppresses nociception.
The aims of the present study were twofold: 1) to determine the involvement of nitric oxide-ergic system in the effects of MIF-1 and Tyr-MIF-1 after immobilization, cold and heat stress (IS, CS and HS); 2) to examine the effects of MIF-1 and Tyr-MIF-1 on NOS expression in PAG after stress models mentioned above.
Groups of male Wistar rats were injected i.p. with MIF-1, Tyr-MIF-1, L-Arginine, L-NAME or SIN-1 after each of the stress models. Nociception was measured by paw-pressure (PP) test and immunocytochemistry was used for determination of NOS expression.
Our results showed differences in obtained results after injection of investigated peptides immediately after IS, CS and HS, which maybe due to peptide structure and properties and its interaction with opioid and non-opioid component on three models of stress. Also increased after stress NOS expression in PAG was significantly decreased by two investigated peptides.
In conclusion, nitric oxide-ergic system is involved in the nociceptive effects of MIF-1 and Tyr-MIF-1 after immobilization, cold and heat stress.

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THERAPEUTIC EFFICACY AND SAFETY OF COX-2 INHIBITORY DRUG CELECOXIB IN LOW BACK PAIN

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Background: Low back pain with its neuropathic and nociceptive aspects is one of the most frequent complications in the neurological practice. Non-steroid anti-inflammatory drugs are useful for the treatment of acute back pain. Side effects occur because of COX-1 inhibition, while COX-2 inhibition is mainly responsible for the therapeutic effect. The aim of this study was to evaluate the therapeutic efficacy and safety of the COX-2 inhibitor celecoxib (Celebrex) in patients with acute low back pain.
Material and methods: 30 patients with acute low back pain of different origin - degenerative, traumatic and inflammatory were enrolled in this study. The drug was taken in 200 mg once daily every day in a 10-day period. Before and after the treatment a neurological examination and pressure algometry of the paraspinal muscles was performed. The spontaneous muscle pain and the pain during palpation were assessed using the Visual Analogous Scale. All patients were instructed not to receive another drug therapy.
Results: Our results revealed in all patients a reduction of the spontaneous low back pain and the pain during palpation after the treatment. At the end of the study, the pressure pain threshold during algometry was increased.
Conclusion: COX-2 inhibitory drug celecoxib (Celebrex) is useful and safety approach for treatment of patients with acute low back pain.

DEFICIENCY OF GLUTATHIONE IN ENRICHED HYPOTHALAMIC NEURONS APPEARS TO BE CONNECTED WITH ETHANOL-INDUCED APOPTOSIS

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The antioxidant glutathione (GSH) is essential for cellular detoxification of reactive oxygen species in brain cells. Recent data demonstrate that besides intracellular function GSH has also important extra cellular functions in the brain. Astrocytes appear to play a key role in the GSH metabolism in the brain, since astroglia export is essential for providing GSH precursors to neurons. We have demonstrated that ethanol caused apoptosis in enriched hypothalamic neurons. We also showed that astroglia protected hypothalamic neurons from ethanol-induced apoptotic cell death.

Aim and Methods: The aim of this study was to determine the effect of ethanol on cellular glutathione and GSH-Px activity. Enriched hypothalamic neuronal cells in culture were treated with or without ethanol (25 and 50 Mm) for a period of 2 and 4 days. Then cells were harvested and glutathione was determined in umol/L/ug of protein by GSH ASSAY (Calbiochem, USA). Additionally, the GSH-Px activity was measured by using the method of Paglia and Valentine (1967). The apoptosis was determined in nucleosomes/ml by ELISA.

Results and Conclusion: The data demonstrate that enriched embryonic hypothalamic neurons contained less glutathione compared to hypothalamic astroglial cells. The results also demonstrate that ethanol decreased the levels of glutathione and increased apoptotic cell death of embryonic hypothalamic neurons. The results suggest that ethanol may cause apoptotic cell death of hypothalamic neurons by inhibiting of neuronal glutathione. Moreover, our data demonstrate that the antioxidant status of neurons plays a role in apoptotic effect of alcohol on brain cells.

EFFECT OF ALCOHOL ON HYPOTHALAMIC GLUTATHIONE AND FREE RADICALS OF RATS

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Reactive oxygen species (ROS) are generated continuously during oxidative stress. Increased production of ROS and/or decreased in the antioxidant capacity of cells caused oxidative stress. The oxidative stress plays a role in pathogenesis of brain diseases as well as in alcohol effects. The antioxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) are present in the CNS i.e. in the cortex, cerebellum, hypothalamus, striatum, and spinal cord, where they are responsible for the brain's basic functions, both physical and cognitive. Moreover, the highest activity of these enzymes is observed in neurons and/or glial cells. There is evidence that alcohol caused oxidative stress on brain cells. The role of glutathione and ROS in alcohol effects on neurons is not established. We have previously published the *in vitro* effects of alcohol on glutathione on astroglia.
The aim of this study was to determine the effects of alcohol on ROS and glutathione in hypothalamus of male rats. Rats were exposed to alcohol for a period of 4 weeks. The Hypothalamic glutathione was determined in umol/L/ug of protein by GSH ASSAY (Calbiochem, USA). Additionally, the GSH-Px activity was measured by using the method of Paglia and Valentine (1967). The superoxide dismutase (SOD) activity was determined by pyrogallol authooxidation method. The results demonstrate that alcohol decreased glutathione and increased ROS in hypothalamus. Our results suggest that the reduction of glutathione in brain cells after alcohol treatment increased the toxic effect of ROS. Moreover, our data support the role of hypothalamic antioxidant system in alcohol effects on neurons.

PROSTATE CANCEROGENESIS AND ADIPOCYTE-DERIVED CYTOKINES

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Prostate cancer commonly affects men in the Western world. Chronic inflammation is associated with processes that contribute to the onset or progression of cancer. Adiposity and adipocyte-derived cytokines have been implicated in both inflammation and prostate carcinogenesis.
The aim of this study was to investigate the relationships between circulating levels of the adipocyte-derived cytokines (adiponectin, TNF-alpha and IL-6) and development of prostate cancer in rats.
Design and methods: Male rats were treated with N-methyl-N-nitrosourea (NMU) for a period of 13 weeks and then injected with testosterone. All rats were monitored daily. The rats were killed after at the end of experiment. Pathomorphological study determined the changes in prostates. The blood was taken and the levels of adiponectin, TNF-alpha and IL-6 were determined by ELISA quantitative.
Results and conclusion: We observed the increased blood levels of IL-6 and TNF-alpha. Increasing levels of both cytokines positively associated with development of prostate cancer. We also determined the low levels of adiponectin in rats with developed prostate cancer. Our study suggests that both inflammatory cytokines TNF-alpha and IL-6 may play roles in development of prostate cancer in rats. Moreover, our data suggest that the increased levels of adipocyte-derived cytokines are associated with the risk of prostate cancer.

OPIOIDS AND DIABETES MELLITUS: ROLE OF BETA-ENDORPHIN AND EFFECTS OF TRAMADOL

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Opioid receptors are best known for analgesia. They couple multiple systems to result in various pharmacological effects. Beta-endorphin is well known endogenous opioid. It is not clear whether opioid agonists affect the development of streptozotocin-induced diabetes (STZ-diabetes) in rats. Moreover, the hypothalamic beta-endorphin in STZ-induced diabetic rats is not well established. We have been suggested that molecules expressed in both the hypothalamus and pancreas, such as μ-opioid receptor 1 could play a role in development of diabetes mellitus.
The aim of this study was to investigate the role of beta-endorphin and opioid receptors in development of STZ-induced diabetes in rats. We employed STZ-induced diabetic rats as type 1 diabetes-like animal model to investigate the opioid-related mechanisms in control of insulin, plasma glucose and beta-endorphin. Plasma glucose, insulin and beta-endorphin were determined during development of STZ-diabetes in male rats. We also determined the hypothalamic beta-endorphin at day 3, 6, 9, 12 and 15^{th} after application of streptozotocin. The μ-agonist of opioid receptors (tramadol) was injected in STZ-induced diabetic rats and plasma glucose, insulin and β-endorphin were determined. The effect of tramadol on peripheral utilization of glucose was determined.
Results and conclusion: Plasma glucose, insulin and beta-endorphin were changed in a time-dependent manner after a single injection of streptozotocin. Tramadol decreased fasted serum glucose levels. Our results demonstrated that tramadol affects peripheral glucose metabolism through central activation of μ-opioid receptors. The data demonstrated that the activation of μ-receptors by β-endorphin might increase the peripheral utilization of glucose. Moreover, our results suggest that beta-endorphin plays a role in regulation of neuronal insulin cascade during development of STZ-induced diabetes in rats.

MEDICAL EDUCATION AND VIRTUAL PATIENT: NEW MODULE FOR TEACHING PHARMACOLOGY

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Medical/Pharmacology education and virtual patient is a new direction for teaching of students, doctors and nurses. The Department of Pharmacology and Toxicology of Medical Faculty in Sofia won as a partner in the European Educational project (MVPS) in 2008.
The aim of our study is to develop multi languages virtual-patient model for teaching of students, medical doctors and nurses. The development team consists of medical doctors, researchers and educational specialists. The module focused on patients with various symptoms and diseases. The module includes seven languages: Bulgarian, English, German, Italian, Spanish, Hungarian, and Portuguese.
In this study, we describe the architecture of virtual multi languages patients in internal medicine and pharmacotherapy. This study demonstrated that an integrative, multi languages virtual patient learning module for medical students could be an effective tool for providing students and physicians needed clinical exposure to patients with various disorders/symptoms treated with various pharmacological drugs. The web-based applications for medical education using the virtual patients were developed. Some future directions for development of the model are also present.

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INVOLVEMENT OF NITRIC OXIDE-ERGIC SYSTEM IN THE EFFECTS OF TYR-CIT-MIF-1 AND TYR-CAV-MIF-1 AFTER STRESS

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Stress is known to exert an influence on neuroendocrine, autonomic, hormonal, and immune functioning. Various stress models have been reported to induce stress-induced analgesia (SIA) - phenomenon, mediated by periaqueductal gray (PAG), endogenous opioid peptides and nitric oxide (NO). Also, many stress models have been reported to affect the opioid receptors within the PAG and expression of nitric oxide synthase (NOS) which activate descending opioid and noradrenaline inhibitory pathways and suppress nociception. On the other hand, Tyr-MIF-1 is neuropeptide/neuromodulator, which are able to inhibit the expression of some forms of stress.
The objectives of the present study were twofold: 1) to determine the involvement of nitric oxide-ergic system in the effects of newly synthesized Tyr-MIF-1 analogues containing amino acids from the urea cycle *citrulline* (Tyr-Cit-MIF-1) and *canavanine* (Tyr-Cav-MIF-1) after immobilization, cold and heat stress (IS, CS and HS); 2) to examine the effects of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 on NOS expression in PAG after stress models mentioned above.
After the completion of each of the stress models male Wistar rats were injected with Tyr-Cit-MIF-1 or Tyr-Cav-MIF-1 (both at a dose of 1 mg/kg, i.p.). Nociception was measured by paw-pressure (PP) test and L-Arginine, L-NAME and SIN-1 were used too. Immunocitochemistry was used for determination of NOS expression.
Our results showed differences in obtained results after injection of investigated peptides immediately after IS, CS and HS, which maybe due to different interaction of both peptides with opioid μ-receptors, different involvement of opioid and non-opioid component in each kind of stress. Also NO-ergic system influenced in different way opioid and non-opioid components of investigated stress models.
On the other hand our results showed that NOS expression in PAG was increased after each of stress models. Application after stress of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 decreased it significantly.
In conclusion, our results suggest that: 1) there is a different kind of involvement of nitric oxide-ergic system in the mechanisms of nociception of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 after immobilization, cold and heat stress; 2) two investigated peptides influenced NOS expression in PAG after three stress models mentioned above.
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DYNAMICS OF THE ACTIVITY OF THE IL-3 SIGNALLING PATHWAY DURING ANTIPSYCHOTIC TREATMENT OF PATIENTS SUFFERING FROM SCHIZOPHRENIA

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Recent data links various abnormalities in the IL-3 signaling pathway are associated with schizophrenia. Drugs in the antipsychotics group influence signalling pathways which share common elements with the IL-3 signalling pathway. The aim of the present study is to study the dynamics of IL-3 signalling pathway activity in patients suffering from schizophrenia during antipsychotic treatment. The activity of the IL-3 signalling pathway is measured indirectly by the expression of *cyclon* – a gene induced by IL-3.
The analysis was performed twice in 10 patients suffering from schizophrenia. The first analysis was performed before initiation of the antipsychotic treatment. The period prior antipsychotic treatment is more than two months. The second analysis was performed after successful remission and after more than 35 days antipsychotic treatment. The dynamics of *cyclon* expression before and after antipsychotic treatment is assessed. The differences of *cyclon* expression with respect to the antipsychotic drug and the PANSS and GAF symptom severity are also assessed.

EFFECT OF Mn(III)TETRAKIS(4-BENZOIC ACID)PORPHYRIN (MnTBAP) ON MARKERS FOR CYTOTOXICITY IN RAT BRONCHOALVEOLAR LAVAGE FLUID AFTER PARAQUAT TREATMENT

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The antioxidant effect of MnTBAP was tested in our study using paraquat pneumotoxic model.
The study was carried out on 96 male Wistar rats divided into four groups: group 1 – controls; group 2 – treated with MnTBAP; group 3 – treated with paraquat, group 4 – treated with paraquat and MnTBAP. Paraquat was administered *per os* as a water solution at a dose of 40 mg/kg. MnTBAP was injected *intraperitoneally* after receiving paraquat at a dose of 10 mg/kg. The activities of lactate dehydrogenase (LDH), alkaline phosphatase (AP), acid phosphatase (AcP), total protein content and total cell number in bronchoalveolar lavage fluid **(**BALF) were investigated.
The isolated application of paraquat significantly increased the activity of LDH on day 5 in comparison with controls. The combined treatment with the toxic agent and MnTBAP, decreased the activity of LDH on day 5 compared to the group 3. AP activity was decreased in group 3 (paraquat) on day 1 and increased on days 5 and 15. The same effect was observed in group 4, but elevation on days 5 and 15 was significantly less in comparison to paraquat group. The activity of AcP was increased in paraquat treated group up to day 15. The combination also elevated the enzyme activity but it remained significantly lower as compared to group 3 at the same time points. Total protein content and total cell number in BALF increased abruptly on day 1. The combined treatment with the herbicide and antioxidant increased the protein content and total cell number on day 1 but they remained significantly below the levels measured in group 3.
It is well known that paraquat selectively accumulates in the lung parenchyma and possesses pneumotoxic effects. The paraquat molecules undergo cyclic oxygenation and reduction in pulmonary cells and toxic oxygen species are formed. Metalloporphyrines are potent antioxidants and they have been shown also to be effective in ameliorating inflammation, and injury in a large number of animal models of human diseases.
In conclusion, the metalloporphyrine MnTBAP reduces the pneumotoxic effects of paraquat. The protective effect is well expressed up to day 5 after treatment.

EFFECT OF Mn(III)TETRAKIS(4-BENZOIC ACID)PORPHYRIN (MnTBAP) ON PARAQUAT-INDUCED PULMONARY FIBROSIS IN RATS

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Lung damage and pulmonary fibrosis are the most widespread injuries due to herbicide paraquat. We tested the effect of novel superoxide dismutase mimetic MnTBAP against paraquat-induced pulmonary fibrosis in the rat model.
Methods: The study was carried out on 64 male Wistar rats, divided into four treatment groups: group 1 – controls; group 2 – treated with MnTBAP; group 3 – treated with paraquat, group 4 – treated with paraquat and MnTBAP. Paraquat was administered *per os* as a water solution at a dose of 40 mg/kg. MnTBAP was injected *intraperitoneally* after receiving paraquat at a dose of 10 mg/kg. Pulmonary fibrosis was assessed biochemically by measuring hydroxyproline (HP) content in lung homogenate (LH) and histopathologically on day 15 and 28 after paraquat administration.
Results: Paraquat altered HP levels on day 15 and did result in significant (with 23.32%) increase on day 28 after treatment in comparison with controls. The combined treatment with paraquat and MnTBAP decreased content of HP (2.5±0.46 mcg/ml LH) compared to paraquat alone (2.88±0.20 mcg/ml LH) on day 28. Paraquat administration resulted in histopahological damage on day 28, as indicated by thickening of interstitial spaces, and these damages were attenuated by combination paraquat and MnTBAP.
Discussion: Lung tissue and particularly the type II pneumocytes accumulate paraquat. The pathogenesis of paraquat-induced pulmonary fibrosis includes endothelial and epithelial cell injury, influx of the inflammatory cells and production of their chemical mediators leading to the proliferation and activation of fibroblasts and progressive accumulation of connective tissue replacing normal functional parenchyma. Metalloporphyrines have been shown also to be effective in ameliorating inflammation, and injury in a large number of animal models of human disease.
Conclusions: The antioxidant MnTBAP can substantially protect animals from paraquat-induced pulmonary fibrosis.

ANALOGUES OF VIT. D - REGULATORY ACTIVITIES ON MINERAL METABOLISM DURING REPLACEMENT OF RENAL FUNCTION AS AN *IN VIVO* EXPERIMENTAL MODEL

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The secondary hyperparathyreoidism, down-regulation in the production of Vit D, disregulation in phosphate and calcium turnover are consequences of the chronic renal failure. Cardiovascular calcificates are connected with this disorders and are the most frequent reason for mortality. Renal osteodystrophy is the main reason for invalidisation. Using replacement of renal function as an in vivo experimental model the regulatory activities of alfacalcidol on mineral metabolism were the object of the investigation.
Volunteers on renal replacement therapy with GFR below 10 ml/min were enrolled in the study. Twice a week alfacalcidol 1 µg was applied orally. The observation period was 3 months.
For statistical analysis of the results SPSS 15 and GraphePadPrism 4.0 software products were applied.
The investigated parameters iPTH, Calcium and Phosphorus were analysed as a recent part of the study. The first parameter was analysed twice during this period. The remaining two parameters were tested three times during the observation period.
The results showed down-regulation of iPTH. Plasma levels after the application of the Vit D analogues decreased significantly with $p<0,05$.
Calcium metabolism was up-regulated. Plasma levels were significantly increased with $p<0.05$.
Phosphorus metabolism did not undergo any significant changes.
The recent results demonstrate the influence of Vit D analogue alfacalcidol on mineral metabolism during the replacement of the renal function.

CHANGES IN HAEMATOLOGIC INDEXES OF ADULT MALE RATS UNDER ACUTE AND CHRONIC TESTOSTERONE TREATMENT

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A few essential problems accompany the aging of men:sexual disfunction, hypogonadism, psychologic changes[1]. There is scarce and contradictory information about the way androgene deficiency influenses the haematologic and clinicochemical indexes. The aim of the present study is to establish the early (15 day) and late (15 week) changes in haematologic indexes that are manifesting after testosterone replacement therapy of male rats, aged 24 months. Old male Wistar rats, aged 24 months were used, whith average weight 358 grams, devided into the following groups: control group, 4 testing, treated with 4 and 8 mg testosterone for a period of 15 days and 15 weeks. Blood was collected by decapitation, which was done under ether narcosis. Samples were sent for analysis by automatic haematologic counter-Coulter T – 660, US in Central clinic laboratory of Medical University – Plovdiv. The following six indexes were traced: number of erythrocytes, leucocytes, thrombocytes, haemoglobine, haematocrite and average volume of the erythrocyte.
It was established that testosterone replacement therapy stimulates the haematopoesis in adult male rats. The number of erythrocytes, values of haemoglobine, and haematocrite are increased. Testosterone treatment of male Wistar rats leads to stimulation of the cell immune response[2]. Leucopoesis is probably connected with the serum levels of estradiol, which supposes a decreased number of leucocytes in untreated animals[3]. The present study did not find out the changes that were supposed above.

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NEUROGENESIS IN THE NEW THERAPEUTIC STRATEGIES IN NEURODEGENERATIVE DISORDERS

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Neurodegenerative disorders are characterized by neuronal loss in specific regions of the central nervous system. The dogma, stating the incapability of nerve cells to divide and reintegrate, which existed until recently, had reduced pharmacotherapeutic efforts to research and creation of ways to compensate mediator and functional deficits to a certain degree. This is one of the reasons why currently available therapeutic options have only symptomatic action.
As new conceptions are introduced and established in modern neuroscience, especially the one concerning the functional integration of new neurons in the CNS, directions for search of new therapeutic strategies are changing. One of the aims of contemporary experimental and clinical studies is the stimulation of endogenous neurogenesis in order to overcome the principal effect of the pathological process, cellular loss.
Technologies for manipulation of stem cell populations in neurodegenerative disorders in vitro and in vivo are being developed. Questions related to the influence of known therapeutic options on neurogenesis are being clarified. At the same time, proofs of the therapeutic action of potential new regulating factors are being sought. The introduction of stem (progenitor) cells from embryonal tissue, bone marrow and other sources, after successful proliferation, differentiation and integration into corresponding functional neuronal systems, could lead to a decrease or cessation of pathological deficits.
The conception for the role of neurogenesis in contemporary therapeutic strategies in neurodegenerative disorders is still subject to research and approval, aiming at quicker clinical benefit.

USE OF PREGABALIN IN PATIENTS WITH PAINFUL DIABETIC NEUROPATHY: MECHANISMS OF ACTION, EFFICACY AND SAFETY

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Introduction: Neuropathy is the most common complication and greatest source of morbidity and mortality in diabetes patients. Despite the advances in understanding of mechanisms of diabetic neuropathy, the agents for pain control are limited both by side effects and lack of efficacy. Recently, Gabapentin and related Lyrica (Pregabalin), new generation AEDs, are emerging as first line treatment for painful neuropathy associated with diabetes mellitus.
Objective: To evaluate efficacy and safety of Lyrica in patients with painful diabetic polyneuropathy.
Material and Methods: Fourteen patients (5 males and 9 females; aged 54.2±8.6 years) with painful diabetic polyneuropathy were included in the study. All patients underwent therapy with Lyrica (150-300 mg/daily) for at least 12 weeks. Drug efficacy was assessed on the basis of changes in pain intensity and safety with reporting the related adverse effects. Neurological examination, glucose control and clinical assessment of pain intensity (Visual analog scale) before and after drug addition were performed.
Results: Lyrica reduced pain intensity in 68 percent of patients, with 26 percent becoming totally free of pain over the treatment period. Decreased values of VAS score from 8.5±1.0 to 5.0±1.0 corresponded to the clinical improvement of patients. The most common side effects were transient mild dizziness (2 p), somnolence (1 p), and asthenia (1 p).
Conclusion: Despite the limited number of patients included in this study, we consider therapy with Lyrica useful and safety in control of neuropathic pain associated with diabetic polyneuropathy. Possible mechanisms of drug action are discussed in accordance with our preliminary findings and literature review.

ANTI-AGE ANTIBODIES AND MARKERS OF OXIDATIVE STRESS IN SPONTANEOUSLY HYPERTENSIVE RATS AT DIFFERENT AGES

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Advanced glycation is a major pathway for the posttranslational modifications of tissue proteins and begins with non-enzymatic addition of sugars to the primary amino groups of proteins. Advanced glycation end-products (AGEs) have been shown to enhance vascular oxidative activity and have been implicated in the pathogenesis of many disorders including diabetes, hypertension and ageing. Excessive accumulation of AGEs on tissue proteins changes their structure, respective function and immunogenisity. Glycated proteins form common immunological epitopes, which result in the formation of populations of anti-AGE antibodies (AGE Abs).
The aim of this study was to investigate AGE Abs and two markers of oxidative stress, copper (Cu) and iron (Fe) in sera of Spontaneously Hypertensive Rats (SHR) and Wistar Kyoto Rats (WKY) at 2, 4 and 8 months.
AGE Abs were assessed with direct enzyme-linked immunosorbent assay (ELISA). Iron was measured with a ferene photometric test and Cu with an endpoint colorimetric assay.
Significant differences were observed between AGE Abs in 4-month and 2-month-old SHR ($P < 0.001$) and between 8-month and 4-month-old SHR ($P < 0.001$). In WKY rats there were no significant differences between the 4 month and 2 month old groups, only the comparison between the 8 and 2 month old groups showed significant differences ($P = 0.017$). In the 8-month-old rats the level of AGE Abs in the SHR was significantly higher than in WKY ($P < 0.001$). The levels of AGE Abs in SHR and WKY correlated positively with age ($r = 0.636$, $p = 0.005$), ($r = 0.420$, $p = 0.015$). The same correlation was observed for the serum content of iron and copper in both the SHR and WKY.
This data shows a positive correlation between increased levels of AGE Abs, as well as the serum levels of Fe and Cu as markers of oxidative stress, with increasing age and hypertension.

COMPARATIVE STUDY OF THE EFFECTS OF CHOLINESTERASE INHIBITORS ON SCOPOLAMINE-INDUCED IMPAIRED MEMORY IN RATS

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Background: Psychopharmacological model of memory dysfunction is scopolamine-induced amnesia in rats, used to study the effectiveness of cholinesterase inhibitors (ChEIs) in the treatment of Alzheimer's disease (1). The aim was to compare the effects of tacrine (Tac), donepezil (Don), galantamine (Gal) and metrifonate (Metr) on scopolamin-induced impaired memory in rats.
Methods: Male Wistar rats were treated with: 1st saline (controls); 2nd Scopolamine (Scop) 1 mg/kg and saline; 3rd Scop and Tac 1 mg/kg; 4th Scop and Don 0.5 mg/kg; 5th Scop and Gal 0.1 mg/kg, 6th Scop and Metr 50 mg/kg. Learning and memory was studied in shuttle-box active avoidance test and step-through and step-down passive avoidance tests. Statistical evaluation was done by ANOVA.
Results: In active avoidance test controls increased the avoidances on 3rd, 4th and 5th days learning and on memory retention. The rats with scopolamine model did not change the number of avoidances during learning sessions and memory tests. The animals with Scop and Tac increased the avoidances on 3rd day learning and on memory test. The group with Scop and Don increased the avoidances on 3rd and 4th day learning and on memory retention. The rats with Scop and Gal increased the avoidances in four days learning. The animals with Scop and Metr increased the number of avoidances on 2nd, 3rd and 4th days learning. In both passive avoidance tests controls increased the latency on 2nd day learning and on short and long memory retentions. In step-through test animals with Scop and saline decreased the latency of reactions on 2nd day learning and on short and long memory tests. The groups with Scop and Tac or Don increased the latency on 2nd day of learning session and memory tests. The group with Scop and Gal increased the latency of reactions on short and long memory retention. The rats with Scop and Metr increased the latency on two memory test. In step-down test the rats with Scop and saline decreased the latency of reactions on learning session and on memory tests. The animals with Scop and Don increased latency on long memory tests. The groups with Scop and Tac or Gal increased the latency of reactions on two days learning, on two memory tests. The group with Scop and Metr increased latency on long memory test.
Conclusion: Our results showed that Gal and Metr improved better rats learning ability in active avoidance test, but Tac in passive avoidance tests. ChEIs Tac, Don and Gal improved short and long memory in rats with scopolamine-induced amnesia.
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PHARMACOKINETICS OF ENROFLOXACIN IN A BLOOD SERUM AND AQUEOUS HUMOUR OF RABBITS

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ITRODUCTION

The aim of this paper was to study the pharmacokinetics of the quinolone enrofloxacin (EFL) and its metabolite ciprofloxacin (CFL) in rabbits after *i.v.* and *i.m.* injection.

MATERIALS AND METHODS

Eight White New Zealand health rabbits [4 male and 4 female; 9 months-old; with body weight 3.10±0.22 kg;] were used. EFL was administered in a dose of 5 mg/kg (Baytril 5 % solution for injection, Germany). The "wash-out" period between the two methods of administration was 2 weeks. Blood samples (1 ml each) were collected from the catheterized *v. auricularis magna* at 0, 0.17, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8 and 12 h after EFL injection. Humor aqueous (300 µl) was obtained (following a preliminary 2 % lidocaine local anesthesia of the eye) by puncture with 0.5 ml disposable sterile syringes and G-28 injection needles from the anterior ocular chamber. EFL and CFL were determined by a validated HPLC method with UV detection. LOQ for EFL was 5 ng/ml and for CFL – 7.5 ng/ml. Pharmacokinetic analysis of the data was performed using the non-compartmental method. Pharmacokinetic parameters were calculated with TopFit, v.2.0. computer program.

RESULTS

Table. 1. Pharmacokinetic parameters of EFL and its metabolite CFL in rabbits after *i.v.* injection

Parameter	Units	Blood serum		Aqueous humor	
		EFL	CFL	EFL	CFL
$T_{1/2}$	h	2.42±0.4	4.41±0.8	5.64±0.8	6.28±0.1
MRT	h	3.37±0.1	5.35±0.8	7.25±0.9	8.53±0.1
V_{ss}	l/kg	3.55±0.1	4.16±0.3	23.5±1.3	11.4±0.2
Cl_B	ml/kg.min	22.5±0.9	48.2±0.2	57.1±0.2	67.5±0.4
$AUC_{0\rightarrow\infty}$	µg.h/ml	3.06±2.6	0.55±0.3	2.24±0.5	0.11±0.1
MR	%	–	18.2±0.3	–	4.73±0.2

Table. 2. Pharmacokinetic parameters of EFL and its metabolite CFL in a rabbits after *i.m.* injection

Paramter	Units	Blood serum	Aqueous humor		
		EFL	CFL	EFL	CFL
$T_{1/2}$	h	2.18±0.6	3.83±0.3	4.76±1.0	5.67±0.7
$AUC_{0\rightarrow\infty}$	µg.h/ml	2.56±0.1	0.29±0.9	1.73±0.9	0.08±0.1
MRT	h	3.96±0.3	4.5±0.7	7.3±0.5	6.8±
MAT	h	0.58±0.0	–	–	–
C_{max}	µg/ml	2.14±0.3	1.23±0.3	0.46±0.4	0.15±0.1
T_{max}	h	0.33±0.0	1.33±0.2	2.00±0.0	2.33±0.2
F	%	83.5±0.2	52.9±0.1	77.2±0.5	72.7±0.6
MR	%	–	11.4±0.5	–	4.80±0.3

$T_{1/2}$– half-life of elimination; MRT – mean residence time; MAT – mean absorption time; Cl_B – total body clearance; C_{max} – maximum serum concentration; T_{max}– time to reach peak serum concentrations; V_{ss} – steady-state volume of distribution; Cl_B – total body clearance; $AUC_{0\rightarrow\infty}$ – area under the concentration-time curve from 0 to ∞; F – absolute bioavailability; MR – metabolite ratio.

PHARMACOKINETICS OF TOBRAMYCIN IN PIGS

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Tobramycin (TB) is a broad-spectrum aminoglycoside antibiotic, which has limited veterinary applications. The purpose of this study was to characterise its pharmacokinetics in pigs following *i.v.* and *i.m.* injection.

MATERIALS AND METHODS

The experiments were conducted with 12 clinically health hybrid pigs (Large White x Landras), 6 male and 6 female, weighing 21-27 kg. The pigs were injected *i.v.* and *i.m.* with a 5 % aqueous solution. Between the two ways of application was provided a 30 days "wash-out" period. The antibiotic was *i.v.* injected in the right *vena auricularis*, and the *i.m.* application – in the neck muscles, near the years. The blood samples (1,5 ml each) were collected before the application of the antibiotic and at the 0.08, 0.17, 0.33, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h after injection. The concentrations of the antibiotic were determined using the agar-gel diffusion and a test-microorganism *Bacillus subtilis* ATCC 6633. The concentrations of the prepared standard solutions were in the range between 12.5 μg/ml and 0.006 μg/ml. The values of LOD and LOQ were 0.012 μg/ml and 0.024 μg/ml, respectively. The pharmacokinetic parameters of each pig were determined using the computer software TopFit v.2.0.

RESULTS

Table. 1. Pharmacokinetic parameters of tobramycin in pigs after *i.v.* and *i.m.* injection (mean±SEM)

Para-meter	Units	*Intravenous*		*Intramuscular*	
		Compart. method	Non-compart.	Compartme thod	Non-compart.
$T_{1/2\alpha}$	h	0.41±0.1	–	0.23±0.1	–
$T_{1/2}$	h	3.22±0.5	2.20±0.2	4.01±0.1	2.63±0.1
MRT	h	3.32±0.3	2.71±0.2	5.51±0.2	4.18±0.2
Vss	l/kg	0.45±0.07	0.36±0.1	–	–
AUC	μg.h/ml	85.37±4.7	79.90±4.0	42.4±4.6	43.0±4.6
C_{max}	μg/ml	–	–	19.1±0.4	20.7±0.5
T_{max}	h	–	–	0.51±0.1	0.43±0.03
$T_{1/2abs.}$	h	–	–	0.22±0.2	–
F	%	–	–	54.4±6.2	58.7±6.4

$T_{1/2\alpha}$– half-life of distribution; $T_{1/2}$– half-life of elimination; $T_{1/2abs}$– half-life of absorption; MRT – mean residence time;
Cl_B – total body clearance; C_{max} – maximum serum concentration; T_{max}– time to peak serum concentrations; V_{ss} – steady-state volume of distribution; $AUC_{0\rightarrow\infty}$ – area under the concentration-time curve from 0 to ∞; F – absolute bioavailability.

COMPARATIVE ANTINEOPLASTIC ACTIVITY OF DECITABINE, DINALINE, ERUFOSINE AND SAPONINE MIXTURE IN BREAST CARCINOMA CELLS IN REGARD TO OSTEOPONTIN EXPRESSION

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During the last few years breast cancer has become the most common malignancy among women. One of the most frequent and quality of life affecting consequences of the advanced disease is the metastasis to the bones. The osteopontin (OPN) glycoprotein is known to be responsible for bone metastasis and poorer survival prognosis when highly expressed in breast cancer and other malignancies. Our study was focused on two cancer cell lines – MCF-7 (estrogen receptor positive) and MDA-MB 231 (lacking estrogen receptor). We investigated the changes in the mRNA level of OPN in both cell lines after treatment with the following cytoreductive agents – dinaline (histone deacetylase inhibitor), decitabine (DNA methyltransferase inhibitor), erufosine (membrane-active alkylphosphocholine) and a mixture of two saponines, derived from the plant *Astragalus hamosus*, L. It was found that MCF-7 cells do not express OPN themselves but only 6 hours after treatment with dinaline a detectable band for OPN mRNA appeared and this OPN induction was dinaline concentration dependent. MDA-MB 231 cells responded in a similar manner to dinaline exposure, having clearly detectable starting level of OPN. On the other hand, no significant changes in the OPN level were found after treatment of both cell lines with decitabine, erufosine and saponine mixture. So, we could assume that the influence of dinaline on the OPN mRNA level is specific. It can be hypothesized that dinaline could modulate the growth of breast cancer cells in an unfavorable manner, also. Therefore, under effective OPN gene silencing the antineoplastic efficacy of dinaline could be significantly increased. In conclusion, our findings indicate that erufosine and the investigated saponine mixture, as well as the demethylating agent decitabine exerted strong promising antitumor activity in vitro and could have clinical relevance in breast cancer chemotherapy.

TWO APPROACHES FOR HPLC QUANTITATION OF ALPHA-TOCOPHEROL IN SERUM SAMPLES

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Aim: To establish a simple and rapid HPLC- procedure with UV-detection for quantification of α-tocopherol (TF) in small quantities of blood serum using different approaches for sample preparation.
Materials and methods: Two approaches for sample preparation were used: 1. liquid/liquid extraction; 2. microcolumn (Chromosorb P NAW 80-100 mesh) extraction. The hexane fraction obtained by both methods was evaporated to dryness, reconstituted in mobile phase and injected in the HPLC system. HPLC conditions: RP-column Nucleosil (25 cm x 0,46 cm) with UV-detection (295nm), mobile phase composed of 100% methanol, flow rate 1,0 ml/min. Standard solutions as well as and biologic standards spiked with known amount of TF were used for quantitative determination.
Results: The average recoveries of both methods for different concentrations varied between 86,6 and 99,4% (method 1); 80.4 and 104,9%, (method 2). The variation coefficients are 11,08 and 8,05% respectively. The percentage of accuracy variation is between 0.25-6.6% (method 1) and 4.4-13.2% (method 2).
Conclusion: Both methods are easy to use, rapid (sample preparation 20 min, HPLC analysis 15 min). Microcolumn extraction (method 2) requires smaller volume of serum (50 ul), than liquid/liquid extraction (method 1) - 200 ul and would be more suitable for TF analysis in pediatric practice. Method 1 was used for determination of TF levels in a group of patients with CVD.

TREATMENT OF NEUROPATHIC PAIN IN PATIENTS WITH DIABETIC POLYNEUROPATHY

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Introduction: Diabetic polyneuropathy is a common complication of Diabetes mellitus, resulting in significant invalidization. There are various clinical forms of diabetic polyneuropathy, but about 30-50% of the patients suffer from sensor or sensomotor polyneuropathy. Symptoms include paresthesia, dysesthesia, and disturbance of vibrating and deep sensitivity, muscle weakness, painful muscle cramps, decreasing or loss of deep tendon reflexes. The most common and disturbing symptom, however, is the neuropatic pain, presented in about ¼ of the patients.
Aim: The aim of this study is to evaluate the effect of different oral treatment to sensory symptoms and especially neuropathic pain. Three drugs influencing neuropathic pain – Lyrica, Carbamazepin, Gabaneural were compared according to their efficiency, adverse event and tolerance.
Materials and methods: 75 patients with diabetic polyneuropathy were physically and neurologically examined. The pain rate was assessed with The Visual Abalogue Scale (VAS). The mean age of the patients is 58±7 years. Forty-five percents are on insulin therapy and the rest 55% - on oral medication. All the patients have had Diabetes mellitus for minimum 5 years. The mean VAS assessment is 8 +/-0.75. All the patients were divided into three groups: first, receiving Lyrica - 450 mg/day; the second - Carbamazepin 600mg/day; and the third - Gabaneural – 900 mg/day. On the 5^{th}, 15^{th} and 30th day, all the patients were clinically and VAS examined.
Results: The results show decreasing of pain level and reducing sleeping disorders in all the three groups. Patients treated with Gabaneural and Lyrica had quick pain relief (on the 5^{th} day) and thus better result compared with the patients treated with Carbamazepin ($p<0,001$). There is not significant difference in decreasing pain-level in patients treated with Gabaneural and Lyrica. The patients tolerate Lyrica better ($p<0.00347$) than Gabaneural and Carbamazepin.

STUDY ON THE EFFECTS OF KETAMINE ON ANALGESIC TESTS AND ON LEARNING AND MEMORY IN RATS

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Background: Ketamine is intravenous anaesthetic with NMDA-glutamate receptors mechanism of action (1). NMDA receptors are involved in the antinociceptive actions of ketamine (2). **Methods:** Male Wister rats (10 per group) were chronically treated once daily with: 1st group saline 0.1 ml/100 g body weight; 2nd, 3rd and 4th groups – Ketamine 10, 15 and 20 mg/kg respectively. For active avoidance test an automatic reflex conditioner (shuttle box) was used. It was observed: number of avoidances, escapes and intertrial crossings. Step-through passive avoidance test was done with 2 days learning session and short and long memory retention test. Criteria were latency of reactions 180 s staying in the light chamber. Step-down passive avoidance test was performed with 2 days learning session and short and long memory retention tests. Criteria were the maximal latency of reaction 60 s staying on the platform. The hot-plate test was performed which evaluates the reaction time of the rats which are dropped on a heated surface and thus confronted with a heat stimulus applied to the plantar surface. The analgesy-meter was used which exerts a force that increases at constant rate. It was measured on the scale the force at which the animal felt pain.
Results: In active avoidance test the control rats learned the tasks and increased the number of avoidances on 3rd, 4th and 5th days ($p<0.05$) on learning session as well as on memory retention test ($p<0.05$). Ketamine in all doses used increased the number of avoidances on 4th and 5th days of learning session ($p<0.05$) as well as on memory retention test ($p<0.05$). Controls did not changed the number of escapes, but ketamine in the highest dose used decreased it ($p<0.05$) on learning and memory tests. The number of intertribal crossings was not changed by controls or ketamine-treated rats during learning and memory tests. In passive avoidance test step-through the controls and ketamine-treated rats (at all doses applied) increased the latency time ($p< 0.05$) during learning and short and long memory retention tests. In passive avoidance step-down test the controls and ketamine-treated rats (at higher doses applied) increased the latency time ($p< 0.05$) during learning and short and long memory retention tests. In hot-plate analgesic test the controls and ketamine-treated rats did not changed the latency of reaction. In analgesy-meter test the controls and ketamine-treated rats did not changed the latency of reaction. **Conclusion:** The obtained results allowed us to suggest that ketamine improved learning and memory processes and has no analgesic effect in doses applied.

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INVOLVEMENT OF A_2-ADRENERGIC AGENTS IN THE EFFECTS OF TYR-CIT-MIF-1 AND TYR-CAV-MIF-1 AFTER THREE MODELS OF STRESS

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Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 are newly synthesized analogues of Tyr-MIF-1 - endogenous peptide with opioid-like and antiopioid-like properties.
Noradrenaline is involved in various CNS functions, including modulation of pain and it's considered as a transmitter of the antinociceptive effects of μ-selective opioids.
Literature data showed that stress elicits antinociceptive effects. This is phenomenon named stress-induced analgesia (SIA). It has been categorized into opioid and non-opioid. It's known that Tyr-MIF-1 is able to inhibit the expression of some forms of SIA and adrenergic system is involved in this effect.
The aim of our study was to examine whether α_2-adrenergic agents (α_2-adrenoceptor agonist clonidine, a_2-blocker yohimbine, noradrenaline re-uptake inhibitor desipramine) are involved in the effects of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 after a 1-hour immobilization (IS), cold (CS) or heat (HS) stress. All drugs were injected intraperitoneally (i.p.) in male Wistar rats. The nociception was measured by paw pressure (PP) test.
Co-administration of clonidine, yohimbine or desipramine with each of peptides decreased significantly the pain threshold but in different way.
The results suggest that α_2-adrenergic agents are involved in the antinociceptive effects of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1. These effects are probably due to different interaction between μ- and α_2-receptors on presynaptic level, interactions of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 with opioid μ-receptors and involvement of opioid and non-opioid component in immobilization, cold or heat stresses.

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EFFECT OF NEW L-VALINE DERIVATIVES ON BRAIN FUNCTION IN EXPERIMENTAL MODEL OF AGGRESSION IN MICE

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Effective protection with amino acids and pyridine derivatives has been reported against memory deficit occurring in aggression and some mental diseases. The newly synthesized compounds include L-valine and nicotinic/isonicotinic acids (symbolized in the text as M6 and P6).
Our previous data demonstrated their low toxicity (over 2000 mg/kg orally and intraperitoneally) and a good therapeutic index (over 8).
The purpose of the study: To evaluate the influence of the compounds M6 and P6 on the cognitive processes in mice with experimental aggression.
Methods: The experimental model of aggression was developed in Albino mice with social isolation syndrome (for a period of 6 weeks). Isolated mice were divided in two groups - aggressive and non-aggressive animals. Both compounds (in single or repeated doses 125 and 250 mg/kg b.wt. i.p., 3 days) were applied. Several tests were performed on the 24th hour and on the 7th day after the last injection – step-through, hole-board, rota-rod, horizontal bar test and acetic acid test. Some differences in biogenic monoamine levels in hippocampus (dopamine-DA, noradrenaline-NA and 5-hydroxy triptamine-5-HT) were established in male Wistar rats treated with single doses of the compounds (250 mg/kg, b.wt. i.p). ANOVA was used for statistical assessment of the experimental data.
Results and discussion:
Our results show significant pharmacological potency of the compounds studied. The mechanism of the stable preventive effect of both compounds on damaged cognitive processes in aggressive animals is still not clear.
P6 demonstrated stronger effect on learning and memory (in normal and especially in aggressive animals), but M6 had a stronger and persisting analgesic effect. The variations in the effects of both compounds can be explained with their positional isomery and difference in some physico-chemical parameters.
The compounds also changed the functional activity of the dopaminergic neurotransmitter system, modulating the levels of biogenic monoamines in hippocampus. In single doses they increased 5-HT levels in rat hippocampus (significant only for P-6) and P-6 also increased NA levels.

NEW L-VALINE PEPTIDOMIMETICS - IN VITRO AND IN VIVO STUDIES

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Four newly synthesized peptidomimetics were studied as potential pharmacological agents. The amino-acid L-valine is bound to nicotinic (m-pyridinic) acid – [M] or isonicotinic (p-pyridinic) acid [P] on one side and to an alkyl spacer containing 3 or 6 methylene groups on the other side. Literature data for similar compounds suggest possible biological activity.
The purpose of the study: To evaluate the toxicity and pharmacological activity of the compounds (in vitro and in vivo). **Methods:** Toxicological studies - in vitro toxicity (on cell cultures F4N) and in vivo, on Albino mice. Antiviral activity (against Herpes simplex virus) and chelating activity to Fe (II) in blood plasma. Compounds were applied in single or repeated (5 days) doses (125 and 250 mg/kg b.w. i.p.) on Albino mice. The processes of learning and memory (step-down test) and nociception (acetic acid test) were studied. Their interactions with some CNS- active compounds (hexobarbital-HB and pentylene tetrazole - PTZ) were examined. For statistics the t-test of Student Fisher and ANOVA were used.
Results and discussion: In vivo and in vitro experiments showed very low toxicity of the compounds (intraperitoneal and oral- over 2 000 mg/kg and cytotoxicity lower than this of vitamin C) as well as good therapeutic index (over 8). The antiviral activity against Herpes simplex virus was moderate and probably is related to the established chelating activity toward Fe (II). Stronger chelating activity in vitro of the compounds with 6 spacers correlated with their better activity (in comparison to the compounds with 3 spacers).
The compounds are neuropharmacologically active agents (especially the 6-isomers). Two of the compounds (M6 and P6) improved the processes of learning and memory and had significant analgesic and anticonvulsive activity. Comparing data in vivo and in vitro allows us to assume that their lipo-solubility and chelating ability are probably important for their pharmacological effects. The compounds were able to modify the effects of some CNS-drugs. HB sleeping time was prolonged by M-6 and was antagonized by P6 in acute treatment, but after 5 days of administration of M-6, it was shortened significantly. The mechanism of interaction is probably not only on CNS level, but on the metabolic level too. The differences and varieties in their effects are obviously due to their positional and structure isomery.

INFLUENCE OF ANTIVERTIGINOUS FIXED COMBINATION OF CINNARIZINE AND DIMENHYDRINATE ON SENSORY-MOTOR AND INFORMATION PROCESSING IN HEALTHY PERSONS

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The frequent adverse reactions associated with the use of antivertiginous drug are the decreases in alertness and vigilance and impairment of performance. The analysis of event related potentials (ERPs) and reaction time (RT) yielded valuable data of attention dependent information processing and executive functions. The present study focused on the effects of Arlevert (a fixed combination of cinnarizine 20 mg and dimenhydrinate 40 mg) on central information processing by investigation of auditory ERPs, RT and psychometric tests after multiple dosing in healthy persons with aim to evaluate adverse reactions of this drug.
Method: 21 healthy volunteers received 4 doses (within 24 h) of Arlevert (ARL), dimenhydrinate (DH) 50 mg, or a placebo, in randomized order at 1-week intervals. EEG was recorded from Fz, Cz, and Pz and computer-generated 100 tones - high and low (3:1) were presented in passive listening series and two discrimination task conditions - oddball paradigm and binary sensomotor setting. Auditory ERPs, RT and psychometric tests were assessed before as well as 60 and 150 minutes after the intake of the 1st (Day 1) and the 4th (Day 2) dose of study medication.
Results: The evaluation of adverse events supports a good tolerability of the three treatments with the lowest rate of ARL. None of the medications affected the latency and amplitude of the sensory ERP component N100, neither under passive listening nor under discrimination task conditions. The latency of P300 in response to the rare target tones (oddball paradigm and binary series), showed significant delays after 4 doses of DH and no significant differences between ARL and either DH or placebo. The P300 amplitude showed the greatest decreases under DH in both active series, with no significant differences between ARL and either DH or placebo. The medications (ARL, DH, and placebo) did not significantly prolong RT nor did they impair the performance of psychometric tests.
In conclusion, the results of the present study gave no evidence for an impairment of central information processing and psychomotor performance after multiple dosing of ARL in healthy volunteers, and showed no significant difference between ARL and placebo.

PREVALENCE OF POTENTIAL CLINICALLY SIGNIFICANT DRUG-DRUG INTERACTIONS INVOLVING STATINS IN THE PRE-HOSPITAL AND HOSPITAL PRESCRIBING

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Drug-drug interactions (DDIs) represent an important clinical problem. They substantially increase the incidence of adverse drug reactions that may be severe enough as to require hospitalization.
Statins are widely used in the primary and secondary cardiovascular prevention. The safety of statins is well documented; yet possible complications due to their muscle and liver toxicity, albeit rare, should not be underestimated. The risks are considerably elevated when these drugs are combined with other drugs capable of potentiating their effects. Since most of the statins are metabolized by the cytochrome P-450 (CYP) enzymes, their plasma levels are sensitive to CYP inhibitors and inducers. Another group of risky interactions are those in which statins increase the likelihood of other drugs' toxicity, such as digitalis and oral anticoagulants.
The aim of the present study was to assess the incidence of potential DDIs (pDDIs) that patients are exposed to at hospital entry and at discharge.
The study was conducted in the 1st Clinics of Cardiology of the University Hospital St Maria in Varna. A total of 1235 patients' charts were examined for the period from July 2007 to April 2008. Of these, 162 patients were identified as receiving statin therapy at admission and 357 at discharge. Results: The statin prescribed most commonly was simvastatin – 34.6% at admission and 58.8 % at discharge; correspondingly, most of the DDIs found are related to this drug. Next were lovastatin (26.5%) and atorvastatin (22.8%) at admission and atorvastatin (16.2%), rosuvastatin (9.5%) and lovastatin (9.2%) at discharge. The drug least used was pravastatin (1.8%) at admission; it did not appear at discharge. The total number of potential clinically significant DDIs was 41 (25%) at admission and 86 (24%) at discharge. The prevalence in both cases is generally the same, but the structures of the exposures differ. Amongst the CYP inhibitors leading to potentially major DDIs with many statins, amiodarone was the most commonly encountered (~4%). There were no combinations with non-DHP calcium antagonists at discharge, while 3.7% of the admitted patients received a statin plus either verapamil (5) or diltiazem (1). The co-prescription with acenocoumarol and cardiac glycosides prevailed in patients leaving the hospital.
The exposure to the pDDIs is discussed in respect to possible causes and outcomes, clinical and laboratory supporting findings, and in comparative manner with other similar studies.

MODEL OF PROSTATE CANCEROGENESIS IN N-METHYL-N-NITROSOUREA (NMU)-TESTOSTERONE-TREATED RATS WITH AND WITHOUT FRUCTOSE-FEEDING

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Dysmetabolic syndrome including carbohydrate and lipid disturbances is increasingly studied as a possible important factor in prostate cancerogenesis.
The aim of our study was to induce prostate cancer in rats with *N*-methyl-*N*-nitrosourea (NMU)-androgen-treatment and to study effect of fructose-feeding in rats (an well established model of dysmetabolic syndrome).
Design and methods. 100 rats were divided in 4 groups – control (C-group), group with induction of prostate-cancerogenesis (P-group), fructose-treated – (F-group) and group with fructose-treatment and induction of prostate-cancer (PF-group), n=25 each.
Starting at 6 weeks of age and continuing for the next 3 weeks, all rats from groups P and PF received daily intraperitoneal injections of the luteinizing hormone - releasing hormone antagonist followed by subcutaneous injections of testosterone. All rats were monitored daily, and rats showing any signs or symptoms of morbidity, including reduced food intake or weight loss, were killed. The remaining rats were killed at the study end (after 1 year). Pathomorphological evaluation of rat prostate was done, as well as biochemical evaluation of blood – glucose, lipid profile, adiponectin, insulin.
For the whole period of the study for treatment of groups F and PF, the rats were given 10% fructose solution in drinking water.
Results. 20 rats from P-group and 12 – from PF-group developed prostate carcinoma. Rats from groups C and F did not developed carcinoma at all. Glucose, triglycerides and insulin were significantly higher in groups F and PF, as compared with groups C and P ($p<0.05$). Adiponectin was significantly lower in P, F and PF-groups, as compared with C group ($p<0.05$).
Discussion. Dysmetabolic syndrome, including hyperinsulinemia, hyperglycemia and hypertriglyceidemia was induced with 10% fructose solution used instead of drinking water (groups F and PF). *N*-methyl-*N*-nitrosourea (NMU)-androgen-treatment induced prostate carcinoma in rats. The incidence was lower when dysmetabolic syndrome was present (PF group) than group P (without fructose treatment).
Conclusion. Fructose feeding in rats could have protective role against prostate cancerogenesis.

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INFLUENCE OF PHYTOADAPTOGENS ON DIAZEPAM WITHDRAWAL IN RATS

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The anxiolytic-like effects of phytoadaptogens Hypericum perforatum L. (St. John's wort) (HYP) and Panax ginseng (PG) were assessed using the elevated plus-maze and forced swimming test in male Wistar withdrawn rats. It is well documented that withdrawal from normal dosage benzodiazepine treatment produced in animals typical anxiety response and can result in a number of symptomatic patterns coming on within 1-4 days of discontinuation. After 21 days of treatment with diazepam (2 mg/kg IP) rats were tested 24-, 48-, 72- and 96 h after the last injection in the elevated plus-maze test of anxiety and the locomotor activity in optovarimex was checked. An increase in the percentage number of entries onto the open arms in the elevated plus-maze and in horizontal and/or vertical locomotor activity in optovarimex is interpreted as an anxiolytic response. Stress-related behavioural alterations were evaluated also by the forced swimming test (Porsolt test) on the 96th h after the last diazepam treatment. Two swim sessions were conducted in the initial 13-min pretest; a 6-min test followed 24 h later. The total period of immobility during the 6-min retesting period was recorded and data were assessed using the Student's t test. All data were analyzed by one-way ANOVA and post-hoc comparisons for statistical significance were made by the Dunett's test.
Compared with control-treated rats, withdrawn animals showed significant decreases in the percent number of entries onto open arms of the plus-maze on the 72nd and 96th hour ($F\ 1.11 = 6.33$; $p<0.03$; $F\ 1,11 = 6.55$; $p<0,03$) and reduced vertical locomotor activity in optovarimex on the 96th h after the last diazepam injection ($F1.11 = 13.19$; $p<0.0025$), indicating an anxiogenic response. HYP (300 mg/kg, PO) and PG (30 mg/kg PO) applied during the 21 – diazepam treatment and 1 h before testing significantly reversed the anxiogenic effects after diazepam withdrawal on the 48th; 72nd and 96th hour in the elevated plus-maze ($F1.11 = 7.4$; $p<0.04$; $F1.11 = 6.2$; $p<0.03$; $F\ 1,11 = 6.8$, $p< 0.03$; $F1.11 = 8.4$; $p<0.05$; $F1.11 = 6.8$; $p<0.03$; $F\ 1,11 = 7.3$, $p< 0.04$)) respectively, and were ineffective in optovarimex. In the forced swimming test chronic administration of both diazepam and HYP ($F\ 1.11 = 9.92$; $p<0.007$) or diazepam and PG ($F\ 1.11 = 19.92$; $p<0.0003$) significantly decreased the duration of immobility as compared with withdrawn rats.
Our present findings are consistent with earlier proposals that concurrent treatment with HYP attenuates nicotine withdrawal in mice and is effective in smoking cessation. We suggest that chronic complement treatment with HYP or PG may prove to be a promising strategy for preventing the development of benzodiazepine withdrawal-induced anxiety.

PHYTOTHERAPEUTICAL APPROACH IN A CASE OF VIGILANT COMA AFTER REPEATED BRAIN SURGERY BECAUSE OF MALIGNANT ASTROCYTOMA

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The phyto therapeutical approach in a case of 35 years old patient in vigilant coma after repeated brain surgery is discussed. The onset of the disease was three years ago with the ordinary symptoms of brain tumour (splitting headache, instability step, daze, impaired vision etc.) and according to the i) pre- and post operative computer tomography, ii) postoperative histological verification and iii) the specific course of the disease, the diagnose "astrocytoma anaplasticum cum recidiva and hydrocephalia" was confirmed. Between March 2003 – May 2006, the patient has been operated five times. Radiological (30 heatings) and chemotherapy treatment with Temodal was almost lack of effect. After the 5th surgical intervention (May 2006 by Prof. Gabrovski, National Institute for Emergency Medical services), the patient fall in a vigilant coma. Complex phytotherapy with the Antioxidant complex Novomin (AC-N) and the phytoadaptogen Rodhiola rosea L. (Rr) started on May 18, 2006. As a result of experimental and clinical studies (over 2.000 cancer patients), the AC-N has been developed 20 years ago by (1). The product displays definite antioxidant (protective) effect only in the healthy tissues, whereas in the cancer cells it exerts prooxidant (tumor-damaging) action. Therefore, Novomin is the first and so far the only phytopreparation having the so-called alternative action. The AC-N normalizes the metabolic processes in the healthy tissues and simultaneously it exerts strong selective tumour-damaging action on the DNA-level in the cancer cells. Rr is has been extensively studied as an adaptogen with various health- and especially brain-promoting effects (2 etc). The common result of this complex therapy was the astonishing prompt recovering process and in the course of six month's the patient was completely rehabilitated. In the moment he works in a furniture factory in Sofia, goes every day into sports for bicycle, skateboard and folk dancing. The retracing with repeated brain magnetic resonance (July'07 and January'08) manifested the total brain recovery without any malignant recurrence, hydrocephaly or other pathomorphological findings.

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MODULATION OF THE PI3K/AKT SIGNAL TRANSDUCTION PATHWAY BY ERUFOSINE IN MYELOMA CELLS

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In Multiple Myeloma the *Akt* pathway is activated by IL- 6 and IGF and acts downstream of the phosphatidyl-inositol-3 kinase (PI3K). The PI3K/Akt signalling pathway is constitutively activated in MM thus rescuing MM cells from PTEN-mediated apoptosis. Alkylphosphocholines (APC) were derived from ether lipids, but their clinical use is limited by side effects, e.g. gastrointestinal toxicity and hemolytic properties. Erucylphospho-N,N,N-trimethyl-propyl-ammonium (erufosine), an APC-derivative with a 22 carbon atom chain and a cis-13, 14 double bond, is the first derivative suitable for intravenous application, because of the lack of haemolytic activity. The aim of this study was to determine if the new alkylphosphocholine erufosine has the ability to modulate one of the most important signal pathways- PI3K/Akt in myeloma cells and does this modulation correlate with the cytotoxicity of erufosine. Two human myeloma cell lines - RPMI-8226 and U-266 were used. The cell survival fraction was determined by the MTT dye-reduction assay. Immunoblot served to detect specific protein changes. On the molar basis, erufosine showed higher antineoplastic activity against RPMI-8226 cells as compared to U-266 cells. After 6h exposure of RPMI-8226 cells their pAkt was up-regulated. Nearly double down-regulation was observed after 14 h at 40 μM concentration in RPMI-8266. In U-266 cells the pAkt expression pattern was quite different. After 6h exposure, pAkt was two times lower at concentrations of 5 μM and after 14 h it disappeared almost completely at the same concentration. Erufosine concentrations of 5-40 μM induced activation of caspases -3 and -9 in RPMI-8226 cells after short exposure. PARP cleavage was recorded as well. Bcl-XL/Bcl-2-associated death promoter (BAD) was found to be activated. Taken together our data indicate that erufosine is a potent cytotoxic drug that effectively kills MM cells. In addition, erufosine was shown to modulate the phosphorylation of pAkt. Erufosine could be a useful drug for MM because of its unusual mode of action by targeting membrane associated signal pathways and having stimulating activity on normal hematopoiesis.

ANTITUMOUR EFFECT OF PROBIOTICS

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Cancer is a significant cause of mortality in all over the world. Compelling experimental and epidemiologic evidences indicate that diet and nutrition are key factors in modulating colon cancer onset and progression. Identifying dietary constituents with antitumour activity and investigating their mechanisms of action may lead to significant advances in the prevention of the neoplasms of the gastrointestinal tract. The human colon can be described as a complex microbial ecosystem, comprising several hundred bacterial species. Some of these enteric bacteria such as lactic acid bacteria or Bifidobacteria are beneficial to the host and have been shown to exert beneficial antimutagenic and anticarcinogenic properties. It has been proved that regular consumption of dairy products fermented with probiotic lactobacili may elicit antitumour effects.
These effects are attributed to the inhibition of mutagenic activity, decrease in several enzymes implicated in the generation of carcinogens, mutagens, or tumor-promoting agents, suppression of tumours, and the epidemiology correlating dietary regimes and cancer. Nowadays there exist numerous possibilities for the development of application schemes of probiotics from lactobacillus for the stimulation of several functions of the immune system, creation a new forms of antitumour drugs and combination of them with oral vaccines for improving their immunogenicity. In this review, several significant issues of the rising role of probiotics in cancer prevention are outlined.

PROFESSOR DR. VLADIMIR ALEXIEFF (1879-1948): A CLASSIC OF BULGARIAN INTERNAL MEDICINE AND PHARMACOLOGY

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Professor Dr. Vladimir Alexieff is a distinguished Bulgarian clinical and pharmacologist, one of the founders of Medical Faculty (1918) in Sofia. Born in Gabrovo, he graduated at the Medical University, Geneva, Switzerland, and continued to work in the Clinic of Internal Diseases until 1911. He served as a head of the Pharmacology and Therapeutic Department (1920-1948). Prof. Alexieff is the author of a series of manuals and textbooks of pharmacology belonging to the Bulgarian classical heritage in medical literature. Developer of many original medicaments for treatment of sepsis, respiratory infections, thyreotoxicosis, etc., his drug Pulmochin in ampullar form was in medical use until 1982 in cases of pulmonary infection.
Prof. Alexieff proposed the so-called “abortive therapy”, i.e. treatment even to the first days of septic state with his original medicament Chromocrysin (Polychrom),
composed of Tripaflavin, Chininum and colloidal gold. He also created the preparation Thinjodin, containing thiosinamine (Allylthiourea), and introduced it in into Basedow’s disease treatment in 1938, i.e. five years earlier than in other countries. So, Prof. Alexieff is the very first clinician having applied allylthiourea as thyreostatic drug for thyreotoxicosis treatment.

EXPERIMENTAL CLINICAL STUDY OF THE EFFECT OF THE PROBIOTIC BIOMILK IN CASES OF LIVER INJURIES

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Probiotics, containing lactobacilli, have beneficial effects proved in the prevention and treatment of gastrointestinal and liver diseases, cancer, infections, etc. Viral hepatitis is an infection, which is of great social importance and it damages mainly the liver and increases the liver enzymes in blood.
The aim of our work is to study the effects of chronic (30 days) per oral administration of the probiotic *Biomilk,* containing original *Lactobacillus bulgaricus,* on the biochemical liver indices (AST, ALT, AP, GGT) in cases of acute liver damages: patients, suffering of acute viral hepatitis and experimental animals with CCL_4-induced hepatotoxicity. 308 patients with acute viral hepatitis have been investigated in this clinical study. These 308 patients were observed in the period from November 2006 to december 2008. Hundred sixty-six of them have been taken 500 g *Biomilk* daily for 30 days. The control group is 142 patients, treated with the standard therapy. We study the dynamics of bilirubin, AST, ALT, AP, GGT, cholesterol and total protein. The clinical and biochemical indices were investigated before treatment and 2 weeks, 1 month and 2 months after starting the treatment.
In patients, treated with probiotics we found faster decrease of the total bilirubin, AST, ALT, AP, cholesterol and total protein as compared to the control group. The hospital stay of the group treated with *Biomilk* group was shortened.
Twenty eight Wistar rats were used in the experimental study, 7 rats in a group. 1600 mg/kg *Biomilk* was given to the rats for 30 days, and the last 2 days of the experiment to the rats was given 0.2 ml/kg carbon tetrachloride (CCL_4). The indices AST, ALT, AP, GGT were studied. The levels of the plasmatic AST, ALT and GGT in the animals, treated in advance with *Biomilk* and then put to the influence of CCL_4 were vastly lower than those of the animals with CCL_4 ($p<0.0001$).
Our information shows that using the probiotic *Biomilk* has a beneficial effect on the biochemical changes in the liver in the two studies (decrease of the increased plasmatic levels of AST, ALT and GGT, which indicate damages of the liver cells).
The results cogently show the capacity of the probiotic *Biomilk* to protect liver of toxic and viral damages.

CHANGES IN INTERPULSE INTERVAL VARIABILITY AFTER APPLICATION OF NOCICEPTIN ANALOGUE [Dap^9]N/OFQ(1-13)NH_2 IN CONSCIOUS RATS

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The aim of our study was to compare the influence of nociceptin analogues N/OFQ(1-13)NH_2 and [Dap9]N/OFQ(1-13)NH_2 on the interpulse interval variability in conscious Wistar rats. The animals were divided in two groups: treated with nociceptin analogue N/OFQ(1-13)NH_2, (n=10) and treated with [Dap9]N/OFQ(1-13)NH_2, (n=10). Under general anaesthesia the femoral vein for drug application and femoral artery for blood pressure wave registration were catheterized. 24 hours after surgical preparation the blood pressure wave was registered directly through pressure transducer Gould Statham P23, connected to data acquisition system MP100 WS. The blood pressure wave registration was performed in control period and 5 min after N/OFQ(1-13)NH_2 or [Dap9]N/OFQ(1-13)NH_2 application (100 nmol/kg) during nine 10 min long consecutive intervals. The interpulse interval (IPI) was determined in terms of time between two consecutive diastolic minimums of the blood pressure wave. In spectrograms for IPI derived by Fast Fourier Transform algorithm (Lab View 3.1.1) the spectral power (P) in low (LF), mid (MF) and high (HF) frequency band were studied. Sympatho-vagal balance was determined by the relation P_{MF}/P_{HF}. The application of N/OFQ(1-13)NH_2 led to a decrease of P_{LF} in the first three 10 min long intervals from 7.76±0.69 to 3.04±0.66 ms^2 in the first, to 2.14±0.92 ms^2 in the second and to 2.71±0.99 ms^2 in the third interval, $p<0.05$). In contrast, the application of [Dap9]N/OFQ(1-13)NH_2 did not change P_{LF}. N/OFQ(1-13)NH_2 led to a decrease of P_{MF} in the first interval from 1.13±0.22 to 0.40±0.13 ms^2, in the second to 0.50±0.07 ms^2 and in the third to 0.58±0.13 ms^2, ($p<0.05$). The application of [Dap9]N/OFQ(1-13)NH_2 also led to a decrease of P_{MF} but only in first and second intervals: from 0.79±0.09 to 0.43±0.07 ms^2 and to 0.44±0.06 ms^2. The P_{HF} did not change after N/OFQ(1-13)NH_2, but after [Dap9]N/OFQ(1-13)NH_2 application decreased from 1.83±0.09 to 1.18±0.08 ms^2 in the first 10 min long period and this effect was remained to the end of the experiment, ($p<0.05$). The application of N/OFQ(1-13)NH_2 led to a decrease of P_{MF}/P_{HF} ratio during first, second and third 10 min interval as a result mainly of a decrease of P_{MF}, ($p<0.05$). However, the established decrease of P_{MF}/P_{HF} relation in the first 20 min after [Dap9]N/OFQ(1-13)NH_2 application, ($p<0.05$) is a result of a decrease of both P_{MF} and P_{HF} but after that to the end of the experiment is a result manly by a decrease of P_{HF} ($p<0.05$).

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TREATMENT OF ELDERLY AND SENILE PATIENTS WITH ACUTE INFECTIOUS DISEASES

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Introduction: Nowadays, one third of all patients are elderly people. Extreme ages are appraised as periods of increased susceptibility to infections due to various causes. Changes in the cell-mediated and humoral immunity caused by ageing process, decreased physiologic functions, multiple chronic conditions, immunosuppressive drugs usage and living in communities are responsible for the high infections frequency among the elderly population. A major part of the treatment expenses realize in the last few months before death.
Methods: A total of 439 patients from 60 to 88 years (median age of 72.82±1.85 years) hospitalized in the Infectious diseases clinics of Saint Marina Hospital of Varna for the period 2000-2007 were investigated. Of them 67.88% (298) were elderly, senile were 32.12% (141). There were 118 patients with diarrhoeal syndrome, 132 with Mediterranean spotted fever, 40 with infections of the nervous system, 43 with erysipelas, 74 with Lymeborreliosis, and 35 with acute viral hepatitis.
Objectives: To propose an algorithm for the treatment of some of the commonest infectious diseases in patients over 60 years of age.
Results: In the investigated age group of 60+ severe course of the disease and of moderate severity were observed as follows: in 87% of the patients with diarrhoeal syndrome; 89.39% of those with Mediterranean spotted fever; 82.50% of the patients with infection of the nervous system; 71.88% of those with acute viral hepatitis; 65.12% of the patients with erysipelas. Thirty eight of the investigated patients died – 18 of them women and 20 men between 60 and 84 years, median age of 71.08±2.13. Lethality was 8.65%**.** Direct cause of death in all of them was cerebral oedema and/or pulmonary oedema with consecutive acute respiratory and circulatory failure. Delayed hospitalization in the deceased patients was observed (3.73±1.47 days after the appearance of neurological symptoms), as well as multiple chronic conditions and poor socio-economic status.
Conclusion: 1. The predominance of severe forms of the infectious diseases and forms of median severity in patients of 60+ supposes hospital treatment.
2. Establishes the necessity of etiologic treatment of all hospitalized patients over 60 years of age with infectious diseases.

NEW DERIVATIVES OF "OLD" SUBSTANCES: 4-HYDRO-XYCOUMARINS (4-HC) AND 8-HYDROXY-2-STYRYLQUINOLINES (8-SQ) WITH ANTI-HIV ACTIVITY IN CELL CULTURE

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Aim: Rational synthesis of new derivatives based on substitutions in general structural formulas of 4-HC and 8-SQ. To check the new derivatives for anti-HIV-1 activity in cell culture.
Materials and Methods: MT-2/MT-4 cells were infected with HIV-1 IIIB and the derivatives were added to growth medium in different concentrations. The antiviral effect was measured by cytopathic effect (MTT test) and reverse transcriprase (RT) activity inhibition. After that, the target and mechanism of action of inhibiting compounds were studied for effect on RT, HIV protease, developing of resistant mutants by increasing pressure of the active derivatives, sequencing and molecular docking.
Results: Three active 4-HC showed anti-integrase activity, two 8-SQ showed RT inhibiting activity and two 8-SQ were with anti-protease plus anti-integrase activity.
Conclusions: Here we show the steps of an algorithm for search and targeting of novel derivatives for anti-HIV activity in cell culture.

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METHYLPHENIDATE MODULATES SENSORIMOTOR INTEGRATION IN HEALTHY ADULTS AS REFLECTED BY NEUROELECTRIC OSCILLATIONS IN A GO/NOGO TASK

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Methylphenidate (MPH) is a psychostimulant drug acting mainly on the dopaminergic and noradrenergic systems. It is widely used in the treatment of attention-deficit/hyperactivity disorder (ADHD). Behavioural and pschophysiological studies have shown that MPH affects executive functions at the level of both sensory and motor systems. Thus suggested effects of MPH on sensorimotor integration can be assessed by means of event-related neuroelectric oscillations (Yordanova et al, 2004).
Fourteen healthy subjects (8 male, aged 20-40 years) performed a visual Go/NoGo task (S_1-S_2 paradigm). MPH (20 mg) or placebo was administered using a randomized, double-blind, cross-over design. Event-related electroencephalographic (EEG) activity after S_2 was recorded. Time-frequency decomposition (wavelet analysis) was applied to evaluate power dynamics in delta, theta, slow and fast alpha, and beta frequency bands.
At the performance level, faster reaction times and a trend towards less impulsivity errors under MPH vs. placebo were observed. MPH effects on EEG were specific for Go-trials at parietal locations. MPH enhanced slow alpha total power (8.29-9.68 Hz) and fast alpha total power (10.32-14.45 Hz) within the first 300 ms post-stimulus associated with motor production.
Conclusions: It might be proposed that a single dose of 20 mg MPH modulates early visual information processing related with motor response generation in healthy adults.

Key words: Methylphenidate, sensory and motor systems, sensorimotor integration, event-related oscillations, Go/NoGo task, Wavelet analysis, EEG

SERUM LEVELS OF CORTISOL AND PROLACTIN AND ANTIPSYCHOTIC TREATMENT

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According many studies, mental disease are connected in one way or another with desynchronization of brain activity therefore with changes in serum levels of two basic hormones – cortisol and prolactin.
In this connection, we made our goal to study the level of serum prolactin and cortisol as well as it's rhythm at the patients suffering from schizophrenia. To establish the influence of antipsychotic drugs for these patients under studied parameters, 25 patients were examined. The study was lead at the period, in which the patients did not take antipsychotic drug for at least 30 days and after remission established clinically and with PANNS.
For objectivity of asynchronization of brain activity, EEG examination was conducted at all patients at the beginning and at the end of study. The examination is consistent with increasing in prolactin levels by antipsychotic drugs.
Conclusion: Homeopathic non-specific hyposensitization therapy in IAR and PAR in childhood is a safe and effective alternative method of sublingual immunotherapy.

SYNERGISTIC DRUG INTERACTIONS OF ERUFOSINE WITH AS_2O_3 OR DECITABINE IN CHRONIC MYELOID LEUKEMIA CELLS

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Chronic myeloid leukemia (CML) is a clonal disorder of the haematopoietic stem cells. It is characterised with proliferation of the malignantly transformed haematopoietic progenitor cells. Erufosine (erucylphospho-N,N,N,-trimethyl-propylammonium, ErPC3) is a new compound with antineoplastic properties. It belongs to the third generation alkylphosphocholines (APCs) that are inhibitors of protein kinase C, protein kinase B and of phospholipase C. It selectively damages malignant cells without affecting the normal haematopoiesis. However, the mechanism of action of erufosine remains rather unclear. Arsenic trioxide (As_2O_3) was reported to exert anti-proliferative and apoptosis-inducing effects in human leukemic and solid tumour cells. Decitabine is a DNA methylation inhibitor, and its use initiates genome-wide demethylation and then leads to reactivation of methylation silenced genes, e.g. tumour-suppressor genes. In order to assess the increase in efficacy, we evaluated the combinatory interaction of erufosine with As_2O_3 or decitabine against two CML derived cell lines - the high resistant K-562 and the more sensitive BV-173 cells. In addition, we focused on the influence of erufosine and As_2O_3 on cell proteins involved in the apoptotic signal pathways. The viability of the cells after single and combined treatment was estimated using the MTT-dye reduction assay. Cytoplasmatic and nuclear lysates.
from treated with erufosine or As_2O_3 K-562 cells were prepared and subjected to Western blot. The consecutive treatment of K-562 and BV-173 cells with the antitumor drugs mentioned above led to additive or synergistic drug interactions. Combination of erufosine with decitabine in BV-173 led to synergism, but the same combination in K-562 cells caused only additive or antagonistic effects. Combination of erufosine with As_2O_3 led to additive or synergistic effect in both cell lines. Erufosine caused increases of Bad, but also of Bcl-XL expression. Bcl-XL is an anti-apoptotic member of the Bcl-2 family that plays a central role in CML-derived cells. Treatment of K-562 cells with As_2O_3 led to decrease in the expression levels of Bcl-XL after 48 h incubation. Our findings suggest that some of the combinations used, eg. erufosine with decitabine or As_2O_3 potentiated synergistically the efficacy of erufosine against CML cells. Therefore, the synergistic drug interactions found could be beneficial for patients with CML, especially for those developing resistance towards conventional treatment. The decrease of Bcl-XL levels after treatment with As_2O_3 could be the explanation for the synergistic drug interaction found in K-562 cells. In conclusion, combinations of erufosine with arsenic trioxide or decitabine could have clinical relevance.

CHANGES IN THE SUBSTANCE P-IMMUNOREACTIVITY OF THE RAT RECTO-ANAL REGION IN AGING

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Background and aim. Substance P (SP) has been first identified in extract of brain and gut by its powerful stimulatory effects on gastrointestinal muscle. The recto-anal region is innervated by extrinsic and intrinsic nerves. A number of neuropeptides have been suggested to participate in the regulation of the intestinal motility. The age-related changes in the distribution of SP-immunoreactive nerve structures in the distal part of the rat recto-anal region were investigated.
Methods. The presence of SP was studied in tissue samples from intestinal wall of the distal rectum, anal canal and internal anal sphincter of 15-day-old, 3-month-old and 26-month-old Wistar rats by using immunohistochemistry.
Results. In 15-day-old rats the myenteric plexus of the distal rectum and anal canal was well outlined by numerous SP-immunoreactive varicose nerve fibres, encircling in a basket-like manner the immunonegative perikarya. In the circular muscle layer nerve fibres and small nerve bundles ran parallel to the muscle cells. In the longitudinal muscle layer, only occasional nerve fibers were seen in-between. At the level of the internal anal sphincter no myenteric ganglia were present. Here, thin varicose fibres ran parallel to the smooth muscle cells. In 3-month-old rats, a large number of intensely stained SP-immunoreactive nerve fibres were found. In the circular muscle layer thicker nerve strands were observed. In 26-month-old rats the density and staining intensity of SP-immunopositive nerve fibers in the myenteric plexus was lower than in 3-month-old rats. Similar changes in the distribution of SP-immunostained fibers in the internal anal sphincter were observed.
Conclusion. The degenerative alterations in SP-containing fibers appear to play a role in the anorectal motility and sphincter control.
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AMANTADINE INFUSIONS FOR HYPOKINETIC "OFF" STATE IN PARKINSON'S DISEASE

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Late stage Parkinson's disease (PD) is characterized by frequent motor fluctuations such as "wearing off", "no on", "unpredictable off" phenomena, freezing of gait. These disabling symptoms necessitate urgent correction of treatment.
We studied the effect of amantadine sulfate infusions on hypokinetic crises in 11 hospitalised PD patients (7 men), aged 68.64 years, with 8.18 years of mean disease duration, at Hoehn-Yahr stage III and IV (3.27). Disease severity was assessed by UPDRS. Mean daily dose of levodopa was 1245 mg and remained unchanged during the study period.
Amantadine sulfate infusions of 500 ml/200 mg were i.v. administered once daily, for 5 days. Infusions were well tolerated and no side effects were observed.
Severity and occurrence of hypokinetic "off" state decreased and after day 5 mean UPDRS total and UPDRS motor scores lowered.
We confirm the importance of amantadine sulfate infusions as a valuable option in the adjunct therapy of hypokinetic "off" state of Parkinson's disease.

TOLTERODINE FOR UROVESICAL AUTONOMIC DYSFUNCTION IN PARKINSON'S DISEASE

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Studies performed in Parkinson's disease (PD) have demonstrated the presence of urovesical autonomic dysfunction in up to 97% of patients, the most frequent symptoms being nicturia, frequency and urgency.
We studied the effect of tolterodine on micturitional disturbances in 17 PD patients (13 men), aged 62.18 years, with 4.41 years of mean disease duration, at Hoehn-Yahr stage II-III (2.29). Disease severity was assessed by UPDRS. During the 3-month period, patients were on stable antiparkinsonian treatment.
Complaints of nicturia (16 patients), urgency (14 patients), frequency (9 patients) and urinary incontinence (2 patients) were registered. Uroflowmetry and ultrasound measurement of residual urine were performed on baseline and after 3 months of treatment.
Four milligrams of tolterodine were administered daily, for 3 months.
During the treatment, 3 patients were withdrawn due to side effects. The remaining 14 patients reported relief of symptoms. Uroflowmetry and ultrasound residual urine measurements also improved.
Our results confirmed the effect of tolterodine on micturitional autonomic dysfunction in PD, especially on urgency and frequency.

DIFFERENTIAL LEARNING AND MEMORY EFFECTS OF VASOACTIVE INTESTINAL PEPTIDE MICROINJECTED INTO THE CA1 HIPPOCAMPAL AREA

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Vasoactive Intestinal Peptide (VIP) is a 28 amino acids peptide, which was first isolated from the porcine small intestine. In the past few years, it has also been detected in the peripheral and the central nervous system. It has been found that VIP is widely and unevenly distributed in the CNS of rat. Hippocampus was chosen as a brain structure with high concentrations of VIP and involved in many behavioural functions. The effects of VIP microinjected unilaterally (left or right) and bilaterally (left and right) at a dose of 50 ng into hippocampal CA1 area of male Wistar rats on learning and memory (shuttle-box) were studied. VIP was dissolved *ex tempore* in saline and 1μl of VIP solution (pH 7.4) was then infused 15 min before learning (1st and 2nd training day) and memory tests (24 h after 2nd training day and on 7th day). Bilateral VIP microinjections impaired learning and memory, i.e. decreased the number of avoidances during the second training day and memory tests, compared to the respective controls. Infused into the left CA1 area, VIP exerted a marked inhibitory effect.
Right side VIP microinjections did not change the number of avoidances during the learning and memory tests. Compared to the right side, left-side VIP infusions provoked a threefold decrease of the number of avoidances on the 2nd training day and on the memory tests (24 h after the 2nd training day and on 7th day). These findings reveal lateralized inhibitory effects of VIP on cognitive processes in hippocampus. In conclusion, the hippocampal lateralized learning and memory effect of VIP depends on the hemisphere of injection. This finding suggests a differential distribution of VIP receptors (VPAC1, VPAC2 or PAC1) mediating learning and memory processes, or VIP interaction with other brain neurotransmitters (serotonin, CCK, GABA, Ach), or a differential distribution of their receptors in the brain hemispheres.

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CLINICAL STUDY ON AMANTADINE SULFATE FOR THE TREATMENT OF FATIGUE IN PATIENTS WITH SECONDARY – PROGRESSIVE MULTIPLE SCLEROSIS, IMPLIES A DEGENERATIVE PROCESS

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Introduction:
Fatigue in multiple sclerosis is defined as an abnormal sense of tiredness or lack of energy, out of proportion to the degree of effort or level of disability, that significantly interferes with routine physical or intellectual functioning. It is one of the most disabling symptoms of the disease.
Although there it is centrally mediated complication of multiple sclerosis, some data for peripheral dysfunction suggest that fatigue in multiple sclerosis is multifactorial.
Some short term studies indicated that fatigue was reduced with amantadine treatment in 20%-40% in patients with multiple sclerosis.
The aim of this study is to investigate the efficacy and safety of long term (8 months) treatment with Amantadine sulfate on fatigue in patients with Secondary-Progressive Multiple Sclerosis (SPMS).
Patients and methods:
Twenty out-patients (11 female and 9 male) with a diagnosis SPMS and fatigue were enrolled in the study. All were treated with amantadine sulfate 300 mg/daily for 8 months. Efficacy was evaluated by EDSS and self rating scales: Fatigue questionnaire, Fatigue Severity Scale (FSS) and Modified fatigue Impact Scale (MFIS).
All patients were evaluated before the treatment, and 8 months later.
Results:
The mean Fatigue Severity Scale (FSS) score was significantly improved after 8 months as compared to the beginning of the study. The mean Modified fatigue Impact Scale (MFIS) score is significantly improved after 8 months as compared to the beginning of the study. These changes in fatigue scales were significant.
No significant abnormalities on blood and urinary assesments were registered.
Conclusions:
The results suggested that Amantadine sulfate 300 mg/daily is safety and well tolerated and significantly improves the chronic fatigue syndrome in patients with SPMS.

Key words: amantadine, fatigue, multiple sclerosis

A CASE OF POST-OPERATIONAL PAIN CURED BY THE ACUPUNCTURE

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In medical practice, very often have been met severe cases of post operational pain syndrome. These patients could not work, their normal life is dramatically lowed and many of them fall down in drug abusive use.
Materials and methods: A 60-year old patient has been unsuccessfully operated and got cut 11and 12 thoracic nerve. As a result, he could not stay in sitting more than several seconds. He did not have temperature, and, when sleeping, he did not feel pain. He had been under treatment in several hospitals and sanatoriums and in two year he could not find relief from the pain. He was invited in the neurological clinic of the "Alexandrovska" hospital for 10 days. The treatment was based on acupuncture methods.
After 9 days of acupuncture procedures, the patient become stronger and the pain decreased sharply.
Results: A strong effect of the acupuncture method on the pain.
Discussion: The used Acupuncture method was not for pain. The variant that have been used is a standard for cutaneous surface inflammation. It corresponded with the fact that the patient does not feel pain during sleeping.
Conclusions: Acupuncture could be used in this kind of cases instead of medicines.

IN VITRO EFFECT OF VISCUM ALBUM AGGLUTININ I ON ISOMETRIC CONTRACTION OF HUMAN MESENTERIC ARTERIES

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Introduction: In spite of the wide use of Viscum album extracts for the treatment of many diseases, there are a few scientific papers, documented the effect of Viscum album on contractile behaviour of arterial vessels. Because of the effectiveness of this agent in the treatment of human diseases, particularly cancer, the importance of physiological and pharmacological study, concerning the nature of this substance rises. Therefore, we started our investigation on the effects of the exogenously applied Viscum album agglutinin I on isometric contraction of human arterial vessels, isolated from mesenteric bed.
Methods: We evaluated the contractile response of isolated human mesenteric arterial strips upon treatment with Viscum album agglutinin I using small wire myograph (DMT, Denmark). Viscum album agglutinin I was applied in increasing concentrations: 1nM, 3 nM and 10nM to the experimental bath. Two kinds of vessel preparations were used: first- with endothelium (referred as "native" preparations) and the second type is deendothelized arterial strip. In the performed experiments, Viscum album agglutinin I slightly potentiated KCl (42 mM) elicited tension in the vessels strips with intact endothelium, compared to the control, treated with vehicle of the substance (PBS). In the case of arterial mesenteric rings with removed endothelium, Viscum album agglutinin I failed to elicit an additional contraction or relaxation of KCl treated preparation, compared to the controls.
Discussion: The ability of Viscum album agglutinin I to influence contractile properties of arterials vessels *in vitro* is consistent with previous finding in the literature but our results showed complex dependence of this effect from: 1) compartments of arterial wall (i.e. endothelium, smooth muscle layer) and 2) spatial and species- dependent characteristics of vascular bed investigated.
Conclusion: In concentrations of 1 nM, 3 nM and 10nM, Viscum album agglutinin I has shown a great potential for pharmacological use because of its mild influence on contraction/ relaxation properties of physiologically important network of mesenteric arterial vessels in humans.

EFFECT OF CARBON MONOXIDE ON CONTRACTILE ACTIVITY OF *A. OPHTHALMICA – IN VITRO* MODEL

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Introduction: Heme oxygenase (HO) catalyzes the initial reaction in heme catabolism. It has been reported that carbon monoxide (CO), which is a product of the HO-catalyzed reaction, may inhibit the contractility of vascular smooth muscle. CO is suspected to be a signal molecule for generation of cGMP in biological systems, like NO and play an important physiological role in vascular smooth muscle homeostasis. The effects of Hemin, a substrate for HO and exogenous CO on precontracted by 42 mM K^+ PSS rat external ophthalmic arteries were investigated.
Methods: The contractile activity of 1.8-2.0 mm long rings of *a. ophthalmica* was registered isometrically with wire-myograph. The vessels were stretched radially to their optimal lumen diameter corresponding to 90% of the passive diameter of the vessel at 100 mm Hg. The reactivity of the vessel was tested with three applications of a solution containing 125 mM KCl. All drugs were applied directly into the experimental chamber. In part of experiments, the endothelium was removed by gently moving a wire through the lumen of the vessel, a procedure that abolished acetylcholine-induced relaxations.
Discussion: Our experiments showed that the relaxatory action of Hemin in the concentration 10 μM on 42 mM K^+ PSS preconstricted rings caused significant relaxation of the vessel. External CO applied into experimental chamber also decreased the vessel tone.
Conclusion: We conclude that Heme Oxigenase is represented in rat *a. ophthalmica* and it may play a role in vasodilatation by producing endogenous carbon monoxide.

MYOCARDIAL PRECONDITIONING ENHANCES LEVELS OF INTERLEUKIN-8 AND STIMULATES ANGIOGENESIS IN RAT HEART

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Coronary angiogenesis and collateral growth are chronic adaptations to myocardial ischemia. Significant expression and angiogenic activity of the pro-inflammatory chemoattractant cytokine interleukin-8 (IL-8) was observed in human coronary atherectomy tissue. The aim of the present study was to introduce a method for measuring of IL-8 levels in rat heart tissue and to study whether ischemic preconditioning enhances IL-8 level in the infarcted heart. We examined a novel method of stimulating myocardial angiogenesis through ischemic preconditioning in the form of *in vivo* 4 repetitive cycles of coronary artery occlusion each followed by reperfusion. All animals used in this study received humane care in compliance with the principles of laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals published by NIH. Rats were randomly divided into 5 groups: baseline control; normoxia + sham surgery; ischemic preconditioning + sham surgery; normoxia + myocardial infarction; ischemic preconditioning + myocardial infarction. Tissue samples from left ventricle were homogenized and sonicated in 1 ml antiprotease buffer consisted of 1 x PBS with 2 mM phenylmethylsulfonyl fluoride, and 1 □g/ml each of leupeptin and pepstatin A. Total protein concentrations were determined using bicinchoninic acid protein assay kits. Aliquots of homogenate supernatants were obtained after centrifugation at 10 000 x g for 10 min and frozen at –70^o C until thawed for assay by specific IL-8 ELISA. Samples were assesed in duplicate using cellular communication assay kit for rat IL-8 (GRO/CINC-1). Arteriolar and capillary density was evaluated by the standard deparaffinization protocol. Concentrations of IL-8 in the left ventricle were significantly elevated after 2, 4, 7, 14 and 28 days of left coronary artery occlusion in the ischemic preconditioning + myocardial infarction group as compared to the normoxia + myocardial infarction and/or baseline control or sham groups, and gradually decreased with time. In our study, ischemic preconditioning increased capillary and arteriolar density. The nonparametric correlation found by plotting of IL-8 levels vs. arteriolar or capillary density showed that IL-8, and capillary density. Therefore, IL-8 could be one of the angiogenesis “promoters” and eventually possess therapeutic potential in the infracted heart.

USE OF KEPPRA® IN STRUCTURAL BRAIN LESIONS ASSOCIATED WITH REFRACTORY SEIZURES: MECHANISMS OF DRUG ACTION AND EFFICACY

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Introduction: Refractory epilepsy is common in patients with structural brain lesions, including brain tumours. The most frequently prescribed anticonvulsants often interact with other medications, complicating their dosing and effectiveness. Recently, significant advances in epileptology are related to the development of new AEDs, as well as to a better understanding of their mechanisms of action and drug interactions. Accordingly, Keppra® (Levetiracetam) is a second generation AED, indicated as adjunctive therapy in adults with partial seizures.
Objective: To evaluate efficacy and safety of Keppra® in patients with structural brain lesions and partial epilepsy intractable to medical therapy.
Material and Methods: Twelve patients (5 males and 7 females; aged 41.6±7.6 years) with refractory seizures, associated with cerebral tumors (3 low-grade astrocytomas and 2 meningiomas) and non-neoplastic brain lesions (3 benign arachnoid cysts, 2 cerebral angiomas, and 2 cerebral trauma with sequel) were included in the study.
They presented either with simple (70%) or complex partial (30%) seizures. Diagnosis was based on the criteria of ILAE. All patients underwent adjunctive therapy with Keppra® (2000 mg/daily) for at least six months. Assessment of efficacy was based on the changes in seizure frequency and safety with reporting the drug-related adverse effects. Anatomical neuroimaging, EEG monitoring, and clinical assessment before and every six months after initiation of treatment were performed.
Results: Keppra® reduced seizure frequency in 85% of patients, with 46% becoming seizure free over the treatment period. The most common side effect was transient somnolence.
Conclusion: We consider Keppra® adjunctive therapy useful and safe for control of epilepsy in patients with refractory partial seizures associated with structural brain lesions. Possible mechanisms of drug action and efficacy are discussed in accordance with our own notices and literature review.

ANTIPSYCHOTIC TREATMENT AND METABOLIC SYNDROME IN PATIENTS WITH SCHIZOPHRENIA

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There are data that patients with schizophrenia are at a greater risk for metabolic syndrome. Most of those patients do not pay enough attention for that problem and their behaviour is similar to the general population. In this site, the beginning and the development of metabolic syndrome is due to genetic characteristics, dietary habits and inactive lifestyle. According to the data, antipsychotic drugs are related to a high risk of development of metabolic syndrome. So, the purpose of this study is to compare the weight of 100 patients that take antipsychotics for one year with 30 healthy controls, mentally healthy, with the same social status and age, never treated with antipsychotics. In this study, we measured a cluster of clinical features: weight, waist circumference, body mass index (BMI), blood pressure, and took lipid profile. In 15 patients we take glycemic tolerance (control). The clinical and metabolic findings were compared between the groups under 45-years old and over 45-years old in matter to find some age-adjusted relation of metabolic syndrome.
The conclusions are that antipsychotic treatment does not change statistically significantly the investigated clinical features.

ANTIHISTAMINES IN THE TREATMENT OF URTICARIA

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Urticaria is a heterogeneous group of 12 diseases. There is a huge number of underlying causes and enormous variety of factors, which can cause urticaria.
Inappropriate activation of the dermal mast cells is thought to be the prime pathological event in urticaria. Histamine is the sole biogenic amine in the human mast cells. Urticaria is starting because of the histamine release from the mast cells. The histamine action on H_1-receptors located on endothelial cells leads to clinical appearance of wheals. The histamine's actions on sensory nerves can also lead to neurogenic flare and pruritus. Hence, H_1-antihistamines are of great importance in the treatment of urticaria.
Antihistamines came out on the marked in the 1950s. Since then most of the patients with urticaria are successfully treated with a very few adverse effects. The 1st generation, so-called "sedating" antihistamines, have a marked influence on the central nervous system which lasts longer than 12 hours, while the antipruritic antihistamine H_1-effect – only for 4 to 6 hours. Also many drug interactions have been described for these sedating antihistamines.
The development of 2nd generation antihistamines offered drugs, which are minimally-sedating antihistamines and free of anticholinergic effects. Further progress with regard on drug safety was achieved by the development of the new generation antihistamines, which are cytochrome P450 independent metabolites of earlier antihistamines.
Nowadays according to the official guidelines the first medication of choice for urticaria is the new generation non-sedating H_1-antihistamines. If standard dosing is not effective it is recommended to increase the dosage up to a four-fold. Patients who do not respond to it, the recommendation is to add drugs of second-line therapies to the antihistamine treatment. UV-A and UV-B treatment for 1-3 months can be added to the antihistamine treatment for chronic urticaria. Also corticosteroids may be helpful to reduce angio-oedema and disease duration. Cyclosporin also has a moderate, direct effect on mast cell mediator release and is the only agent of this type to inhibit histamine release from basophilic cells.
Furthermore, urticaria has a deep influence on the quality of life and effective treatment is therefore required. The goal of the treatment is the same for all types of urticaria: complete symptom relief.

LARGE INTESTINAL MOTILITY AND REFLEXES NEAR COLORECTAL CARCINOMA - IN VITRO HUMAN STUDY

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Introduction: The colorectal cancer takes third place in mortality of men and women and also takes the third place in frequency for men after prostate and lung cancer and for women's after breast and lung cancer. Often it is clinically represented with the large or small intestine obstruction and motility disorders.
Methods: We evaluated spontaneous, pharmacologically- or electrically-elicited contractions and/or relaxations of the circular and longitudinal muscles of the segments from colon and rectum, isolated near colorectal cancer surgically removed from human patients. The motor activity of preparations of circular, longitudinal and taenia coli smooth muscles was recorded simultaneously *in vitro* as a display of functional coordination of autonomic reflexes underlying the smooth muscle motility in the colorectal region.
Results and Discussion: We used field electrical stimulation (1.0 ms pulse duration, 50 V, 2, 5 or 10 Hz for 10 sec) and pharmacological agents to define the control and drug-induced motor activity after blocking of adrenergic and cholinergic receptors.
Spontaneous high-amplitude contractions, but not relaxations, were appeared synchronously in the longitudinal, circular and taenia coli smooth muscles of colon and rectum. The electrically induced motor responses were frequency-depended. The contractions of the longitudinal layer and taenia coli muscle were more pronounced than those of the circular layer, suggesting a dominant role of the longitudinal muscles in the coordinated motor activity. In the presence of adrenergic and cholinergic receptor blockers the electrically-elicited motor activity was less expressed. In preparations isolated closer to the adenocarcinoma, a considerably less expressed spontaneous and electrically-evoked motor activity was observed.
The present results give ground to believe that the electrical stimulation applied to the preparations isolated near to colorectal adenocarcinoma could be a useful model for the characterization of the neuro-muscular communications underlying motility in the "carcinoma" region.

GLOBAL IMPACT OF UVEITIS: NEW TRENDS IN DIAGNOSIS AND TREATMENT

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Uveitis is a major cause of blindness and low vision worldwide. The incidence and types of uveitis depend on geographic and social factors. In developed countries, the frequency of non-infectious uveitis increases, while in developing ones, infections remain the major cause for intraocular inflammation. Masquerading syndromes demonstrating as uveitis are also diagnosed more often, due to widespread oncologic diseases even in younger age groups. Recently many previously accepted as idiopathic types of uveitis were proven to be a result of infection. The frequency of uveitis caused by tuberculosis and syphilis is also increasing. The introduction of many new diagnostic methods – serological testing, specific interferon measurement, PCR contribute for precise diagnosis and application of etiologically targeted medications. Treatment of uveitis is undergoing a change – corticosteroids are replaced with drugs that directly influence the pathogenesis of the immune reaction in uveitis: Immunosuppressants, immunomodulators, anti-TNF, monoclonal antibodies and immunoglobulin. Studies are being conducted on intravitreal application of anti-TNF with promising results.

SLEEP SIGNATURES OF CHILD PSYCHIATRIC DISORDERS: IMPACT ON NEUROTRANSMITTER MODELS AND TREATMENT CHOICE

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Disturbed sleep is very frequently reported in child psychiatric disorders. In a series of studies, we examined sleep patterns in children with attention-deficit/hyperactivity disorder (ADHD), Tic disorder (TD) and ADHD/TD co-morbidity. We consistently found that whereas children with ADHD display sleepiness and REM sleep overdrive, those with TD have disturbed sleep expressed by less sleep efficiency, delayed sleep onset and latency to slow wave sleep. Importantly, in the co-morbid condition, these sleep patterns distinguished ADHD and TD as separate neuropsychiatric entities. Building on these observations, we conclude that the mechanisms laying psychopathology of these child psychiatric disorders and sleep regulation have common neurochemical backgrounds, which appeals for a more precise evaluation of sleep problems and treatment choice in child psychiatry.

BRAIN PLASTICITY AND MEMORY PROCESSES DURING WAKE: NEUROBIOLOGICAL CONSIDERATIONS

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Slow cortical oscillation (SO) during sleep is proved as a mechanism for sleep-dependent declarative memory consolidation. Here we tested whether this mechanism can consolidate memories during both quiet wake retention period and active wake of learning by application of slowly oscillating trans-cranial direct current stimulation. Stimulation did not enhance SO EEG power, nor did it affect memory. However, stimulation produced a strong increase in theta EEG power and did improve encoding memory.
We conclude that memory processes during sleep and wake distinguish different modes of information processing, reflecting state-dependent brain plasticity. Since sleep is dominated by cholinergic over-activity with absence of aminergic projections to cortex, during wake noradrenaline, serotonin and acetylcholine orchestrate to balance cortical excitability.

ACE-INHIBITORS INDUCED ANGIOEDEMA

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ACE-inhibitors are frequently used class of medicinal products, especially for treatment of arterial hypertension. There are more than 20 medicinal products of this group, registered and marketed in Bulgaria. ACE-inhibitor induced adverse drug reactions are well-known; however, angioedema is often underestimated although it is potentially lethal.
The aim of the present study is to describe the clinical characteristics and epidemiological profile of ACE inhibitors-induced angio-oedema, based on the patients, treated in the Toxicology Clinic of UMHATEM "Pirogov" over two years period of time. Additionally a detailed bibliographic data on the frequency, characteristics and lethality of ACE-inhibitor- induced angioedema is provided.
Materials and methods: We have studied, both prospectively and retrospectively, the patients with ACE- inhibitors-induced angioedema, treated in the Toxicology Clinic of UMHATEM "Pirogov" for the period 2007- 2008 because of severe angioedema, as result of treatment with ACE-inhibitors. The most probable mechanism of pathogenesis of angioedema is presented, as well as our reach experience with this adverse drug reaction.
Results: A total of 114 patients above 18 years of age with ACE inhibitor-induced angioedema have been treated in the Toxicology Clinic for two years period of time (2007- 2008), 60 of which are men and 54 women. The clinical outcome in all these patients is favourable as result of the timely and complex treatment. Possible treatment protocols are also presented.
Knowledge of adverse drug reactions and careful consideration of patients' condition is essential for the prevention of this potentially life threatening adverse drug reaction.

EFFECT OF 2-SUBSTITUTED-[1,3]THIAZOLO[3,2-a]-BENZIMIDAZOLE-3(2H)-ONES WITH ANTIHELMINTIC ACTIVITY ON ISOLATED RAT HEPATOCYTES

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In this study was investigated the effect of new thiazolo-benzimidazoles with known antihelmintic activity on isolated rat hepatocytes.
For isolation of hepatocytes two-stepped collagenase perfusion was used.
Cell viability, Lactate dehydrogenase (LDH) activity and reduced glutathione (GSH) depletion were measured as signs of cytotoxicity.
The effects of the compounds were compared to the effects of Albendazole – a known antihelmintic drug.
In vivo all compounds had shown similar antihelmintic activity against *Trichinella spiralis*, compared to Albendazole.
All tested substances had statistically significant toxic effect compared to the control group (untreated cells) on the cell viability, LDH activity and GSH level.
Compounds KA-164, KA-163, KA-165, KA-120 and KA-109 had more statistically significant cytotoxicity on the exammed parameters, while the toxicity of KA-112 was statistically significantly weaker compared to Albendazole.
KA-112 increased statistically significantly LDH activity with 13%, while Albendazole – with 82%, compared to the control.
The cell viability and level of GSH in the hepatocytes were decreased by KA-112 statistically significantly with 49% and 56%, respectively; while by Albendazole – with 56% and 62%, compared to the control.
The differences in the hepatotoxic effect of Albendazole and the new compounds might be due to differences in their structure and metabolism.
Based on the results about weak cytotoxicity and similar antihelmintic activity of KA-112, compared to Albendazole; we can suggest that only KA-112 from the compounds is suitable for further investigation as perspective and potential antihelmintic drug.

DOSE-DEPENDENT ANALGESIC EFFECT OF CHRONIC ORALLY TREATED RATS WITH TRICYCLIC ANTIDEPRESSANT AMITRIPTYLINE

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Introduction: Antidepressants have been found to be of value in the treatment of pain of various aetiologies. Tricyclic antidepressants have analgesic effect in addition to their antidepressant properties. The analgesic effect is independent of theirs antidepressant activity. The analgesic effect of acutely administrated amitriptyline is well investigated in different doses and pain stimuli (1).
The aim of our study is to investigate the dose-dependent analgesic effect of chronically administrated amitriptyline at doses of 5, 10, and 20 mg/kg p.o. in two physical nociceptive tests (hot plate and Randall-Selitto test).The antinociceptive effect was measured on the 15th day of the treatment and was compared to those of metamizole sodium. Methods: Male Wistar rats were divided in five groups (12 animals): 1st group treated with saline, 2nd - metamizole sodium 150 mg/kg, 3rd, 4th and 5th - amitriptyline 5, 10 and 20 mg/kg. For detection of antinociceptive effect were used hot plate test (thermal test) and Randall-Selitto test (analgesy-meter). Statistics: SPSS 11.0 for windows; independent sample test. Results: Analgesic effect was observed in the rats treated with Metamisol sodium and Amitriptyline. The antinociception induced from Amitriptyline at doses of 5 and 10 mg/kg is similar and comparable to those induced from Metamizole sodium. Analgesic effect of 20 mg/kg Amitriptyline is stronger than the other groups with statistical significance ($p<0,001$). Discussion: This results support the analgesic effect of amitriptyline. The antinociceptive potency of antidepressants varies to their monoamine specificity and the nature of stimulus. Serotoninergic inhibitors are effective in the thermal nociceptive tests and in the treatment of chronic pain (2). Amitriptyline is a mixed antidepressant, which acts stronger on the 5-HT reuptake and less on the noradrenaline reuptake. This can explain its antinociceptive effect during the chronic administration. Conclusion: In chronically treated rats the analgesic effect of Amitriptyline become stronger with the increases of the doses. In low (5 mg/kg) and medium (10 mg/kg) doses, it is similar to those of metamizole sodium but in high doses (20 mg/kg p.o.) Amitriptilyne seems to be more effective than non-opioid analgesics in the treatment of chronic pain.

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IMMUNOPROPHYLAXIS AND IMMUNOTHERAPY WITH "UROSTIM" IN PATIENTS WITH CHRONIC URINARY TRACT INFECTIONS

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Urinary tract infections (UTIs) are among the most widely spread infections in humans and are serious problem in medical practice, because of frequent use of antibiotics which leads to appearance of polyresistant bacteria. This fact points the attention on new alternative approaches for prevention and control of UTIs, as mucosal vaccines and immunomodulators. The oral polybacterial preparation **Urostim (U)** composed of killed whole cells and lyzates from *E. coli, P. mirabilis*, *K. pneumoniae* and *E. faecalis* has been successfully applied in clinical practice for immunoprophylaxis and therapy of UTIs. **In this study** the immune and clinic effects of U (50 mg daily for 3 months) in 88 patients with chronic pyelonephritis were investigated in dynamics. **Immunological results** obtained demonstrate a significant improvement of non-specific and specific lymphoproliferative responses, confirmed by electron-microscopic data. At the same time the levels of serum proinflammatory cytokines decreased with the improvement of chronic inflammation. U stimulated humoral systemic and mucosal immunity, with production of antibacterial antibodies (Abs) with specific dynamics: in patients with low pre-treatment levels an increase of salivary S-IgA, IgG, IgM and serum IgA, IgG, IgM Abs was found; in patients with high initial levels a drop of Abs was registered followed by an increase. **Clinical results**: Before and after treatment with U for a period of 12 months the clinical/laboratory picture of the disease was observed: frequency and severity of UTIs exacerbations, laboratory characteristic for activity of infection, microbiological data for causative agents and laboratory parameters for basic renal functions. A significant decrease of the number of active UTI episodes for 12 months after the beginning of U application in comparison with the previous year was found in all patients, with milder clinical picture of exacerbations and no changes in basic renal functions. No considerable differences were observed in patients with additional night prophylaxis compared to patients treated with U only. **In conclusion**, our data show that U as a powerful immunogen and immunomodulator stimulates the immune system for intense cellular and humoral systemic and mucosal immune responses and has undoubted positive clinical effect in patients with chronic UTIs, demonstrated by a significant decrease of number of active episodes for 1 year after the beginning of treatment and milder clinical picture of exacerbations.

LOCAL RENIN-ANGIOTENSIN SYSTEMS – A NEW TARGET FOR ANTICANCER THERAPY

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The renin-angiotensin system (RAS) has been associated with systemic regulation of cardiovascular homeostasis. Knowledge of the RAS has increased dramatically in recent years with the discovery of local tissue RASs, new enzymes, peptides and functions. A functioning RAS has been identified in cardiovascular organs, adipose tissue, brain, kidney, liver, pancreas, bone marrow and reproductive organs. Experimental and epidemiological studies suggest that the local RASs may contribute to the paracrine regulation of tumourigenesis. The mechanisms of this regulation include modulation of angiogenesis, cellular proliferation, immune response and extracellular matrix formation. The components of the RAS are differentially expressed in various cancers including brain, lung, prostate, pancreatic, colon, skin, breast and cervical carcinomas in comparison with their corresponding non-malignant tissue. In particular, over-expression of the angiotensin-II type1 (AT1) receptor is common. This receptor is often up-regulated during the progression from normal to malignant phenotypes, indicating a correlation between the RAS and tumour progression. Changes in the expression of RAS components appear to correlate with tumour grade and there is evidence of tissue- and tumour-specific differences. Angiotensin-II is a main effector peptide of the RAS and its molecular mechanisms have been elucidated, especially in cardiovascular cells, where it acts as a growth factor through the AT1 receptor. Surprisingly, similar functions of AT-II occur in several kinds of cancer tissue. ACE inhibitors and AT1 receptor blockers have antiproliferative activity against cancers. Blockade of the RAS with ACE inhibitors and AT1 receptor blockers may have the potential to reduce cancer risk or retard growth and metastases. Much evidence has accumulated that ACE inhibitors suppress the growth of cultured cancer cells *in vitro* and in animal models *in vivo*. An apparent low prevalence of cancer in hypertensive patients receiving ACE inhibitors is reported. Experimental data and clinical studies suggest that AT1 receptor blockers may inhibit the growth of various cancer cells and tumours trough the AT1 receptor. The mechanism of this inhibition has been considered to be suppression of signal transduction pathways activated by growth factors. This review focuses on the mechanisms of the RAS manipulation that may provide useful, safe and inexpensive adjunctive anticancer strategy.

Key words: RAS, malignancy, cancer, ACE inhibitors, AT1 blockers

INVESTIGATIONS ON CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITIES OF CO(II) COMPLEXES WITH SUBSTITUTED SALICYLALDEHYDES (X-SALOH) AND THE NITROGENOUS BASES ENR

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The 2-hydroxy-benzaldehydes (salicylaldehydes, Hsalo), are known to have strong coordinating properties with transition metals. Due to these properties they have stimulated research interest that find applications in both the pure and applied chemistry. Knowledge of the structural features and the parameters influencing bond formation and chelate stability would help in the design of ligands with predetermined properties. On the other hand, the chelates with bidentate heterocyclic nitrogenous bases, such as 2, 2´-bipyridine (bipy) and 1, 10´ phenanthroline (phen) have important biological properties with 3d transition metals and especially with cobalt. When, however, there is a competition between two different ligands for coordination bonds to the same metal, the formation of mixed-ligand coordination compounds $[M^{II}(ligand)(bipy)_2]^+$ or addition compounds $[M^{II}(ligand)_2bipy]$ gives new and interesting properties. Specifically, the Lewis acidity of $[Co^{II}(salo)_2]$ chelates renders them susceptible to nucleoplilic attack from heterocyclic nitrogenous bases and addition compounds can be obtained. Recently, we initiated a research project of Co(II) addition compounds with enR and substituted salicylaldehydes (X-salo), where X = 3-OCH_3, 5-CH_3, 5-Cl or 5-NO_2. The resultant octahedral compounds $[Co^{II}(X\text{-salo})_2(enR)]$, with CoN_2O_4 chromophore, are stable in air at room temperature, in the solid state and CH_3CN solution, but they gradually oxidized to Co(III) in MeOH and DMF solutions. The aim of the study presented here was to evaluate the influence on cell viability and proliferation of three complexes under the general formula $[Co(X\text{-salo})_2(enR)]$: $[Co^{II}(3\text{-}OCH_3\text{-salicylaldehyde})_2(bipy)]$, $[Co^{II}(5\text{-}NO_2\text{-salicylaldehyde})_2(bipy)].H_2O$ and $[Co^{II}(5\text{-}NO_2\text{-salicylaldehyde})_2(phen)]$. The experiments were carried out by MTT test and colony-forming method using cultured human permanent cell lines established from glioblastoma multiforme (8 MG BA), breast cancer (MCF-7) and cervical carcinoma (HeLa). The compounds were applied at concentrations of 1 – 100 µg/ml for 24 h, 48 h and 72 h. The complexes examined decreased cell viability and proliferation in a time- and concentration-dependent manner. The most pronounced cytotoxic and antiproliferative properties was found to express $[Co^{II}(5\text{-}NO_2\text{-salicylaldehyde})_2(phen)]$.

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BLOOD-PRESSURE VARIABILITY AFTER APPLICATION OF NOCICEPTIN ANALOGUE [Dap9]N/OFQ(1-13)NH_2 IN CONSCIOUS RATS

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The aim of this study was to investigate the influence of nociceptin analogues N/OFQ(1-13)NH_2 and [Dap9]N/OFQ(1-13)NH_2 on the blood pressure variability in conscious Wistar rats. Experiments were carried out on two groups: firs group (n=10) treated with nociceptin analogue N/OFQ(1-13)NH_2 and second group (n=10) treated with [Dap9]N/OFQ(1-13)NH_2. The arterial blood pressure wave was registered directly by previously implanted in femoral artery catheter through pressure transducer Gould Statham P23, connected to data acquisition system MP100 WS. The blood pressure wave registration was performed in control period and 5 min after intravenously application through femoral vein catheter of N/OFQ(1-13)NH_2 or [Dap9]N/OFQ(1-13)NH_2 in equal doses (100 nmol/kg) during nine 10 min long consecutive intervals.
The spectrograms for systolic (SAP), diastolic (DAP) and mean (MAP) arterial blood pressure were derived from 512 successive values through virtual instrument developed in graphical programming environment Lab VIEW 3.1.1, by using Fast Fourier Transform algorithm. The spectral power (P) in low (LF: 20-195 mHz), mid (MF: 195-605 mHz) and high (HF: 605-3000 mHz) frequency band was studied. The application of N/OFQ(1-13)NH_2 led to a decrease of P_{LF} in the spectrograms of SAP, DAP and MAP in the first three investigated ten-minute long intervals. In SAP spectrograms P_{LF} decreased from 2.37±0.31 to 1.46±0.34 $mmHg^2$ in the first interval, to 1.38±0.33 $mmHg^2$ in the second and to 1.55±0.23 $mmHg^2$ in the third investigated interval, ($p<0.05$). In DAP spectrograms P_{LF} decreased from 2.17±0.39 to 1.29±0.24 $mmHg^2$; to 1.02±0.20 $mmHg^2$ and to 1.31±0.20 $mmHg^2$ in the first, second and third interval respectively, ($p<0.05$). P_{LF} in MAP decreased from 2.24±0.35 $mmHg^2$ to 1.42±0.25 $mmHg^2$ in the first; to 1.14±0.10 $mmHg^2$ in the second and to1.42±0.15 $mmHg^2$ in the third interval, ($p<0.05$). It also reduced P_{MF} in the spectrograms of SAP by 34.5%, 47.9%, 43.7%; DAP by 46.9%, 41.6%, 43.1% and MAP by 42.3%, 40.4%, 36.8%, ($p<0.05$) during the same intervals. The arterial blood pressure variability in the high frequency band was not affected. The application of [Dap9]N/OFQ(1-13)NH_2 did not change the variability of SAP, DAP and MAP.
Hence, the replacement of lysine with diaminopropanoic acid in the 9th position abolished the effects of nociceptin analogue N/OFQ(1-13)NH_2 on blood pressure variability in Wistar rats.
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ROLIPRAM AND THEOPHYLLINE INHIBIT PHOSPHORYLATION AND ACTIVATION OF ERK1/2 MAP KINASE SIGNALLING PATHWAY

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Phosphodiesterase (PDE) enzymes catalyze the breakdown of cAMP and thus may have potent immune modulatory activity.
We are interested to investigate whether PDE4 inhibitor rolipram and a non-specific PDE inhibitor theophylline modulate ERK1/2 signalling pathway in freshly isolated human PBMCs. To determine the effect of rolipram and theophylline on the phosphorylation and activation of ERK1/2 signalling pathway, human PBMCs ($5x10^6$) ml were serum-starved and treated with 1 μg/ml Der f. After a 15-min incubation, cells were harvested and lysed in cell lytic buffer.
Equal amounts of lysates (40μg) were subjected to SDS-PAGE and Western blot analysis using an antibody phospho-p44/42 MAP kinase (Thr 202/Tyr 204), specific for the activated form of ERK1/2. This antibody recognizes ERK when phosphorylated on both threonine and tyrosine residues. Cells treated with Der f exhibited detectable phosphorylation of ERK1/2 at 15 min. Our present study demonstrates that PDE4 inhibitor rolipram (10^{-5} M) and non-specific phosphodiesterase inhibitor theophylline (10^{-5} M) decreased ERK phosphorylation induced by Der f.

SUCCESS IN ORAL TREATMENT OF ONYCHOMYCOSES – CRUCIAL FACTORS

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New oral antifungal drugs have excellent in vitro antimycotic effect. However, this does not correspond to reported results from clinical practice: 15-40% of the patients with onychomycosis are not cured. After onychomycosis treatment, 15-30% of the cured patients develop re-infection or relapse.
Factors responsible for these failures could be classified in 6 groups: fungus properties, nail disorders, doctor's lapses, patient's factors, environmental factors and drug properties.
We might believe in contemporary antimycotics, but we have to bear in mind that in vivo, majority of them have lower antimycotic activity than expected. For instance, a realistic bioassay corneofungimetry shows lack of fungicidal effect of terbinafine ex vivo; in contrast to itraconazole- its broad antimycotic spectrum is confirmed.
Doctors have to take into consideration all these factors during every phase of the onychomycosis treatment. It is also important for the patients to be informed about predisposing factors. So that, they cooperate with the specialists to achieve better result in the treatment of onychomycosis.

BIOLOGICAL ACTIVITY OF NOVEL HEXAPEPTIDE DERIVATIVES SYNTHESIZED WITH Cα,α-DISUBSTITUTED CYCLIC AMINO PHOSPHONATES

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After the discovery of nociceptin and ORL1 (NOP) receptor, different kind of synthetic ligands have been identified. For better understanding of physiological functions, in which nociceptin is involved, potential and specific agonists and antagonists are needed. By the combinatorial chemical libraries a series of hexapeptides as Ac-R-Y-Y-R-W-K-NH_2 was synthesized, having significant NOP-receptor affinity and selectivity. Based on Ac-R-Y-Y-R-W-K-NH_2 as a template in structure-activity relationship studies, 6 novel NOP-receptor short-chain derivatives were synthesized by incorporating of Cα,α-disubstituted cyclic amino phosphonate at position 1, using SPPS Fmoc-chemistry. The haxapeptide analogues were tested for agonistic activity *in vitro* on electrically stimulated (0.05 Hz frequemcy, 1 ms pulse duration, sub-maximal voltage) rat vas deferens smooth-muscle preparations. The investigated compounds were cumulatively administered ($1x10^{-8}$M-$1x10^{-4}$M). In order to block the classical opioid receptors, but not NOP-receptor, $1x10^{-6}$M naloxone (NAL) was applied 10 min before the tested substance. Since it is known that some hexapeptides produce strong tachyphylaxis, in another experimental series, the preparations were pretreated with $3x10^{-5}$M naloxone benzoylhydrazone, which blocks both opioid and NOP-receptors. The effects of the novel ligands were compared to Ac-R-Y-Y-R-W-K-NH_2, a peptide, which was reported as a potent and specific partial agonist for NOP-receptors. The removal of the acetylic group abolished the agonistic activity of the newly-synthesized compound P6 (pEC_{50}=4.84). When the Ac-group was replaced with cyclicoamino phosphonic residue (compound P1) the agonistic activity was reduced slightly, compared to Ac-R-Y-Y-R-W-K-NH_2 (the referent compound). In case that Arg at position 1 was substituted with Cα,α-disubstituted cyclic amino phosphonates, the agonistic activity decreased significantly. Furthermore, the enlargement of cycle (5-8 C-atoms, compounds P1-P4) additionally diminished both the activity and the selectivity for NOP- receptor.
In conclusion: incorporation of Cα,α -disubstituted cyclic amino phosphonates in position 1 decreases activity of newly-synthesized peptides.

This study was supported by Grant L-1510/05 of the National Science Fund, Sofia, Bulgaria.

THE INFLUENCE OF ANTIOXIDANTS ON CYTOTOXIC AND APOPTOGENIC ACTION OF MYOSMINE

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Myosmine was one of the first structural identified tobacco alkaloids besides nicotine. The occurrence of myosmine is not limited to solanaceae like nicotine. Myosmine has been identified in various foods including staple foods like wheat, maize, rice, and milk as well as in different fruits and vegetables [1]. Myosmine nitrosation and peroxidation yield the oesophageal carcinogen *N*-nitrosonornicotine, classified as "carcinogenic to humans" by the IARC, and 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). Some researchers connect the rapidly increasing incidents of an adenocarcenome of the oesophagus in Europe with the possible cancerogenic activity of the myosmine [2]. The role of myosmine as a dietary risk factor is supported by the studies demonstrating a highly significant positive correlation between HPB-releasing adducts in the mucosa of the lower oesophagus and body mass index, a major risk factor for oesophageal adenocarcinoma.
M**aterials and Methods**. Exponentially growing murine leukemia cells were incubated with compounds and after 24, 48 stained with trypan blue and counted hemocytometrically. For detection of the cell fraction undergoing apoptosis a morphological and cytofluorimetric analysis were made using fluorescent dye propidium iodide.
Results and Discussion. The results of our investigation demonstrated the ability of myosmine to inhibit cell proliferation. The mode of cytotoxic action depends on concentration of myosmine: at low concentration it was growth inhibition, at concentrations 200-300 μM – cytostatic action, at 350-400 μM – induction of apoptosis, at 1 mM – induction of necrosis. This activity of myosmine was significant enhanced when cell culture was incubated with inductors of oxidative stress. Antioxidants exert moderate effect on cytotoxic and apoptogenic action of myosmine.
Conclusion. The elevated oxidative stress in cells may be an important contribution to the risk assessment of myosmine exposure. The role of myosmine as a dietary cancerogenic risk factor is the subject of ongoing studies of our group.

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EFFECTS OF GABAPENTIN, LAMOTRIGINE AND TOPIRAMATE ON PENTYLENETETRAZOL-INDUCED SEIZURES IN MICE

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Background: It was studied the effects of anticonvulsant drugs gabapentin, lamotrigine and topiramate on pentylenetetrazol-induced seizures in mice.
Method: Pentylenetetrazol (PTZ) seizure model of epilepsy with single injection of 50 mg/kg s.c. was used. The mice divided in a 9 groups (n=6) were pretreated i.p. 30 min before PTZ injection with: 1) saline 0.1ml/10g body weight; 2) gabapentin 20 mg/kg; 3) gabapentin 40 mg/kg; 4) topiramate 15 mg/kg; 5) topiramate 20 mg/kg; 6) saline 0.1ml/10g b.w.; 7) lamotrigine 10 mg/kg; 8) lamotrigine 15 mg/kg; 9) lamotrigine 20 mg/kg. It was observed the seizure intensity and latency to the seizures (in min) 60 min after PTZ injection. The following scale for seizure observation was used: 1) excitation; 2) body tremor; 3) clonic seizures of forelimbs; 4) clonic seizures with rotations; 5) tonic seizures of forelimbs; 6) tonic seizures of limbs.
Results: Gabapentin in both doses used did not decrease PTZ seizure intensity, but at lower dose used decreased latency to the first seizure. Lamotrigine in lower doses used did not influenced seizure intensity, but in the highest dose used decreased the seizure intensity ($p<0.05$). Lamotrigine in all doses studied increased the latency to the first seizure ($p<0.05$). Topiramate in the lower dose used have no effect on seizure intensity, but in higher dose used decreased seizure intensity ($p<0.05$). Topiramate in both doses used did not change the latency to the first seizure in mice.
Discussion: PTZ model of epilepsy is largely used for studying the anticonvulsive drugs (1). Its mechanism of action is through GABAergic transmitter system in the brain (2). Gabapentin as GABA analogue acting also on GABAergic transmission. Lamotrigine and topiramate inhibited glutamate transmission through influencing sodium channels (3). Our results permitted conclusion that the anticonvulsive drugs studied gabapentin, lamotrigine and topiramate in some extent have anticonvulsive effect on PTZ seizure model used, suppressing seizure intensity and influencing the latency to the first seizure.

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APPROACHES TO HOMEOPATHIC TREATMENT OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER (ADHD). CLINICAL CASES.

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Attention-Deficit Hyperactivity disorder (ADHD) is a neurobehavioural developmental disorder, most commonly affecting children with symptoms starting before seven years of age. It is diagnosed twice as frequently in boys as in girls by a persistent pattern of impulsiveness, hyperactivity and inattention, with or without a component of hyperactivity.
Homeopathy is a reliable therapeutic method, successfully controlling symptoms in 63% of cases.
Individual characteristics of clinical symptoms are the key for achieving maximum similarity between the patient and Materia medica.
In monotherapy, homeopathic drug is administered in low dilutions for long-course treatment. (5 CH, 9 CH).
Combined treatment is another homeopathic approach in ADHD. Several homeopathic drugs, corresponding to the four quadrants of Hering crest are administered: symptomatic, corresponding to modalities; for sensitive type; for patient terrain and clinical manifestation of disease.
Most commonly prescribed homeopathic drugs for ADHD in inattention, mental tiredness and irritability in growing-ups are: Kalium phosphoricum, Phospharicum acidum, Anacardium. If symptoms of exhaustion, anger outbursts and fidgeting are dominating, drugs of choice are: Nux vomica, China, Chamomilla. Patients with impaired concentration and attention, with feelings of insecurity, anxious of forthcoming situations, restlessness and nervous exhaustion reply well to Gelsemium and Argentum nitricum.
Reported are clinical cases, illustrating both approaches in homeopathic treatment of ADHD.

COURSE IN PHYTOTHERAPY FOR PHARMACY STUDENTS IN THE MEDICAL COLLEGE – VARNA

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The course ”Phytotherapy” is introduced in the Medical University of Varna in the academic year 2008/2009. It is included in the teaching plan for the pharmacy students in the Medical College.
The primary aim of the course is to provide a sufficient knowledge about the main groups of medicinal plants, their most important phyto-biochemical features and their correct and evidence-based medical applications.
The course contains up to date information on the most widely used medicinal plants and phytoproducts, mostly labeled in Bulgaria. The products are discussed in groups according to their therapeutic use. Special attention is paid to the use of phytoproducts in the therapy and prevention of diseases of major social impact – cardiovascular, gastrointestinal, infections and neoplastic diseases.
A common problem in Bulgaria is the lack of adequate, sufficient and reliable information in the field of phytotherapy. There are no specific student-oriented text sources for learning. To fill this gap, an educational CD-ROM is created, including multimedia presentations of each lecture and topic, and a variety of additional text and graphic materials, enabling the learning process of students.
At the end of the course, a questionnaire was made and the obtained results will be taken into consideration for the next 2009/2010 year course updates.

Key words: phytotherapy, education, pharmacology

PHYTOTHERAPY IN THE TREATMENT OF NEOPLASTIC DISEASES

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In the present review an attempt is made to summarize and analyze the vast information, related to the problem of the place and role of phytotherapy and phytoprophylaxy in the treatment of malignant diseases. The review contains data about plants with antineoplastic and antimetastatic effects and about some new and promising substances of plant origin, possessing same effects. Unfortunately, at present, very little is known about the precise molecular mechanism of action for most of them.
As basic data a large number of papers and publications from scientific centers and groups are used.
There is a vide variety of newly discovered potential antineoplastic substances, isolated during the last few years from medicinal plants, such as Curcuma longa, Helleborus niger, Zizyphus jujuba, species Phyllanthus and Terminalia, Rhus verniciflua, and many others. Some of these are in early stages of detailed investigation. From the huge number of candidates, a small group of plants and substances are chosen to be presented here.
The presented antineoplastic plants and agents are selected to fit the following general criteria:

- Available data about antitumor activity (*in vitro* and in experimental models)
- Available data (incomplete in many cases) about molecular mechanism of action
- Relatively easy to obtain from the plant source
- Relatively low toxicity; promising use as antineoplastic clinically in near future.
- Participation in clinical trials (in some cases)

The presented plants and the substances, isolated from them, are showing efficiency against models of prostate, lung, ovary, breast and other types of tumors. Some of them exhibit activity in such malignancy as ALL, AML and some types of lymphomas.

Key words: phytotherapy, antineoplastic activity, antimetastatic potential, plant origin

ONCOPHARMACOLOGICAL SIGNAL TRANSDUCTION MODULATION BY ORGANOMETALLIC COMPOUNDS, ALKYLPHOSPHOCHOLINES AND L-AMINOACID MIMETICS

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A concise overview of the recent preclinical oncological collaborative projects of the Lab for Experimental Chemotherapy will be presented. Several classes of novel metal-based antineoplastic agents have been designed, synthesized and evaluated for antineoplastic activity, in vitro. Complexes of platinum, gold, palladium and ruthenium with various organic ligands showed prominent antineoplastic activity in vitro and some of them lack of cross-resistance to cisplatin and analogues. Pharmacodynamic investigations revealed that the novel metal complexes bind DNA, forming guanine adducts, which are recognized by the HMGB proteins and modified by the nucleotide excision repair pathways. Unlike cisplatin most of these complexes displayed only insignificant toxicity against cultured renal epithelial cells and neurons. Within another ongoing research co-operation the influence of the novel alkylphosphocholine drug erufosine on proteins involved in signal transduction pathways (Rb, PKB/Akt, pAkt, BCR-ABL and p27) in leukemic cells was studied. The consecutive treatment of CML derivedcells with erufosine and imatinib mesylate led to synergism (MTT-dye reduction assay). Erufosine decreased pAkt and BCR-ABL expression, but induced Rb expression in K-562 cells. Our findings suggest that erufosine acts through induction of changes in protein signaling and especially through Rb induction. This unique mode of action makes it an attractive partner for combination therapies, e.g. in combination with imatinib for treatment of CML. Three L-aminoacid mimetics (nitrocanavanine, nitroarginine and methylnitrocanavanine) were synthesized and characterized. Their anti-angiogenic potential was evaluated against VEGF-stimulated human umbilical vein endothelial cells (HUVECs). All agents inhibited the proliferation of VEGF-stimulated HUVECs. The established inhibitory activity of the novel amino-acid mimetics give us reason to consider these compounds as prospective leads for development of antiangiogenic agents.

STRUCTURE-ACTIVITY STUDIES OF NOVEL NOCICEPTIN RECEPTOR LIGAND ANALOGUES

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The peptide sequences Ac-Arg-Tyr-Tyr-Arg-Trp-Lys-NH_2 (S1) and Ac-Arg-Tyr-Tyr-Arg-Ile-Lys-NH_2 (S5) were proved the shortest Nociceptin receptor ligands (1). *The aim* was to investigate the activity of novel S1 and S5 analogues as well as deacylated homologues (H1, H5). The new compounds were synthesized substituting Orn (S2, H2, H6), Dab (S3, H3, S7, H7) and Dap (S4, H4, S8, H8) for Lys. *Methods:* A series of novel analogues was synthesized as described previously (2). *Pharmacological studies:* Rat vas deferens (Wistar, 200-250g bw) was cut (25mm long) and placed horizontally between two ring electrodes (4mm diameter) located at a distance of 10 mm and 26 mm from the outlet of a 1 ml plastic double-jacketed tissue chamber (37° C). The tissue was supperfused with modified Krebs medium at flow rate of 0.6 ml/min with the front end tied to a force displacement transducer for registration of isometric changes in tension. The preparation was stretched until it reached the approximately *in situ* length and allowed to equilibrate for 45 min. The preparation was repeatedly stimulated for 3s with trains of rectangular pulses of 40 V, 0.6 ms, 10 Hz at 120 s. Test substances were supperfused for 25min at concentrations of 1, 3, 10, 30 and 100 μM each supperfusion being followed by a 25-min wash-out period. *The results* showed that novel compounds could be distributed into two groups: one (S1-4, H1-4) with a strong inhibitory effect on the neurogenic contraction without significant effects on the muscle tone, which is typical effect of NOP receptor agonists with efficacy rank order of S3>H3>S4>S1=S2=H2>H1 and another (S5-8, H5-8) with a strong inhibitory effect on muscle tone and negligible effects on the amplitude of neurogenic contraction, which is characteristic effect of NOP receptor antagonists with S7 being most potent. Alike activity was estimated for acylated peptides and corresponding deacylated homologues except for S1/H1 pair where S1 was more potent. *Conclusion*: The study revealed that Dab- but not Dap- or Orn-substitution of Lys at position 6 increase the agonist or antagonist properties of NOP receptor ligands.

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DEXTRAN SULFATE SODIUM (DSS)-INDUCED INFLAMMATION IN RAT COLO-RECTAL GUT REGION

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The aim of this study was to evaluate the contractile activity of intestinal smooth muscles isolated from rat colo-rectal gut region after dextran sulfate sodium (DSS)-induced inflammation. The dextran sulphate sodium (DSS) model, originally reported by Okayasu *et al*, 1990 has been used. Colo-rectal inflammation was induced in male rats by seven consecutive days of 5 % DSS (MW 36,000 ± 50,000) oral administration followed by five days of tap water only. Controls were fed with water only. Symptoms such as weight, faeces consistency and diarrhoea were recorded daily. After a total period of fourteen days rats were killed by cervical dislocation and samples of intestinal muscles were obtained. Contractile activity of these samples was studied using a modified method of the electrical field stimulation *in vitro* (Paton and Vizi, 1969). The effects of *α*- and *β*- adrenergic (*prazosine*, *propranolol*) and cholinergic (*atropine*) receptor blockers on the contractile activity of isolated preparation was studied. Histological observation showed that DSS administration produced submucosal erosions, ulceration,
inflammatory cell infiltration and crypt abscess as well as epithelioglandular hyperplasia. DSS also produced shrinkage of colon length and increased the relative colon weight/length ratio accompanied by mucosal oedema and bloody stool. Significant decrease of the contractile activity of circular and longitudinal muscles has been observed in the presence of receptor blockers prasozine, propranolol and atropine. The contractile activity of the longitudinal muscle was more expressed as compared to that of the circular muscle suggesting a key role of longitudinal muscle in the contractility during inflammation.

BETA-ADRENORECEPTOR BLOCKADE WITH A HIGHLY SELECTIVE B_1-BLOCKER ACCELERATES CONVERSION OF RECENT-ONSET ATRIAL FIBRILLATION

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Background: Atrial fibrillation (AF) is the most common cardiac arrhythmia encountered in clinical practice with an incidence of around 5% in those >65 years of age rising to around 10% in the population >80 years. Propafenon Class IC antiarrhythmic is an agent with proven efficacy for pharmacological cardioversion of recent-onset AF (< 48 hours). β-Blockers are mainly used to regulate heart rate. However, they also have modest efficacy on pharmacological cardioversion of recent-onset AF.
Aim: The aim was to study the effect of co-administration of propafenon (IC) and a highly selective β_1-blocker bisoprolol on earlier conversion of recent-onset AF.
Methods: 81 patients (aged 59.1±12.6) with AF of recent onset (<48 hours) were enrolled in this study. They had no structural heart diseases or COPD and were assigned randomly in two groups. Group A (n=48; 14 women, 34 men) was treated with propafenon (IC) as monotherapy and Group B (n=33; 9 women, 24 men) was treated with propafenon plus bisoprolol. Propafenon was administered i.v., 2 mg/kg bolus for 10 minutes, followed by maintenance infusion of 0.0078 mg/kg/min for 120 min. Bisoprolol 5 or 10 mg p.o. was administered at the very beginning of the propafenon treatment. Patients were monitored and conversion to sinus rhythm (SR) was evaluated on the 3^{rd}, 6^{th} 12^{th} and 24^{th} hour. The data were processed by SPSS for Windows, version 12.0. Descriptive statistics, correlation analysis, t-test and Chi-square were used.
Results and Discussion: In Group A, SR was restored in 45 patients and in Group B in 31. In Group A, conversion to SR on 3^{rd}, 6^{th} and 12^{th} was established to almost one and the same degree 33.3% (n=15) , 28.9%(n=13) and 33.3%(n=15), respectively, whereas on 24^{th} hour only 4.4% (n=2) restored SR. In Group B, still on the 3^{rd} hour, 93.5% of patients (n=29) restored SR and the rest 6.5% (n=2) on the 6^{th} hour. A strong and statistically significant inverse correlation was observed between the combination treatment (propafenon plus bisoprolol) and the time of SR restoration ($r = - 0.527$; $p = 0.001$). An important finding was that Group A and Group B in their LA diameter at Echo (39.4±3.8 vs. 39.3±4.2, respectively; $p>0.05$), mean age (60.1±11.4 vs. 57.7±14.3, respectively; $p>0.05$) and gender showed statistically no significant differences. In both groups, no side effects were registered.
Conclusion: Compared to propafenon (IC) monotherapy, the combination therapy IC and bisoprolol demonstrates a favourable outcome in earlier SR conversion and appears to be as safe as IC monotherapy.

EFFECTS OF GALANTAMINE AND PYMADIN IN EXPERIMENTAL MODEL OF LEARNING AND MEMORY

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Alzheimer's disease (AD) is characterized by cognitive decline due to deficient cholinergic innervation of the cerebral cortex and subcortical structures. Cholinesterase inhibitors, which enhance cholinergic neurotransmission in the central nervous system, are widely approved for the symptomatic treatment in patients with mild to moderate AD and for the last decades remain, the standard approach to the symptomatic treatment of AD.
In the present study, the first goal was to evaluate the effects of galantamine and 4-aminopyridine (alone or in 3 different combination) on locomotor activity in mice and learning/memory task in rats. In the first part of the study galantamine in a dose of 1 mg/kg did not affect locomotor activity in mice, while a dose of 5 mg/kg significantly depressed it. 4-Aminopyridine in dose of 1 and 5 mg/kg also did not affect significantly the locomotor activity in mice. In the second part of the experiment, the effects of different combinations of galantamine and 4-aminopyridine on short- and long-term learning and memory was assessed using active avoidance learning and memory task in Wistar rats. The combination of galantamine 1.65 mg/kg and 4-aminopyridine 0.8 mg/kg significantly enhanced the processes of learning ($p<0.01$) compared to controls. Memory processes were especially enhanced after the 5th day of treatment.
In conclusion, galantamine and aminopyridine favourably affect learning and memory processes, that makes this combination a perspective for symptomatic treatment of AD.

SYNTHESIS AND BIOLOGICAL EFFECTS OF TYR-MIF-1 ANALOGUES

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Four analogues of neuropeptide Tyr-MIF-1 were synthesized: Tyr-Orn-MIF-1, Tyr-Cit-MIF-1, Tyr-Cav-MIF-1, and Tyr-Can-MIF-1.
The aim of our study was to investigate the analgesic effects of newly synthesized peptides in male Wistar rats using paw pressure (PP) test All drugs were injected intraperitoneally (i.p.) in a dose of 1 mg/kg.
The obtained results showed that Tyr-Orn-MIF-1, Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 increased significantly the pain threshold compared to the control. Compared to Tyr-MIF-1 only, Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 showed significant analgesic effects. Tyr-Can-MIF-1 did not show any analgesic effect.
Substitution with citruline and canavanine in the molecule of Tyr-MIF-1 leads to analogues with pronounced analgesic effects.

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IN VITRO SCREENING OF NEW INHIBITORS OF ANGIOTENSIN-CONVERTING ENZYME

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Angiotensin-converting enzyme (ACE) is a zinc-containing metalloprotein. It releases dipeptides from the C-terminus of the peptide substrates as angiotensin I and bradykinin leading to the synthesis of angiotensin II and breakdown of bradykinin thus inducing vasoconstriction. ACE inhibitors are among the most powerful drugs for regulation of blood pressure.
In the present communication, the first results of in vitro testing of new inhibitors of ACE are presented.

Materials and methods.
An original method was applied for ACE activity determination. Rabbit serum was used as enzyme source. Serum aliquot was incubated in buffered medium with the ACE substrate analogue Hippuryl-Histidyl-Leucine (HHL). The hippuric acid produced is extracted and quantified relative to an internal standard by High Performance Liquid Chromatography (HPLC). The amount of hippuric acid formed reflects the ACE activity.
Two new piperidine derivatives (**T-68** and **T-31**) and two new tetrahydroisoquinoline derivatives (**PyHA-PhAla** and **PyHA-Meth)** were tested for their inhibitory potency compared with the well known ACE inhibitors: Lisinopril (pyrrolidine derivative which is not metabolized and excreted unchanged in the urine) and Quinaprilat (active metabolite of quinapril, an isoquinoline derivative).
The IC50 and IC100 values were determined by non-linear regression analysis of enzyme activity/inhibitor concentrations curves using software package GraphPad Prism 5.0.

Results.
From the four compounds studied, the highest inhibitory potency possessed **PyHA-PhAla** with IC50 around 370 ng/ml, followed by **T68** (IC50 around 530 ng/ml), **T31 (**IC50 around 590 ng/ml) and **PyHA-Meth** (IC50 around 1 mcg/ml). The inhibitory effect of Lisinopril and Quinaprilat is 100 times greater than that of **PyHA-PhAla.** The enzyme activity is 100% inhibited after 20 ng/ml.

Conclusion.
The significant inhibition of ACE activity by some piperidine and tetrahydroisoquinoline derivatives is a good base for the synthesis of new compounds with greater inhibitory potency.

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DOSE-DEPENDENT EFFECTS OF CAFFEINE IN A CHRONIC MILD STRESS MODEL OF DEPRESSION IN ICR MICE

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The present study was carried out to elucidate the effect of acutely administered caffeine on male ICR mice exposed to unpredictable chronic mild stress (UCMS) model of depression. The forced swim test (FST) to determine depressive-like and the elevated plus maze (EPM) test to evaluate anxiety-like behaviours, were used. Caffeine injected at stimulant dose of 40 mg/kg, i.p. was effective in the FST. In contrast, the lowest dose of 2 mg/kg caffeine had an opposite effect by increasing the immobility time compared to non-stressed controls. The UCMS regimen induced a degradation of the state of the fur and exerted a depressive-like effect in FST. Caffeine (20 and 40 mg/kg) reversed UCMS-induced increase of the immobility time in FST.
Concerning anxiety-related behaviour, UCMS-treated mice exposed to the highest dose of caffeine displayed an increase in anxiety-like behaviour and reversed UCMS-induced hyper-locomotion in the EPM. These finding indicated that caffeine exhibit dose-dependent antidepressant-like and anxiogenic activity in ICR mice exposed to UCMS.

Acknowledgements

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A PILOT STUDY ON CYP2D6*4 GENETIC POLYMORPHISM IN OUTPATIENTS WITH DEPRESSION AND ANTIDEPRESSANT TREATMENT

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Antidepressant treatment was found to fail in 30-40% of patients with depressions. Individual drug response as well as intolerable side effects may affect the positive outcome of therapy, resulting in lack of efficacy; often switch to another antidepressant and insufficient patient compliance. CYP2D6*4 is the most frequent CYP2D6 non-functional allele in Caucasians, resulting in "poor metabolizer" (PM) phenotype and predictably affecting pharmacokinetics of antidepressants – substrates of the enzyme.
Aim: To investigate the frequency of CYP2D6*4 polymorphism in a group of Bulgarian outpatients with depression, receiving antidepressants.
Methods: Outpatients with ICD-10 criteria for depression were enrolled in a six-month opened prospective study. Hamilton Rating Scale for Depression (HAM-D 21) was applied to assess presence and severity of depression. Blood specimens for DNA isolation were collected after obtaining informed consent. PCR followed by allele-specific PCR was performed for CYP2D6 genotyping and inactive CYP2D6*4 allele identification. Genotype and allele frequencies were estimated.
Results: Caucasian patient n=100 (31% males and 69% females at average age of 49.53±15.64). HAM-D 21 scores=33.21±7.77 on day first. CYP2D6*4 allele was identified in 41 individuals, with estimated allele frequency of 0.245. Genotype frequency of 8% was determined for the mutant allele. Homozygotes for CYP2D6*4 were classified as PM. Fifty nine individuals were homozygous for the wild type allele CYP2D6*1, giving rise to "extensive metabolizer" phenotype. Heterozygotes (n=33) for CYP2D6*4 allele were considered as "intermediate metabolizers".
Conclusions: Study results were comparable to the published data for Caucasian population. Genotype and allele frequencies were higher, but not significantly different from the estimated for the Bulgarian population. Further studies are necessary to follow up the influence of CYP2D6*4 on antidepressant drug response.
Keywords: CYP2D6*4, outpatients, depression.

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CHANGES IN RATS' LEARNING AND MEMORY AFTER A LONG TREATMENT WITH EXTRACT OF *HABERLEA RHODOPENSIS*

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The research project is focused on the influence of the total extract of Haberlea Rhodopensis (HR) on the experimental animals' learning and memory. There are no clinical and experimental data in the available literature related to the above topic.
The **aim** of the current study is to compare the influence of the extract from HR over the rats' short and long term memory with the placebo group.
Material and methods: 24 sexually immature white male "Wistar" rats were divided in three groups of eight. The 1st group – Placebo, received 2 ml distillate water. The 2nd group received extract of HR – 1ml/kg b.w. The 3rd group received extract of HR – 5ml/kg b.w. We conducted the training and the tests for explicit memory, using the Ugo Basile (Italy) equipment: 1. Shuttle box, registering the number of active avoidances, passive escapes, intertrail crossings and latency time. 2. Step trough, registering the latency time. 3. Step down registering the latency time.
Results: The rats from the 2nd group considerably increased the number of active avoidances in shuttle box on 5th day of the learning compared to the Placebo group (9.95±1.37 v/s PL 5.9±1.24, $p<0.05$). The 3rd group considerably increased the number of passive escapes in the shuttle box compared to the Placebo group (0.88±0.1 v/s Placebo 0.57±0.1, $p<0.05$).
Conclusion: Long-time treatment with HR improved to a greater extend the short memory compared to the rats' long memory. The extract of HR promotes the conditioned and unconditioned reflexes.

SOCIO-EMPIRICAL INVESTIGATION OF THE IDEA THE BULGARIAN MEDICAL STUDENTS HAVE OF HOMEOPATHY

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Material and methods. A socio-empirical investigation was held on among 1018 students of medicine, pharmacy and dentistry by inquiry. It included 15 questions of administrative and cognitive character about homeopathy, questions on the interrelation between homeopathy and modern medicine, as well as on risk/benefit of homeopathy. **The aim** of this study is to investigate the idea, understanding and the way the medical students in Bulgaria take homeopathy as a whole.
Results: Students willing to study homeopathy were as follows: 94.6% pharmacists, 94.3% doctors, 70.1% dentists. About 35% of them had chosen homeopathy as a non-compulsory subject before. All the students suggested that Homeopathy should be included in the curriculum of clinical pharmacology or clinical pharmacy. Many of the inquired students 861 (84,6%) define homeopathy as a method of treatment with very low doses, 165 (16.2%) consider it a non-traditional method and 72 (22%) identify it with phytotherapy.
Very few 29 (2.8%) students do not know what homeopathy is. Boys knew less about homeopathy than girls. Students from upper courses had better idea of the means of homeopathy. There was not significant difference in the idea of homeopathy between the students of medicine, pharmacy and dentistry. As of the effect of the treatment with homeopathy - 414 (40.7%) girls and 170 (16.7%) boys consider it significant. Negative answer gave only 9 (0.9%). The question "Is Homeopathy safe?" was given a positive answer by 711 (69.8%), 225 (31.7%) of them were boys, 486 (68.3%) girls, 365 (51.3%) of them had passed the homeopathy course.
Conclusion: The students from the medical universities in Bulgaria are highly motivated to study homeopathy. Their knowledge of the means of homeopathy is rising.

NEUROPROTECTIVE EFFECT OF LAMOTRIGINE AGAINST SODIUM NITRATE INDUCED HYPOXEMIA IN RATS

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Antiepileptic drugs suppress brain excitability and block the toxic mechanisms leading to necrotic and apoptotic neuronal death.
The aim: To evaluate the effect of Lamotrigine (LTG 0.5 mg/kg) on learning and memory in immature rats with reversible hypoxemia induced by sodium nitrate (SN) 40 mg/kg s.c.
Methods: 30 immature Wistar Rats (50 days old) were divided in three groups of ten: 1st (placebo-saline+SN), 2nd (LTG+SN) and 3rd (20 days LTG pre-treatment, then LTG+SN). The tested groups (2nd and 3rd) were compared to the control group (1st) using different behavioural appliances: open field, step-through, step-down and automatic shuttle-box for passive and active avoidances, escapes, inter-training crosses and latency.
Results: The number of active avoidances of 3rd group is significantly higher than the controls during the first day of investigation with SN (χ^2±SEM, resp. 0,035 ±0,006 v/s 0,017 ±0,005). Significant differences were found between the tested groups and controls during the training and retention tests (χ^2±SEM, resp. 0.2296 ±0.019; 0.4377 ±0.067 v/s 0.224 ±0.021 and 0.35 ±0.0248; 0.306 ±0.025 v/s 0.220 ±0.021; $p<0.05$). The tested groups revealed also some significant differences compared to the controls in regards of the other studied parameters of used tools.
Conclusion: LTG significantly increases the short and long-term memory of hypoxemic immature rats compared to the placebo. The received behavioural results suggest that treatment and pre-treatment with LTG could protect the brain in the state of global hypoxemia.

***Key words**:* Lamotrigine, antiepileptic drugs, memory, hypoxemia, sodium nitrate, rats.

COMPARATIVE STUDY OF THE FREQUENCY OF CHROMOSOME ABERRATIONS IN GAMMA IRRADIATED CULTURES OF RABBIT'S LYMPHOCYTES AFTER TREATING WITH EXTRACT OF *HABERLEA RHODOPENSIS* AND VITAMIN C

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Ionizing radiation produce deleterious effects in the living organisms and the rapid technological advancement has increased human exposure to ionizing radiation enormously. There is a need to protect humans against such effects of ionizing radiation. In this study, the effect of Haberlea Rhodopensis and Vitamin C on the frequency of chromosome aberrations in rabbit peripheral blood lymphocytes after *in vitro* gamma irradiation was compared. Results demonstrated that extract of Haberlea Rhodopensis in concentrations 1.0 µl/ml, 2.0 µl/ml, 4.0 µl/ml and 8.0 µl/ml decrease the frequency of chromosome aberration, especially double chromosome fragments and dicentrics, as well as polyploidy. Haberlea Rhodopensis in concentrations 1.0 µl/ml and 4.0 µl/ml was found to be more effective in reducing of chromosome aberrations per cell than Vitamin C (1.0 µl/ml). The effect of Haberlea Rhodopensis and Vitamin C on the frequency of dicentrics was similar, but on double acentric fragments, Vitamin C was more effective than Haberlea Rhodopensis.
From the results obtained, it can be concluded that the extract of Haberlea Rhodopensis in tested concentrations showed radioprotective potential.

Key words: Haberlea Rhodopensis, Vitamin C. chromosome aberrations, radioprotection, rabbit lymphocytes

SEARCHING FOR METAL COMPLEXES WITH PROMISING ANTIMICROBIAL PROPERTIES

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The searching for new and efficient antimicrobial agents have an increasing medical significance. This fact is not surprising - it is well known that nowadays more strains of pathogenic microorganisms, including staphylococci, streptococci and enterobacteria, become resistant to many of the available antibiotics and chemotherapeutic agents, creating a serious problem in the treatment of infectious diseases. The antimicrobial activity of metals and metal compounds has been known for thousands of years. The aim of the study presented here was to evaluate the antimicrobial activity *in vitro* of two groups metal compounds: 1) Twenty complexes of Cu(I,II), Co(II), Ni(II) and Fe(II,III) with Mannich type ligands N,N'-bis(4-antipyrylmethyl)-piperazine (BAMP) and N,N'-tetra-(antipyryl-1-methyl)-1,2-diaminoethane (TAMEN); 2) Six complexes of Cu(II), Zn(II) and Co(II) with cholic acids. Pure cultures of 16 pathogenic bacterial strains, isolated from animals, as well as control strains, were used in the experiments. Eight of them were Gram-positive (4 strains of *Staphylococcus aureus* and 4 of *Streptococcus pyogenes*), the rest 8 were Gram-negative (4 strains of *Escherichia coli* and 4 of *Pseudomonas aeruginosa*). The antimicrobial properties were studied by the agar-diffusion method of Bauer-Kirby and the method of minimum inhibitory concentrations. MTT test was carried out to determine the influence of the compounds on viability of cultured bovine kidney (MDBK) cells. The results obtained revealed that the tested metal complexes with cholic acids showed no or very low antimicrobial activity whereas some of the complexes with Mannich bases [especially $Ni(TAMEN)(ClO_4)_2$, $Ni(TAMEN)(NCS)_2$, $Cu(TAMEN)(CH_3COO)_2$ and $Cu_2(BAMP)(ClO_4)_4$] were found to express pronounced antibacterial and antifungal activity *in vitro*. Applied at concentrations of 1-200 µg/ml for 24 h (the duration of antimicrobial tests) the compounds were shown to be relatively non-toxic for MDBK cells – the viability of the cells was found to be ≥ 85% ($P > 0.05$ as compared to the untreated control). Further experiments are needed to clarify better safety and possible mechanism(s) of action of these compounds.

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LIFE-THREATENING OVERDOSES AMONG DRUG ADDICTS AND SOME CHANGES OF IMMUNE REACTIVITY

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Objective: To establish the changes of some parameters of cell mediated and humoral immunity, observed in the course of severe acute poisoning in a group of drug addicts patients.
Material and methods: The study includes 8 patients, with acute heroin and mixed with other psychoactive drugs intoxications at the age between 16 and 38 years, hospitalized in the Clinic of Toxicology, MHATEM "N.I. Pirogov", Sofia. We have used clinical, clinical-laboratory, immunological, chemical-toxicological, instrumental methods.
Results: In severity and prolonged intoxications with heroin and other psychoactive drugs there are observed multiorganic damages and dysfunction - some injuries from nervous, respiratory, excretory, cardiovascular systems, as well as liver disorders, anaemia, sepsis. Death is registered in 3 persons with multiorgan failure.
some changes in the immune reactivity were found – slight tendency to decrease level of IgG, IgA and C4 complement, statistically significant increase of the level of haptoglobin in heroin abusers. The study of the lymphocyte receptors showed statistically significant decrease of CD4 as well as significantly lower level of CD56 lymphocyte population, natural killer (NK) cell.
Conclusion: The results show that the acute heroin and mixed with other psychoactive drugs intoxications leads to multiorgan dysfunction syndrome and multiorgan failure, and changes of some parameters of cell mediated and humoral immunity.

Key words: life-threatening overdoses, intoxication, drug addicts, psychoactive drugs, immune reactivity

INVESTIGATION ON ANTIBACTERIAL ACTIVITY OF TOTAL EXTRACT OF MEDICAL PLANT *HABERLEA RHODOPENSIS*

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The present work is a preliminary study of the antibacterial activity of the total extract of the medical plant Haberlea rhodopensis. A total extract of Haberlea rhodopensis was used. The extract was prepared by 48 hours maceration of the leaves in 70% water-ethanol solution and subsequent distillation of the ethanol in vacuum vaporizer to a drug/liquid phase proportion of 5:1, has been used. The establishment of the extract's antibacterial activity was performed on some standard and wild pathogenic bacterial strains. The testing was done by the disc-diffusion method using filter paper discs impregnated with the different concentration of the initial extract: undiluted or diluted 1:1 or 1:2.
The results show that the inhibition of the bacterial growth is more pronounced on Staphylococcus aureus than on Gram –negative strains - Pseudomonas aeruginosa and Escherichia coli. According to the received results, the presence of a possible antibacterial activity has been discussed.

Key words: medical herb, antimicrobial activity, Staphylococcus aureus, Escherichia coli

ANTIOXIDANT ACTIVITY ON TOTAL EXTRACT OF *HABERLEA RHODOPENSIS*

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The aim of the present study is to establish an antioxidant activity on the total extract of endemic plant *Haberlea rhodopensis (Friv.)* The extract was prepared by maceration of the leaves of the plant for 48 hours in 70% water-ethanol solution and the following evaporation of the ethanol up to the drug / liquid phase ratio 1:1 (extract 1) and 3:1 (extract 2). The antioxidant effect was assessed by determination of total (Cu-Zn and Mn) superoxide dismutase (SOD) activity according to the method of Sun et al.[1] which is based on the inhibition of NBT (nitro – blau – tetrazol) reduction by the hypoxanthine - xanthine oxidaze system as a superoxide generator. One unit of SOD activity was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate in the absence of SOD. SOD activity was expressed as Units per gram hemoglobin. Spectrophotometer Specol 11 was used to measure the extinction at 560 nm wave.
The results of experiment show higher SOD-like activity for 1 ml extract 1 (305.4±8.4 U SOD) and for 1ml extract 2 (373.3±12.2) compared to a referent compound Trolox™ (water- soluble vitamin E analog) which antioxidant activity is 81 U SOD per 1 mg (Davies, M et al.[2]). This facts can be explain with probable existence of the some phytochemicals as flavonoids and antocianines (cianidine and. quercetine) into the total extract of *H. rhodopensis* which are known as strong scavenging and antioxidant agents.

Key words: Haberlea rhodopensis, SOD activity, antioxidant activity

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A STUDY OF THE SPASMOLYTIC ACTIVITY OF CUPRUM METALLICUM, MAGNEZIA PHOSPHORICA AND COLOCYNTHIS IN ISOLATED ORGANS OF EXPERIMENTAL ANIMALS

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Homeopathic agents Cuprum metallicum, Magnezia phosphorica and Colocynthis have common therapeutic effects and are indicated of spasm of the gastrointestinal, billiary and urinary tracts in different modality for any of them. Cuprum metallicum has in addition a spasmolytic activity in the case of muscular cramps mainly in the legs as in conditions of bronchial and laryngeal spasm. A spasmolytic activity of the medications was studied in isolated organs (ileum and urinary bladder) by an equipment of the company "Experimetria" that permit a contemporaneous registration of five isolated preparations. The spastic conditions were accomplished by cholinergic agents: Acetylcholine and the cholinesterase inhibitor Neostigmin. The results of the present study show the greatest influence of Cuprum metallicum and Colocynthis in reference to the spontaneous motor activity while marked spasmolytic effect in the model of spastic state was observed
for Cuprum metallicum and Magnesia phosphorica and it was weaker for Colocynthis. Similarly, was established that the magnitude of the effect is in relation with the time of application of the spasmogenic substances and this finding has a practical importance for the clinical use of the studied homeopathic medications.

Key Words: isolated organs, Cuprum metallicum, Magnezia phosphorica, Colocynthis

EFFECT OF SELENIUM SUPPLEMENTATION ON LIPID PEROXIDATION IN SPONTANEOUSLY HYPERTENSIVE RATS

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Selenium (Se) is an essential trace element that performs its biological role via selenoproteins. Tissue expression of these enzymes depends on daily Se intake. It is evaluated that diet containing 0.1 μg Se/g of food is enough for normal growth and reproduction in mammals.
The aim of this study was to investigate the influence of Se supplementation on the production of lipid hydroperoxides in spontaneously hypertensive rats (SHR).
Sixteen male, 2 months old SHR were divided into 2 groups: the first group (G1) received a dietary Se supplementation and the second group (G2) was fed in an adequate Se content diet for 8 weeks. The Se nutritional status was assessed by measuring glutathione peroxidase (GPx -1) activity in whole blood, using "Ransel" kit of "Randox Laboratories LTD". The serum lipid hydroperoxide concentration was evaluated by the method of Yagi.
The results showed an increased GPx -1 activity in whole blood in G1 as compared to G2 ($p=0,02$), while the lipid hydroperoxide concentration was significantly reduced in the Se supplemented SHR ($p=0,002$).
Se-dependant cellular glutathione peroxidase (GPx-1)
is the most abundant intracellular isoform of the GPx antioxidant enzyme family and plays a major role in the control of reactive oxygen species, which participate in atherogenesis and in pathogenesis and development of hypertension. Lipid peroxidation and high blood pressure are the primary causes for early development of atherosclerotic changes that may lead to disturbance of important organs blood supply. Hypertension-induced target organ damage is one of the leading causes of morbidity and mortality. Se supplementation has a positive effect in reducing oxidative stress. Our results have revealed the important evidence for this hypothesis, because the therapeutic benefit of Se administration in prevention and treatment of cardiovascular diseases still remains insufficiently documented.
In conclusion, selenium supplementation reduces oxidative stress in SHR.

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EFFECT OF CYTISINE ON SOME BRAIN AND HEPATIC PARAMETERS IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

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Tobacco smoking is a risk factor for variety of cardio-vascular diseases, such as hypertension, myocardial infarction, stroke and many others. It is of great importance for the hypertensive patients to stop smoking. One of the medicines widely used for smoking cessation is the original Bulgarian product Tabex®.
Spontaneously hypertensives rats (SHR) are a good model for investigation not only the cardio-vascular diseases, but also drug metabolism and drug toxicity in this pathological condition. There are literature data that SHR are more prone to liver and brain injury, provoked by some compounds in comparison to their normotensive controls (NTR). Regarding this information, the aim of the following study was to investigate the effects of cytisine on some biochemical parameters in liver and brain from SHR, compared to NTR. For the experiments male Wistar rats (NTR) and male SHR - strain Okamoto-Aoki), were divided into four groups (n = 6): 1- control NTR; 2- untreated SHR ; 3 and 4– NTR and SHR, treated with cytisine 5 mg/kg p.o. for 14 days. The effects of cytisine were assessed by serum ALAT and ASAT activity, reduced glutathione (GSH) level, lipide peroxidation product malone dialdehyde (MDA) in liver and brain. At the same time, the total cytochrome P450 quantity and activity of some hepatic microsomal enzymes, i.e. ethylmorphine-N-demethylase (EMND) and aniline-hydroxilase (AH), were assessed, as well by spectrophotometric methods.
The results of our study showed that liver microsomal EMND activity was increased in untreated SHR without any difference in cytochrome P-450 content and AH activity compared to NTR. MDA level was greater in liver (38%) as well as in brain (25%), while GSH quantity was lower in liver (26%) and brain (30%) in untreated SHR. Administration of cytisine in SHR did not affect the quantity of cytochrome P450, AH and EMND activities, as well as serum transaminase activities in both strains. In the same time MDA level was increased in liver by 25% and in brain by 22% in cytisine treated SHR. GSH level was unchanged in liver, but decreased in brain by 25% in SHR, after cytisine administration.
On the basis of this study, we could conclude that some pathophysiological factors, derived from the chronic hypertensive status in SHR, might affect cytisine toxicity.

PROGNOSTIC IMPACT OF WILMS' TUMOUR PROTEIN AND SURVIVIN EXPRESSION IN *DE NOVO* ADULT ACUTE MYELOID LEUKEMIA PATIENTS

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To evaluate the incidence and clinical impact of Wilms' tumor protein (Wt1) and survivin expression in adult patients with acute myeloid leukemia, Western blot and reverse transcriptase polymerase chain reaction (RT-PCR), respectively, were performed using diagnostic bone marrow samples from 27 patients. All cases were treated with standard chemotherapy regimens, including combinations of Cytosine-arabinoside (Ara-C); anthracycline – Idarubicin or Epirubicin with or without Etoposide. Wt1 expression was detectable in 51.8% (14/27), while survivin – in 77.7% (21/27) of AML patients. High Wt1 and survivin expression were detected in 29.6% (8/27) and 22.2% (6/27) patients, respectively. Based on co-expression of Wt1 and survivin, three groups AML patients can be defined – patients with no or low Wt1- and survivin expression (group 1), high expression of Wt1 or survivin (group 2), high Wt1 and survivin expression (group 3). Complete remissions were achieved in 57.1 % (8/14) of group 1 AML patients. In addition, partial remissions were observed in 2 AML patients (14.3%) of group 1, leading to therapy response of 71.4% (10/14). Only one AML patient in group 2 entered partial remission state, which led to therapyresponse of 8.3% (1/12). AML patient in group 3 was therapy resistant. Thus, Wt1 and survivin expressions are associated with therapy response. The presence of these molecular markers allows determination of prognostic groups of patients with high predictive value for achievement of complete remission and long-term outcome in AML.

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MEDICAL TREATMENT OF PRIMARY OPEN-ANGLE GLAUCOMA

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Primary open angle glaucoma is a slowly progressive, chronic optic neuropathy, in which apoptosis and death of ganglion cells axons leads to loss of optic nerve tissue along with its supporting glia and vasculature. This phenomenon is referred as optic nerve "cupping", or excavation. If sufficient axonal loss occurs, peripheral vision insidiously declines, but loss of central vision happens much later.
Glaucoma can be diagnosed before clinical apparent peripheral vision loss via ophthalmoscopic examination of optic nerve head cupping and retinal fibre layer assessment. This is especially important in patients with one or more of the four main risk factors: advanced age, black race, positive family history and elevated intraocular pressure.
There is no proven direct treatment for the optic neuropathy, so the treatment is focused on lowering intraocular pressure, the only one risk factor that can be modified.
Normotensive glaucoma is similar to primary open angle glaucoma by optic nerve damage and characteristic visual field loss, but IOP is normal. In this disease is important to rule out other reasons for optic nerve atrophy. There is evidence for beneficial role of IOP lowering in reducing glaucomatous progression in normotensive glaucoma.
IOP is reduced by decreasing the aqueous humour production or by increasing its outflow through trabecular meshwork, through uveoscleral pathway, or both.
This review presents the last tendencies in medical therapy for glaucoma, separates the main groups of medicaments by their mechanism of action, and describes the indications for use according the stage of glaucomatous progression. Some drugs have been suggested to have direct neuroprotective effect. Medicaments, used as adjunctive therapy in glaucoma surgery are listed, including antimetabolites and intravitreal drugs. Problems with adherence and compliance to prescribed therapy are discussed and two clinical cases are presented.

EFFECT OF PEPTIDE AND NONPEPTIDE ANTAGONISTS OF ANGIOTENSIN II RECEPTORS ON NORADRENALINE RELEASE IN HYPOTHALAMUS OF RATS WITH ANGIOTENSIN II-INDUCED INCREASE IN WATER INTAKE

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The effect of Ang II-receptor ligands (losartan, EXP 3174 and sarmesin) administered in water-repleted rats on the [^{3}H]NA release from rat hypothalamus was investigated. Male Wistar rats weighing 160-180 g were used for the experiments. The rats were injected i.c.v. with Ang II in the right lateral cerebral ventricle at a dose of 2.5 nmol/rat. Animals were initially screened for Ang II-induced drinking. Only those animals which drank immediately after Ang II injection were chosen for experiments. Forty-eight hours were allowed between screening drinking tests and drinking tests after drug treatment. Ten minutes before Ang II injection, the rats were injected i.c.v. with losartan (17.2 nmoles/rat), EXP 3174 (6.0 nmoles/rat), sarmesin (7.6 nmoles/rat) or with vehicle (0.9% saline). The drug doses were equivalent to the ID_{50} value for each of the antagonists. After injections all animals were allowed a 30-minute access to water and were then decapitated by guillotine. After decapitation the hypothalamus was cold removed. The [^{3}H]NA release from slices of rat hypothalamus was estimated. Ang II administered i.c.v. at a dose inducing drinking behavior in rats significantly increased K^+-stimulated release of [^{3}H]NA in hypothalamus without affecting basal [^{3}H]NA release. The observed difference between the effects of Ang II on basal and K^+-stimulated [^{3}H]NA release may possibly be due to the fact that peptides are released after increased neuronal activity. It can be suggested that Ang II play role mainly in pathological states and proved a substantial role for NA in brain Ang II-induced drinking response. The imidazolic non-peptidic compound DuP 753 (losartan), its active metabolite EXP 3174 and peptide Ang II analogue sarmesin antagonized Ang II-induced effect on [^{3}H]NA release restoring its levels to normal. Thus, acting via AT_1 receptor subtype the drugs tested inhibited K^+-stimulated [^{3}H]NA release in hypothalamus. The neurochemical mechanism for modulatory role of the these drugs on NA-ergic system can be suggested. Ang II receptor antagonists studied by us may become important therapeutic agents acting preferentially on pathologically activated systems and may be of use for the prevention of excessive ingestion of water in some neuropsychotic diseases.

PROTECTIVE EFFECTS OF 21-AMINOSTEROID U-74389G AGAINST AMIODARONE-INDUCED PNEUMOTOXICITY IN RATS

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Amiodarone (AM) is an antidysrhythmic drug that can cause pneumonitis and pulmonary fibrosis. We investigated the protective effect of U-74389 G on amiodarone-induced pneumotoxicity in rats.
Methods: The experiment was carried out on 72 Wistar rats. The rats were treated with AM water solution at a dose 6.25 mg/kg (or water vehicle) intratracheally (i.t.) on day 0 and 2. U-74389G was injected on day 0, 1 and 2 at a dose 15 mg/kg. Total protein content and cytological assays of bronchoalveolar lavage fluid (BALF) were performed on days 3, 7 and 28. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) were measured in lung homogenate (LH). Serum samples were tested for the levels of hydroperoxides (ROOH) on the same points.
Results: AM treatment resulted in increased protein content, total cell count, polymorphonuclear cells and alveolar macrophages. The treatment with AM and U-74389G attenuated significantly these changes in BALT. AM instillation decreased SOD on day 3 and elevated CAT and GP activity on day 3 and 7; combined treatment did not show the significant changes in above mentioned markers in LH. The combination elevated ROOH level significantly less than the alone application of AM on day 3 and 7.
Discussion: AM, instilled i.t., produces rapid damage to the alveolar-capillary barrier and parenchymal cells and may give rise to oxygen species. In vitro and in vivo studies have proven the antioxidative and membrane stabilising potency of U-74389G. U-74389G possesses affinity for the lipid bilayer, is incorporated into and enhances resistance of membranes, interacts with fatty acids, and inhibits peroxidative damage.
Conclusion: U-74389G showed a protective effect on early inflammatory response, assessed by markers in BALT. At the dose used, U-74389G exerted mild effect on activity of studed antioxidant enzymes. U-74389G decreased level of ROOH by inhibition of lipoperoxidation processes.

PAIN RELIEF AFTER CYCLOCRYODESTRUCTION FOR GLAUCOMA

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Introduction: Glaucoma is a serious ocular disease and the leading cause for irreversible blindness in patients over 65 years. When the progression of glaucomatose damage cannot be stopped with medications alone surgical intervention is needed. One possible option is cyclocryodestruction of the ciliary body – an effective procedure, which decreases aqueous production, but can be accompanied by significant postoperative pain.
Aim: To analyze and assess the pain therapy of patients, who underwent cyclocryodestrucion.
Materials and methods: The medical history of all patients who were treated by cyclocryodestrucion between April 2008 and April 2009 was reviewed. During this period, 31 patients (18 males, 13 females) with high intraocular pressure (30-54 mmHg) were treated. Standard cryocoagulation was performed under retrobulbar anesthesia. Information about the postoperative analgesics applied was gathered, as well as any patient feedback reported during the hospital stay and at follow-up visits.
Results: No perioperative complications were recorded. Retrobulbar anaesthesia with lidocaine and levobupivacaine was successful in most patients in blocking intraoperative pain and in four cases even ensured comfortable recuperation without significant complaints. All other patients received postoperatively intramuscular injection of tramadol. While effective, this monotherapy was not sufficient in 14 (52%) of these cases and, on patient's request, a second line of analgesics - NSAIDs - was used. Eight patients (29.5%) received complimentary metamizole and six (22%) - nimesulide. Four patients were prescribed nimesulide for several days after discharge because of continuing pain on the day after cryoapplication. Despite the use of tramadol and in about half of the cases co-medication with NSAIDs most patients reported discomfort and/or mild pain, which in single individuals persisted for several days.
Discussion: The nociceptive stimulation to the ciliary ganglion resulting from cryoapplication causes intense pain, which, while comparatively short-lasting, is very intense and difficult to cope. Preoperative anaesthesia plays an important role in reducing and even eliminating it (13%). Its effective alleviation often requires use of antinociceptive drugs from different groups (e.g. opioid analgesics and NSAIDs).

STREPTOZOTOCIN-INDUCED DIABETES NEUROPATHY: GLUTAMATERGIC TRANSMISSION AND PHARMACOLOGICAL MODULATION

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A large body of evidence suggests that the glutamatergic excitation and NMDA receptors are the most intimately involved in both inflammation- and nerve injury-induced central sensitization. The discovery of glial NMDA receptors indicates further the role of spinal microglia in the pathogenesis of pain hypersensitivity following nerve injury. Current research suggests that the minocycline exerts anti-inflammatory and neuroprotective actions through an effect on microglial cells activation.
The aim was to evaluate the effect of NMDA antagonists (ketamine and MK-801) and minocycline on allodynia and hyperalgesia in the streptozocin-induced diabetic neuropathy in rats.
Materials and methods: Diabetes was induced in adult male Wistar rats by a single i.p. injection of streptozotocin (70 mg/kg; plasma glucose concentrations ≥14 mmol/l). Mechanical and thermal nociceptive thresholds were measured 3 weeks after streptozotocin treatment by paw pressure (PP), plantar heat (PH) and von Frey filament (VF) tests. Light and electron microscopy of spinal cord have been employed. The acute analgesic effects of ketamine (5, 10 and 20 mg/kg, i.p.), MK-801 (0.1 mg/kg, i.p.) and minocycline (50 mg/kg, i.p.) were examined. The experimental protocols have been approved by the Ethics Committee of Medical University Sofia.
Results: Diabetic animals displayed marked mechanical
hyperalgesia and allodynia but PH threshold was not changed significantly. The quantitative image analysis reveals a considerable increase of the astrocyte density in diabetic versus control rats. Ketamine was effective against mechanical allodynia at low and intermediate doses. At higher dose, adverse effects were observed. Mechanical hyperalgesia was reversed in dose-dependent manner by ketamine with significant effects at 5 and 10 mg/kg. MK-801 displayed significant activity against mechanical allodynia but no significant alleviation of mechanical hyperalgesia was found. Minocycline tended to attenuate mechanical allodynia at this treatment regiment.
Conclusion: These data suggest that NMDA-channel blockers have been active against streptozotocin-induced diabetes neuropathy in rats. It agrees with the finding that the astrocyte density increased in the spinal cord of the diabetic rats.

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NEUROPATHIC AND INFLAMMATORY PAIN: THE ROLE OF GENDER AND SEX HORMONES

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Sex differences in pain perception exist due to its differential modulation by estrogens and androgens with females typically reporting higher sensitivity to noxious stimuli and higher incidence of various painful conditions. These differences suggested that gonadal steroid hormones such as estradiol and testosterone could modulate drug-induced analgesia. The conventional treatment of hormone-sensitive cancers includes application of estrogens and androgens, which could modulate the effect of analgesics applied. *The aim* of the study was to investigate gender-related differences in behavioural and morphological manifestations of neuropathic and inflammatory pain. *Methods and materials*: Peripheral neuropathic pain was induced by loose ligation of the sciatic nerve (chronic constriction injury, CCI). Inflammatory hyperalgesia was evoked by intraplantar injection of 0.1% carrageenan. Adult Wistar rats were used divided into following treatment groups: (1) gonadally intact males, (2) castrated males, (3) ovariectomized females, (4) ovariectomized, 17-β-estradiol treated females (0.5 mg/kg, 11 s.c. injections through 21 days). The nociceptive thresholds were determined by paw pressure (PP), plantar heat (PH), hot plate (HP), von Frey filament (VF) and incapacitance (weight bearing) test. Paw oedema was determined by plethysmometry. Electron microscopy was used to study the morphological changes above and under the sciatic nerve ligation. The experimental protocols were approved by the Ethics Committee of the Medical University Sofia.
Results: No significant differences in allodynia between genders were found. Mechanical allodynia was more pronounced in castrated males in comparison to intact males. Mechanical hyperalgesia predominated in ovariectomized females as compared with castrated males. Thermal hyperalgesia was more pronounced in ovariectomized ligated females, which was alleviated by estradiol. The inflammatory oedema was increased after estradiol treatment. The myelin and axonal destructions of sciatic nerve were more expressed in castrated males than in ovariectomized females. However ovariectomized and estrogen treated females showed greater changes than gonadally intact males. *Conclusion:* The data revealed sex related differences in the nociceptive thresholds and morphological changes after CCI of the sciatic nerve.

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MECHANISMS DIRECTED PHARMACOLOGICAL MODULATION OF NEUROPATHIC PAIN IN FEMALE RATS

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Numerous clinical and experimental studies suggested that estrogens play a significant role in pain sensitivity, endogenous pain modulation and analgesia. Females are more vulnerable than males to development of various chronic pain conditions such as fibromyalgia, headache, back pain, arthritis. Evidence has been also presented that estrogens exerted an anti-nociceptive effect. Little is known about influence of estrogens on drug management of neuropathic pain.
The aim was to study the influence of 17β-estradiol replacement on: (i) chronic constriction injury (CCI)-induced hyperalgesia and allodynia; (ii) effect of analgesic drugs and adjuvants, clinically applied for neuropathic pain management.
Methods and materials: Adult female Wistar rats were ovariectomized and 30 days later CCI of the sciatic nerve was performed. Rats were randomly assigned into groups and received through 21 days 11 s. c. injections of estradiol (0.5 mg/kg) or vehicle (0.1 ml/kg). The nociceptive thresholds were determined by paw pressure (PP), hot plate (HP), plantar heat (PH), dynamic plantar (von Frey filaments) and incapacitance (weight bearing) analgesia tests. The acute effect of i. p. applied gabapentin (100 mg/kg), metamizole (150 mg/kg), amitriptyline (10 mg/kg) and tramadol (30 mg/kg) was examined.
The experimental protocols were approved by the Ethics Committee of the Medical University Sofia.
Results: No significant differences in pain sensitivity were estimated between estradiol-treated animals and controls. Tramadol displayed significant antihyperalgesic and antiallodinyc effect in all tests. It was found however more effective in the control group against mechanical hyperalgesia (PP test). Gabapentin and metamizole were found to relieve significantly thermal hyperalgesia (HP, PH) and alleviate moderately the mechanical allodynia (von Frey filament test). Differences between two groups in the analgesic response to gabapentin and metamizole were estimated by various tests. Amitriptyline failed to reverse thermal hyperalgesia and allodynia.
Conclusion: The results suggest that estradiol could have modulatory effect on the activity of analgesic drugs in CCI model of neuropathic pain.

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METAMIZOLE EFFECTIVELY ALLEVIATES PAIN IN TWO ANIMAL MODELS OF NEUROPATHIC PAIN

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The treatment of neuropathic pain continues to be a challenge. Neuropathic pain relief by COX inhibitors has varied widely with the type of neuropathy and agent used. Evidence is accumulating that Metamizole (MTZ) inhibits the release of glutamate, Ca^{2+} uptake and peripheral cyclooxygenases and stimulates β-endorphin release. However, the therapeutic efficacy of MTZ in neuropathic pain needs further characterization.
The aim of present study was to investigate the effect of MTZ and PTC on neuropathic allodynia and hyperalgesia in different pathogenetic models of neuropathic pain.
Methods and materials: Chronic constriction injury (CCI) and streptozotocin-induced diabetes in adult male Wistar rats were employed as models of neuropathic pain.
Adult male Wistar rats (200-250g) were used. Peripheral neuropathy was induced by unilateral loose ligation of the sciatic nerve (CCI) or by streptozotocin (70 mg/kg, i.p.)-induced diabetes (groups of 8-10 rats).
Changes in pain threshold were evaluated by paw pressure (PP), plantar heat (PH), von Frey filament (VF) and incapacitance (weight bearing) tests. Both experimental models show a significant development of hyperalgesia (PP and PH) and tactile allodinya (VF).
The drugs used were metamizole (MTZ, 150 mg/kg, i.p., Sopharma), paracetamol (PCT, 200 mg/kg p.o., GSK).
The experimental protocol was approved by the Ethics Committee of the Medical University Sofia.
Results:
It was found that tactile allodynia was significantly alleviated by MTZ and PCT. Metamizole increased the thermal nociceptive threshold in CCI rats with no effect in diabetic rats.
The weight-bearing deficit was reversed by metamizole in CCI model. Treatment with PCT attenuated the thermal hyperalgesia in diabetic rats. The drugs were found ineffective in reversing the mechanical hyperalgesia in both neuropathic models.
Conclusion: It is suggest that metamizole is effective analgesic in neuropathic pain alleviating both allodinya and hyperalgesia. The drug is less effective in diabetic neuropathic pain compared with PTC, which proved potent analgesic in diabetic rats.

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MODULATION OF LEARNING AND MEMORY AFTER VIP ADMINISTRATION INTO CA1 HIPPOCAMPAL AREA OF OLFACTORY BULBECTOMIZED RATS

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The vasoactive intestinal peptide (VIP) is a neuropeptide with a wide distribution in the peripheral and central nervous systems. VIP has been suggested to participate in the pathophysiology of disorders such as Parkinson, Alzheimer and depression, but its function in CNS disorders has not been clarified. We examined the effects of VIP and VIP antagonist (VIP_{6-28}) infused uni- or bilaterally into hippocampal CA1 area on learning and memory of rats with a model of depression: bilateral olfactory bulbectomy (OBX). VIP microinjected in CA1 hippocampal showed differential modulatory effect on learning and memory abilities of OBX rats in both active and passive avoidance tests. In the active avoidance test (shuttle box) the impairment of the acquisition and the retention of the avoidance task, in OBX lesioned rats, was reduced by VIP when administered bilaterally and into the right CA1 area (on 1st, 2nd training day and on the retention test (24th h after the 2nd training day).
On the passive avoidance task, upon VIP administration bilaterally or unilaterally (left or right) into CA1 area, OBX rats showed increased latency time at the retention tests (3rd and 24th h after the acquisition trial), the effect being more pronounced at right side VIP injection. Although VIP influenced positively learning and memory abilities of OBX rats, the scores of the VIP-treated OBX rats did not reach these of sham-operated controls. VIP_{6-28} microinjected into CA1 area failed to/did not produce any significant effect in OBX rats. Our data point to a modulatory effect of VIP into hippocampal CA1 area, expressed as a significant improvement of passive or active avoidance deficits in the OBX rats. In conclusion**,** our findings suggest the involvement of hippocampal VIP receptors (VPAC1, VPAC2, or PAC1) in learning and memory of OBX rats and unequal distribution of VIP receptors in the right and left CA1 hippocampal areas.

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BEHAVIOURAL ALTERATIONS IN WISTAR AND SPONTANEOUSLY HYPERTENSIVE RATS IN KAINATE MODEL OF EPILEPSY

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A broad number of affective behavioural measures serve as a functional correlates for neurobiological changes in different models of acquired epilepsy as well as in epileptic clinical populations. The present work was focused on strain-specific behavioural characteristics of Wistar (WIS) and spontaneously hypertensive rats (SHRs) during the latent and chronic phase of kainic acid (KA) model of temporal lobe of epilepsy (TLE). Unlike normotensive WIS rats, control SHRs were more active and exhibited lower anxiety level compared to WIS rats in both open field (OF) and the elevated plus-maze (EPM) test. However, WIS rats appeared to be hyperactive during the latent phase of epileptogenesis. Unlike SHRs which did not demonstrated changes in their anxiety level during the latent period, WIS rats were characterized with lower anxiety scores in both OF and EPM test. In addition to the chronic phase hyperactivity, both epileptic
SHRs and Wistar rats were less anxious compared to their respective controls. The findings support the view that in KA model of TLE there are strain specific behavioural dysfunctions developing shortly after status epilepticus (SE) i.e. in the latent phase of epileptogenesis and in the chronic phase of epilepsy.

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ENDOGENOUS β-ADRENERGIC RECEPTOR ANTAGONISTS – A COMPOMENT OF THE AUTACOID SYSTEM

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The purpose of the present communication is to further characterize a new group of endogenous beta adrenergic receptor antagonists (EBARAs) as a component of the autacoid system.
Since 1994, we continuously developed and confirmed a conceptual approach for EBARAs as a system that control β-adrenergic receptor activity in living organisms when noradrenaline and adrenaline are functionally engaged in interaction with β-adrenoceptors.
We accept that "under physiological conditions three out of four active β-adrenergic receptors are under the control of beta-arrestins-1 and 2" in agreement with data of the group of R. Lefkowitz (Lohse at al.; 1990) via final inactivation phosphorylation by 5 specific β-adrenergic kinases. Beta-arrestins play a role of co-factors of those enzymes. Both arrestins are members of relative endogenous specific beta adrenergic receptor antagonists (RESBARAs).
However, according to our concept the fourth functionally active β-adrenergic receptor is under control of ENBARAs. The known EBARAs described so far are: urea, manganese, hydrogen peroxide, l-taurine and maybe still others. Therefore, it can be postulated that, in the absence of exogenous β-adrenergic receptor antagonists (of synthetic, semi-synthetic, plant or animal origin) it is this endogenous system that is probably operating. This system may represent an important subject of the Autacoid Pharmacology.

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TREATMENT OF ACUTE WATERY DIARRHOEA WITH RACECADOTRIL IN CHILDREN

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Racecadotril is an antisecretory drug that exerts its antidiarrhoeal effects by inhibiting intestinal enkephalinase, thereby preventing the breakdown of endogenous enkephalins in the gastrointestinal tract and reducing secretion of water and electrolytes into the gut.
Our study involves 106 children aged 1 to 36 months with watery diarrhoea, who had 5 diarrhoeic stools or more within 24 hours, and dehydration.
The first group of 56 children was treated with Racecadotril and rehydration therapy (RT). The second group of 50 children received only RT.
The mean total stool output was lower in the Racecadotril group than in the group treated only with RT ($p < 0.05$). More patients, who received Racecadotril, were cured by 5 days vs. patients who received RT only ($p < 0.15$). There were no adverse effects in both groups.
It was concluded that, in children with acute watery diarrhoea, Racecadotril as an adjuvant to RT reduced stool output and duration of diarrhoea.

EFFECT OF TROLOX ON BODY WEIGHT, APPETITE AND NUTRITIONAL STATUS OF RATS EXPOSED TO ETHANOL AND DIURNAL RHYTHM DISTURBANCE

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Diurnal Rhythm Disturbance (DRD) occurs in 36 to 72% of alcoholic patients, being significantly associated with alcoholism in community surveys. The nutritional status and Body Weight (BW) are not well studied in animals with DRD exposed to alcohol. In the present study, the effects of DRD and prolonged ethanol intake on the Body Weight (BW) and nutritional parameters were determined. Moreover, the effect of Trolox (TR, soluble form of Vitamin E) on these parameters was evaluated.
Materials and Methods: Eight groups of male Wistar rats (BW of 110±15 g) were used. Two "Control" groups (C, CT) lived under normal light/dark cycle (12/12 hrs). The Diurnal rhythm Disturbance of animal groups D and DT was developed by exposure of rats to a constant light for 24 hours, for a period of 3 weeks. The rats from groups A and AT (Alcohol intake without or with Trolox) were treated with 10% ethanol for three weeks and lived in normal light/dark cycle conditions. Two groups of rats were exposed to DRD and ethanol intake with or without Trolox (groups AD, ADT). Food (standard rodent chow) was provided ad libitum. BW, food, water and alcohol consumption were measured every day. Food, water and alcohol consumption were calculated in grams/kg BW. The total energy consumption was monitored by estimation of the daily calories intake (Calories/kg). Relative differences within data were statistically evaluated using one-way ANOVA, followed by a Bonferoni post-test.
Results: Alcohol intake increased BW for a period of 2 weeks. DRD decreased both alcohol consumption and BW of rats. Trolox increased the appetite, calories/kg, BW in control animals as well as in rats exposed to DRD.
Conclusions: Our results suggest that DRD and chronic alcohol intake may cause changes in nutritional status, appetite and BW. In addition, rolox may change the effect of alcohol intake and DRD in a time-dependent manner.

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EFFECT OF TROLOX ON THE ANTIOXIDANT ENZYMES IN RAT HYPOTHALAMUS IN A MODEL OF PROLONGED ETHANOL INTAKE

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Damage due to oxidative stress (OS) might alter the function of hypothalamus. Hypothalamic dysfunction profoundly alters the homeostasis. Pharmacological control over the OS might help to diminish the Oxidative damage of hypothalamus. In the present work, the effect of Trolox (water soluble form of Vitamin E) on the OS level in hypothalamus due to a prolonged alcohol intake was investigated by using of rat model.
Materials and methods: Four groups of 5 male Wistar rats were named Control (C, CT) and Alcohol (A, AT) and lived under normal light/dark cycle (12/12 hours). The Control groups were provided with tab water ad libitum. The Alcohol consuming groups were adapted to a voluntary use of 10% water solution of ethanol, and then were provided with this solution for 24 hours, during 3 weeks. Animals of groups CT and AT were treated p.o. with 200 mg/kg Trolox for a period of 30 days. Then the animals were anesthetized and hypothalamuses were taken for determination of the OS status. MDA and overall free-radicals formation were used as OS markers. The Antioxidant (AO) defense was estimated by measuring of the activities of the main AO enzymes – SOD and CAT. MDA was determined by the Fe/H_2O_2 method, using the Absorbance at 245 nm. The overall free-radicals formation was monitored by the MTT method. The SOD activity was determined using the pyrogallol authooxidation, while CAT was estimated by its ability to transform H_2O_2 at pH=7.
Results: After the adaptation to ethanol, the average voluntary daily alcohol consumption of group A was at about 8±1 g/kg. The OS level in the hypothalamus of group A was higher compare to OS level of group C. At the same time, the activities of, SOD and CAT decreased. The effect of ethanol was stronger on CAT than on SOD. Application of Trolox decreased the MDA level and the overall free-radicals formation in the hypothalamus. The effect of Trolox on CAT activity was higher than this on SOD.
Conclusion: Our data suggest that Trolox inhibits the Oxidative stress in hypothalamus of rats exposed to a prolonged ethanol intake.

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ANTIOXIDANT EFFECTS OF TROLOX AND GALANTAMINE IN RAT MODEL OF DIURNAL RHYTHM DISTURBANCE

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Many natural and synthetic antioxidants inhibiting lipid peroxidation have been reported to retard oxidative damage and disease progression. Galantamine hydrobromide (GAL) [1] and Trolox (TR) [2] are antioxidants, scavenging free-radicals via different mechanisms [3,4]. In the present study, the antioxidant effects of Galantamine and Trolox on parameters of the Oxidative stress (OS) level were investigated, using rat model of Diurnal rhythm Disturbance (DRD) due to a constant light exposure. MDA level and total free-radicals formation were used as markers of the OS.
Materials and methods: Four groups of male Wistar rats (5 animals each) were used. The animals were exposed to a constant light for three weeks. GAL was administered i.p. (SDD 2.5 mg/kg), while TR was provided via gastric tube (SDD 200 mg/kg). The animals received food and water ad libitum. After decapitation under anaesthesia (ether), the blood plasma was separated and the OS markers were analyzed. The MDA level was estimated by using the $Fe(II)/H_2O_2$ method, while MTT test was used to evaluate the overall free-radicals formation.
Results: GAL decreased both free-radicals formation and MDA level in the blood plasma of the animals of the control group living under normal light/dark cycle. TR did not significantly change the OS markers. Both GAL and TR exhibited antioxidant effect in the blood plasma of the rats exposed to DRD. The effect of Trolox was stronger than this of Galantamine.
Conclusions: Our data suggest that DRD increased the free radicals in blood. Furthermore, the results demonstrate the antioxidant activity of GAL and TR in conditions of DRD.
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INHIBITION OF 5-HT UPTAKE BY CONSTITUENTS OF HYPERICUM ANNULATUM SSP. MORIS *IN VITRO*

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Behavioural models in rodents confirm the antidepressant – like effects of hydro alcoholic extracts from different Hypericum species. 5-HT plays a key role in pathogenesis of depressive disorders.
The aim of the study was to investigate the effects of hyperatomarin, annulatophenon and gentisein, constituents isolated from Hypericum annulatum ssp Moris at 5-HT uptake in rat brain sinaptosomes. Radioligand techniques with [3H]-5HT were used in order to determine a profile of pharmacological activity at serotonin uptake in vitro.
All tested compounds inhibit 5-HT uptake in micromolar concentrations. Gentisein showed the most potent 5-HT uptake inhibition with an IC_{50} value of 4.73×10^{-4} M. Annulatophenon inhibits 5-HT uptake with IC_{50} value of 5.4×10^{-4} M, while hyperatomarin inhibits with weaker potency IC_{50} 16.8 x 10^{-4}M.
We, therefore, hypothesize that inhibition of %-HT uptake might be responsible for antidepressant effect of gentisein, hyperatomarin and annulatophenon isolated from Hypericum annulatum ssp Moris.

Key words: Hypericum, Receptor, Serotonin, Gentisein, Hyperatomarin, Annulatophenon

POTENTIALLY TOXIC DRUG INTERACTIONS WITH HERBAL AND DIETARY SUPPLEMENTS

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Pharmacokinetic herb-drug interactions can affect the activity of xenobiotic metabolizing enzymes (human cytochrome P-450) or transport proteins (e.g. P-glycoproteins), human organic cation transport proteins (OCTP), human organic anion transport proteins (OATP). Thus, herbal drugs and dietary supplements that modulate drug metabolizing enzyme activity and/or transporter function can affect bioavailability and clearance for drug that are substrates for the affected proteins. Pharmacodynamic interactions appear to result from phytochemicals, the action of which either diminishes or exacerbates the effects of conventional medication by mechanisms unrelated to altered metabolism or transport.
The present study represents an overview on several dietary supplements and herbals known or suspected of interacting with conventional medications. It illustrates some aspects of the complex nature of botanical supplement/drug interaction and possible mechanisms of these interactions. The variables that must be considered when evaluating the likelihood of such interactions are mentioned. The discussion is focused on drug interaction with widely used herbals like Grapefruit, St. John's Wort (Hypericum perforatum), Garlic (Allium sativum), Ginkgo Biloba, Panax Ginseng.
In conclusion, the high interest in natural products is mainly motivated by wish to produce a desirable effect with a fewer side effects. In reality of toxicology, all substances in high amounts, even herbals, could be toxic. Prospective investigations into pharmacological mechanisms underlying herb-drug interactions have been delayed and confounded by a variety of factors. They includes rapid influx of phytochemical products into market, high trends of consumer self-medication with dietary supplements, lack of premarket testing of safety and efficacy, problems with product quality. A deeper investigation of drug interactions with herbal and dietary supplements is needed.

PHARMACOTHERAPY OF ISOLATED DEMYELINATING OPTIC NEURITIS

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Acute isolated demyelinating optic neuritis (ON) is a frequent initial manifestation of multiple sclerosis (MS). While most ophthalmologists prescribe low dose oral or local steroids for ON, high dose corticosteroid pulse therapy followed by oral prednisolone is now established as standard treatment of ON presenting as clinically isolated syndrome (CIS) of MS. It is proven to accelerate recovery of vision, though unaffecting its final degree.
AIM: To evaluate the efficacy of corticosteroid therapy in patients with isolated demyelinating ON.
PATIENTS AND METODS: A review of subjects admitted to the Ist Neurological Clinic, Varna University Hospital, was made. Cases with isolated demyelinating ON suggestive of MS and MRI evidence of demyelination were selected. All patients had received i.v. methylprednisolone followed by oral prednisolone. Assessment of visual acuity at 100% and low contrast (3% and 1.5%), colour vision (Ishihara tests), visual field examination and ophthalmoscopy had been performed on days 1 and 14.
RESULTS: Eight women and 3 men aged 18 to 43 years had unilateral decreased vision. Ophthalmoscopy showed normal optic disc in 10 patients and papillitis in 1 patient. Visual acuity in the affected eye was 0.8 (20/25) to 0.5 (20/40) in 6 patients (55%), 0.4 (20/50) to 0.1 (20/200) in 3 patients (27%) and worse than 0.1 (20/200) in 2 patients (18%) at 100% and low contrast levels. Visual acuity at 100% contrast improved on day 14 in 10 patients.
DISCUSSION: Our study demonstrated quick recovery of vision at 100% contrast after intravenous corticosteroid treatment followed by oral prednisolone, thus complying with literature data. The above-cited treatment scheme has proved useful in multiple large studies, which have justified its establishment as a standard, though it is not widely accepted and used in ophthalmologic clinical practice.

KEY WORDS: MS, CIS, optic neuritis, corticosteroid treatment

STUDIES OF CYTOTOXICITY AND ANTIOXIDANT CAPACITY OF N/OFQ(1-13)NH_2 AND ITS STRUCTURAL ANALOGUES

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The potential toxicity and antioxidant capacity of N/OFQ(1-13)NH_2 and its structural analogues [ORN^9]N/OFQ(1-13)NH_2, [Dab^9]N/OFQ(1-13)NH_2 and [Dap^9]N/OFQ(1-13)NH_2, all being synthesised in the Department of Organic Chemistry, University of CTM, Sofia, were studied. The staurosporine- and H_2O_2-induced damage of SH-SY5Y neuroblastoma cells was not changed in the presence of N/OFQ(1-13)NH_2 and [Orn^9]N/OFQ(1-13)NH_2, but it enhanced strongly in the presence of [Dab^9]N/OFQ(1-13)NH_2 and [Dap^9]N/OFQ(1-13)NH_2; alone, the latter two analogues led to cell injury. Neuropeptide-dependent differences in SH-SY5Y cell viability were also observed; a cytoprotective effect was found only in the presence of N/OFQ(1-13)NH_2 and [Orn^9]N/OFQ(1-13)NH_2. Compared with [Dab^9]N/OFQ(1-13)NH_2 and [Dap^9]N/OFQ(1-13)NH_2, N/OFQ(1-13)NH_2 and [Orn^9]N/OFQ(1-13)NH_2 possessed more beneficial effects in systems, generating free oxygen radicals (O_2^- and $^{\cdot}OH$), as well as on the antioxidant status of rat brain and liver. Taken together, all above findings show that N/OFQ(1-13)NH_2 and [Orn^9]N/OFQ(1-13)NH_2 are more beneficial to the cells, than the other two nociceptin analogues. The present results suggest that the shortening of nociceptin side-chain length might contribute to the increase in damage and to reduction in SH-SY5Y cell viability, as well as to the changes, observed in free-oxygen generating systems and in antioxidant status of animal tissues.

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EFFECTS OF ARONIA MELANOCARPA FRUIT JUICE IN BEHAVIOURAL TESTS IN RATS

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The main constituents of Aronia melanocarpa fruit juice (AMFJ) are polyphenolics – procyanidins and flavonoids, mainly anthocyanins. Flavonoids including anthocyanins are reported to cross the rat blood-brain barrier.
The aim of the present study was to investigate the effects of AMFJ at doses of 5 and 10 ml/kg in behavioral tests in rats.
The effect of AMFJ on locomotor activity was evaluated in the open field test (Bronstein, 1972). The object recognition test (Ennanceur and Delacour, 1988), based on discrimination between a familiar and a new object presented at 1 h interval, was chosen to study the effect of AMFJ on working memory. AMFJ was also evaluated in a test for anxiety utilizing social interaction (Sandra and Hyde, 1978). The test was carried out under conditions of high light, unfamiliar arena and unknown test partner to create a high anxiety level. In this test AMFJ was compared with diazepam (1 mg/kg).
The results showed that AMFJ did not significantly change both horizontal and vertical locomotor activity. It did not also adversely affect the working memory. AMFJ dose-dependently increased the time of active social contacts between the test partners. The effect of both AMFJ doses was comparable to that of diazepam.
The results of the present study demonstrate an anxiolytic-like effect of AMFJ in the test of rat social interaction. This effect could be due to its active polyphenolic ingredients. Other plant extracts containing flavonoids, amongst which anthocyanins have been shown to reduce anxiety in rats through binding to $GABA_A$ receptors (Barros et al., 2006). The potential advantage of AMFJ might be that its anxiolytic-like effect is not accompanied by sedation and disruption of memory.

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HYPOLIPIDEMIC EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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The increased risk for developing premature atherosclerosis in diabetes is due to increase in plasma triglycerides (TG) and low-density lipoproteins (LDL) and decrease in high-density lipoproteins (HDL). The aim of the present study was to investigate the influence of Aronia melanocarpa fruit juice (AMFJ) on plasma lipids in diabetic rats.
Diabetes was induced by an intraperitoneal injection of streptozotocin (50 mg/kg). AMFJ was applied by gavage at doses of 10 and 20 ml/kg for six weeks. Streptozotocin application resulted in a significant elevation of plasma glucose by 141%. This effect was accompanied by a significant elevation of plasma TG by 64%, statistically insignificant elevations of total cholesterol and LDL-cholesterol, and a reduction of HDL-cholesterol. In diabetic rats, the two AMFJ doses reduced significantly plasma TG by 35% and 39%, respectively, to levels that did not significantly differ from those of normal control rats and counteracted the influence of streptozotocin on total cholesterol, LDL-cholesterol and HDL-cholesterol. This effect of AMFJ on plasma lipids was probably due to improvement of the diabetic condition estimated by the significant reduction of plasma glucose by 44% and 42%, respectively.
High levels of TG, which are typical of the diabetic condition, were also found in our rat model of diabetes. Hypertriglyceridemia in streptozotocin-diabetic rats is reported to be caused by slower removal of lipoprotein triglycerides from the plasma space, owing to reduced lipolytic activity in the peripheral tissues. AMFJ reduced the abnormalities in blood lipids in diabetic rats probably due to its polyphenolic active constituents and might be useful in prevention of diabetes-associated atherosclerosis.

INHIBITION OF SMALL INTESTINAL TRANSIT RATE BY *ARONIA MELANOCARPA* FRUIT JUICE IN RATS

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Aronia melanocarpa Elliot is a bush grown mostly in Eastern Europe and in North America. Its fruits are rich in phenolic substances, mainly flavonoids and tannins. The aim of the present study was to investigate the influence of *Aronia melanocarpa* fruit juice (AMFJ) at doses of 5 and 10 ml/kg on the small intestinal transit of charcoal meal in rats.
The black marker (a suspension containing 10% charcoal) was applied orally 30 min after AMFJ. Intestinal transit rate (part of the small intestine travelled by the charcoal head for 30 min) was determined. AMFJ dose-dependently reduced the rate of intestinal transit and the effect was statistically significant at the dose of 10 ml/kg.
This effect of AMFJ might be due to the flavonoids and tannins, which are its most abundant active ingredients. These results are in accordance with other experiments, demonstrating the slowing of intestinal transit by quercetin, procyanidin and plant extracts, containing tannins and flavonoids. Mechanisms proposed for the explanation of the effect of plant extracts on intestinal motility are inhibitory effects on alpha$_2$-adrenoceptors (Akindele and Adeyemi, 2006; Nwafor and Bassey, 2007; Mbagwu and Adeyemi, 2008) and on cholinergic systems (Nwafor and Bassey, 2007) and facilitation of inhibitory enteric pathways (Gharzouli and Holzer, 2008).

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INFLUENCE OF INDUCTION ON AMPHETAMINE METABOLISM AND TOXICITY IN RATS

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Amphetamines are widely used drugs of abuse. Their multiple administrations are connected with neuro- and hepatotoxicity. In rats, amphetamine metabolizes to hydroxyamphetamine, through a toxic epoxide. This metabolic pathway, mediated by CYP 2D predominantly, is thought to be responsible for amphetamine hepatotoxicity in rats. Regarding this information, the aim of the following study was to trace the influence of induction by nifedipine and by phenobarbital on amphetamine metabolism and toxicity, after multiple administrations in rats.
For the experiments male Wistar rats were divided into six groups (n = 6): 1 – control; 2 – Nifedipine control (5 mg/kg i.p. 5 days); 3 – Phenobarbital control (75 mg/kg i.p. 4 days); 4 – amphetamine (5 mg/kg i.p. 5 days); 5 - treated with amphetamine (5 mg/kg i.p., for 5 days) after 5 days induction with nifedipine; 6 - treated with amphetamine (5 mg/kg i.p., for 5 days) after 4 days induction with phenobarbital. The evaluation of the biochemical parameters was carried out in the six groups of animals, 24 hours after the last administration of the compounds.
The results were presented as Mean ± SD of 6 animals in each group. Student's t-test was used. Probability values less than 0.05 were considered significant.
Amphetamine, per se, multiply administered, led to a significant reduction of cytochrome P 450 quantity and EMND activity, by 27 % ($p < 0.05$) and by 40 % ($p < 0.05$), respectively, without changing AH activity. GSH level was depleted by 28 % ($p<0.05$). Both nifedipine and phenobarbital induction resulted in additional decrease as in cytochrome P 450 quantity, as well as in EMND activity. In Phenobarbital induced rats, amphetamine administration led to a significant GSH depletion by 42 % ($p<0.05$), which is by 21 % ($p<0.05$) more, compared to amphetamine effect in non-induced animals. Nifedipine induction also resulted in additional GSH depletion, caused by amphetamine, but it was not as prominent as in Phenobarbital induced-rats.
On the basis of this study we could conclude that in amphetamine metabolism and toxicity other than CYP 2D isoforms, namely CYP 3A and CYP 2B might be involved.

TREATMENT WITH VITAMIN E OF PATIENTS WITH CHRONIC HEART FAILURE: IS THERE CONTRIBUTION TO THE CONVENTIONAL THERAPY?

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The aim of study is to investigate the role of antioxidant vitamin E in treatment and prognosis of HF.
Material and methods: Double blind placebo, controlled trial on 62 patients of II – IV NYHA functional class (FC), hospitalized in the Clinic of Cardiology, Medical University of Sofia. The ratio between groups is 1:1. The follow up period was 24 weeks (6 months). Dose regimen was defined as 800 mg α-tocopherol per day. Levels of both brain natriuretic peptide (BNP), a biomarker of HF, and α-tocopherol (AT) in serum were also analyzed. Vitamin E concentration was determined by HPLC with UV-detection.
Results: At study entry, patients' characteristics do not differ significantly between groups, except for the observed distribution by FC. There were significantly more patients of the III -NYHA FC in the experimental group as compared to placebo group (48.4% vs. 25.8%), althoug the mean FC was the same in the both groups. Serum concentrations of vitamin E are almost the same in both groups (10.3±1.9 vs. 9.6±3.0 μg/ml) and considerably lower compared to the found in other studies. At the end of the follow up mean concentration of vitamin E increase up to 24.9±10.8 μg/ml in experimental group ($P < .001$) and remains unchanged in placebo group (11.6±4.8 μg/ml). BNP–values significantly increase at the end of follow up in both groups (14.3±23.0 vs. 13.3±1.9 pg/ml, but the observed increase in BNP is six fold than the baseline values (2.2±3.4) in placebo group, whereas in vitamin E group the observed increase is approximately only twice than the started values (5.4±1.2). A trend of increase is also observed in vitamin E group for functional indices of HF and therapy with diuretics.
Discussion: The observed increase in BNP in both groups exclude to some extend the consideration that it could be due to vitamin E treatment. The lack of difference in the final BNP values in both groups corresponds to the results of prognostic analysis. Other studies, investigating changes in BNP in HF patients treated with vitamin E, have not been conducted yet.
In conclusion, treatment with vitamin E is related to improvement in functional indices, as well as to lower degree of increase in BNP values, sugesting a slower progression of HF in studied patients.

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EXPERIMENTAL MODELS OF NEUROPATHIC PAIN: MECHANISMS DIRECTED PHARMACOLOGICAL MODULATION IN MALE RATS

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Neuropathic pain is a common clinical syndrome caused by nerve injuries. Animal models of pain have added to the understanding of neuropathic pain and can spur development of understanding of pathogenesis and novel therapy approaches. In addition to physical nerve injuries model (mostly used chronic constrictive injury, CCI), another extensively studied animal model of pain is streptozotocin (STZ)-induced diabetes (aetiologies include metabolic disorders).
The purpose was to evaluate the analgesic effect of different class of drug for treatment of neuropathic pain (morphine, gabapentin and amitriptyline) in two distinct experimental neuropathic pain models: CCI and STZ-induced diabetic neuropathy.
Methods and materials: The experimental protocols were approved by the Ethics Committee of the Medical University Sofia. Adult male Wistar rats (200-250g) were used. Experimental peripheral neuropathy was induced by unilateral loose ligation of the sciatic nerve or by STZ-induced diabetes (70 mg/kg, i.p.; plasma glucose concentrations >14 mmol/l). The effects of morphine (5 mg/kg, s.c.), gabapentin (100 mg/kg, p.o.) and amitriptyline (10 mg/kg, i.p.) was examined in both models. The nociceptive thresholds were determined by paw pressure (PP), plantar heat (PH), von Frey filament (VF) and incapacitance (weight bearing) tests.
Results: Mechanical allodynia (VF) was developed in both neuropathic models. Mechanical hyperalgesia (PP) was more pronounced in diabetic rats whereas thermal hyperalgesia (PH) dominated in CCI model. Morphine reduced mechanical allodynia in ligated rats and alleviated the thermal hyperalgesia in both models. Amitriptyline alleviated neuropathic hyperalgesia (PH) and allodynia in diabetic rats and blocked the thermal hyperalgesia in CCI model. Gabapentin had significant antiallodynic effect in diabetic rats but did not alter hyperalgesia in both models. CCI induced weight bearing disturbance was reversed by Morphine only.
Conclusion: Present data demonstrated different symptomatic manifestations of neuropathic pain, which probably depend on animal model and drugs pharmacodynamics. The analgesic effect of tested drugs (morphine, gabapentin and amitriptyline) suggests the implication of mechanism-based combination of analgesics in the treatment of neuropathic pain.
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NEW BIOMARKERS FOR DIAGNOSIS AND PROGNOSIS OF POISONING WITH SARIN AND SOMAN

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INTRODUCTION. Changes in primary biomarkers (ACHE and BCHE activities in plasma, liver and brain) of rats poisoned with soman and tabun (i.m. 1xLD50) were compared with the changes in immune system and detoxication liver capacity as well as with the signs of oxidative stress.
RESULTS. Increased nitric oxide production in lung macrophages in rats treated with soman and most significantly with tabun, have been found. Significant signs of oxidative stress were observed in the early phases of soman and tabun intoxication (increased corticosterone levels in plasma, decreased glutathione levels in liver and brain, decreased catalase, superoxide dismutase and glutathione peroxidase activities in erythrocytes, brain and liver). The signs of oxidative stress were more pronounced in tabun poisoned animals than in soman. That could be explained by the fact that soman poisoning developed very quickly with symptoms mainly of neurotoxicity. After soman treatment, unchanged blood and liver GSH-S-transferase and brain hydrolase activities were observed. Liver and brain carboxylesterase and blood GSH-S-transferase activities were also unchanged after tabun poisoning; however some more changes in the peripheral organs were observed for longer period of time (increased inhibition of hydrolase, ACHE and BCHE activities). Moreover, for the first time is shown a selective, significant and long lasting (up to 20% from the control after $1xLD_{50}$ at day 7^{th}) decrease of acylpeptide hydrolase activity in blood. The inhibition is selective for the tabun poisoning and not demonstrated after soman.
CONCLUSION. Blood acylpeptide hydrolase inhibition could serve as good long lasting prognostic sign of poisoning with tabun. In general, the present study show that more essential parameters changes, tested in rat blood, liver and brain are observed after tabun poisoning.

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ERUFOSINE, A NOVEL ALKYLPHOSPHOCHOLINE, IN CHRONIC LYMPHOCYTIC LEUKEMIA: IN VITRO COMPARISON WITH BENDAMUSTINE AND FLUDARABINE

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Background: Alkylphosphocholines represent a new class of cytostatic drugs with a novel mode of action. Erucylphospho-N,N,N-trimethylpropylammonium (erufosine - ErPC3), the first compound of this class that can be administered intravenously, has recently been shown to be active against human tumor and leukemic cell lines and is currently undergoing clinical trials in leukemia patients. Erufosine has shown high activity against leukemic cells without affecting the normal hematopoiesis.
The aim of the study was to evaluate in vitro the antileukemic potential of ErPC3 in chronic lymphocytic leukemia (CLL) compared to fludarabine and bendamustine, that are routinely used drugs for the treatment of CLL.
Materials and Methods: The cytoreductive activity of ErPC3, fludarabine, and bendamustine was evaluated in primary cell cultures of mononuclear cells isolated from peripheral blood from newly diagnosed patients with CLL using the MTT-dye reduction assay. The levels of bcl-2 and NF-kB proteins were measured by Western blot.
Results: Erufosine showed clear concentration-dependent efficacy against CLL cells that exceeded in some cases the efficacy of the reference drugs fludarabine and bendamustine (IC50 values ranging from 12 to 30 μM). Subsets of cell cultures showed greater sensitivity towards fludarabine or bendamustine as estimated by the corresponding IC50 values. Immunoblot analyses revealed NF-kB positivity in 70% of the samples while Bcl-2 protein was positive in 90% showing a decrease of expression after cytoreductive treatment.
Conclusions: Based on these data ErPC3 appears as a novel promising antileukemic candidate drug for the treatment of lymphoproliferative neoplasms such as CLL due to its remarkable antitumour activity in vitro and its ability to intensify normal hematopoiesis.

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THE ALKYLPHOSPHOCHOLINE ERUFOSINE IS A POTENTIAL ANTIMYELOMA DRUG

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Multiple myeloma (MM) is a frequent hematological malignancy (about 1 % of all human neoplasias) which remains incurable despite recent pharmacological developments such as proteasome and angiogenic inhibitors. Erufosine is an i.v. injectable alkylphosphocholine which is active against various leukemias and lymphomas in vitro. Clinical trials are ongoing in CLL patients. In the present study, the antineoplastic activity of erufosine against three MM cell lines (U-266, RPMI-8226 and OPM-2) was investigated. Cytotoxicity was determined by the MTT-dye reduction assay and anti-migratory activity was evaluated by a modified Boyden-chamber assay. Bcl-2 and Bcl-X_L expression level, activation of caspases as well as cleavage of PARP were studied by Western blotting, using specific antibodies. Apoptosis induction was additionally studied by DAPI-staining and agarose gel electrophoresis. RT-PCR was employed to compare IL-6 mRNA levels among the three cell lines. The haematological toxicity of erufosine was assessed using a clonogenicity assay with human haematopoietic progenitors from umbilical cord blood. Erufosine exerted pronounced cytotoxic effects on MM cells, RPMI-8226 being most and U-266 being least sensitive (IC_{50} values of 3 and 17 μM, respectively). Comparison of the characteristics of erufosine-induced cell death in these cell lines revealed a complex mode of action with apoptotic elements of cell death prevailing in OPM-2 cells and non-apoptotic elements prevailing in U-266 cells. The sensitivity of the MM cell lines to erufosine-induced apoptosis correlated inversely with the respective expression level of Bcl-X_L, as well as with the level of IL-6 mRNA. Additionally, erufosine showed potent migration-inhibiting activity in RPMI-8226 cells. Erufosine was not toxic for normal haematopoietic progenitor cells from human umbilical cord blood and even stimulated them to form granulocyte/macrophage colonies. Taken together, our data indicate that erufosine has potential as an antimyeloma drug and deserves further detailed preclinical and clinical development. Moreover, the stimulating effect on normal haematopoiesis could be beneficial when erufosine is used in combination with conventional myelotoxic cytostatic drugs.

ANTAGONISTIC EFFECT OF SR 48692 ON NEUROTENSIN-INDUCED CONTRACTIONS IN SMOOTH MUSCLES OF GUINEA-PIG ILEUM

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Mechanical activity was recorded from isolated guinea-pig ileum segments. Neurotensin (NT) (10^{-11}M-10^{-8}M) produced dose-dependent tonic contractions. Recently, the characterization of the first a high-affinity selective non-peptide neurotensin receptor antagonist "type 1" (NTR1) 2-(1-7-chloro-4-quinolinyl)-5-(2,6-dimethoxyphenyl)pyrazol-3yl)carbonylamino tricycle (3.3.1.1.^{3,7})decan-2-carboxylic acid (SR 48692) was reported. The antagonist was shown to inhibit competitively and selectively the interaction of NT with high affinity binding sites in brain structures. However, the existence and the activity of the antagonist in smooth muscles remain unclear. In our experiments SR 48692 has been studied for its ability to antagonize the contractions produced by NT on isolated guinea-pig ileum muscles. At concentrations of 10^{-8}M to 10^{-6}M SR 48692 has no effect on the spontaneous tone and the phasic activity of the ileum. At the same concentrations the compound antagonized dose-dependently the NT-induced contractions in the ileum segments. In the presence of SR 48692 10^{-6} M the concentration-effect curves for NT were shifted to the right without changing the maximum of the effect. Analysis of the data (Arunlakshana and Schild, 1959) gives the pA_2 values of NT for ileum 6.28, and the slope of the Shild plot 1.05. This data suggests that SR 48692 is a competitive antagonist. Moreover, the antagonistic action of SR 48692 appeared to be a specific one since at concentration 10^{-6}M the compound had no antagonistic effect on the responses of the ileum induced with acetylcholine and histamine. This favours the suggestion of existence of neurotensin "type 1" (NTR1) receptors in the ileum and that the stimulant effect of NT in this muscle is mediated by NTR1 receptors.

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BIOLOGICAL ACTIVITY OF NEWLY SYNTHESIZED AMINOPIPERIDINONES WITH AMINOACID- AND/OR PHOSPHONATE RADICALS

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It is known that piperidines and piperazines exhibit antihistaminic and analgesic activity. The piperidine CP 99,994, that incorporates amino-group in the side chain is a potent neurokinin NK1 receptor antagonist. **The purpose** of this investigation is to study the antihistaminic and anti-SP-activity of 5-aminopiperidinones, with amino group as a part of either a piperazine moiety or a peptide bond as a substitute to the piperidinone ring. The following compounds named according to the IUPAC rules (with their codes in brackets) are studied: (±)-*trans*-1-benzyl-5-((4-(3-chlorophenyl) piperazin-1-yl)methyl)-6-phenylpiperidin-2-one**(NB-165**); tert-butyl 4-((±)-*trans*-1-benzyl-6-oxo-2-phenylpiperidin-3-yl)methyl)piperazine-1-carboxylate **(NB-166**); S-2-amino-N-((±)-trans-1-benzyl-6-oxo-2-phenylpiperidin-3-yl)methyl)-3-phenylpropanamide **(T-31)**; S-2-amino-N-((±)-trans-1-benzyl-6-oxo-2-phenylpiperidin-3-yl)methyl)-3-(1H-indol-3-yl)propanamide **(T-34)**; S-2-(2-aminoacetamido)-N-((±)-trans-1-benzyl-6-oxo-2-phenylpiperidin-3-yl)methyl)-3-(1H-indol-3-yl)propanamide **(T-66)** The synthesis of the aforementioned compounds is carried out from the key acid 1-benzyl-2-phenyl-6-oxopiperidine-3-carboxylic acid via multi-step carboxylic group transformations.
The antihistamine and anti-SP activity of newly synthesized compounds has been studied on guinea-pig smooth-muscle preparations - ileum (for antihistamine) and trachea (for anti-SP). The contractions, induced by cumulatively applied spasmogens alone (HA 10^{-8}M-10^{-4}M; SP 10^{-10}M-10^{-6}M) were compared to those obtained after incubation of muscle segments with compounds tested (10^{-6}M, 10^{-5}M, 10^{-4}M). EC_{50} and E_{max} for each compound have been calculated. All compounds have shown well-expressed, concentration-dependent inhibitory effect on HA-evoked contractions. Applied 5 min before SP, T34, NB165 and NB166 did not change, but T66 increased the contractions, induced by SP 10^{7}M-10^{-6}M, the effect being most probably due to nonspecific interactions.
In conclusion, the substances studied possess antihistamine (with different potency) but not SP-antagonistic activity.

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EFFECT OF ISOTEOLINE IN A RAT MODEL OF OROFACIAL DYSKINESIA

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Motor side effects induced by the typical antipsychotics represent a serious clinical problem. Atypical neuroleptics are much less prone to cause extrapyramidal effects. The mechanism underlying this difference is still not completely understood. It is thought that the substantial blockade of 5-HT2 receptors with the newer drugs counteracts the antidopaminergic effect of the classical drugs in the nigro-striatal system.
Vacuous chewing movements (VCM) in the rat are regarded as an experimental model of orofacial dyskinesia – an early onset type motor disturbance. 5-HT2C receptors have been shown to mediate VCM in several models of dopamine hypofunction. It has been recently demonstrated that subchronic administration of haloperidol leads to adaptive increase in 5-HT2C signaling thus augmenting the ability of 5-HT2C receptor agonists to cause VCM.
Isoteoline (IST) is an aporphine derivative with behavior of a 5-HT2C antagonist in different experimental sets. The objective of this study was to see whether IST could prevent the effect of repeated haloperidol treatment on the induction of VCM.
The following experimental designs were used: 1) Acute experiment: naïve rats were given pretreatment with either water or IST (1 and 4 mg/kg, i.p.) and 20 min later they received mCPP 1 mg/kg s.c. After 10 min the rats were observed for VCM during a 10 min session. 2) Sub-chronic experiment: three groups of rats (8 per group) were repeatedly treated daily for 21 days with (a) haloperidol (1 mg/kg), (b) haloperidol (1 mg/kg) and (c) water i.p. During the last week the rats received additional daily co-treatment: group (a) – IST (4 mg/kg, i.p.); group (b) and group (c) – water. One day after the last pre-treatment administration, rats were given an initial challenge with water and scored for VCM. On the next day, after another challenge with mCPP, they were tested for VCM again.
The results from the acute experiment confirmed the induction of VCM by the 5-HT2C agonist mCPP. IST was inactive by itself, but reduced significantly the number of mCPP-induced VCM in the both doses used. In the sub-chronic experiment the repetitive haloperidol treatment increased both the spontaneous and mCPP-induced VCM. The spontaneous increase was borderline significant and IST pretreatment tended to reduce it. VCM produced by mCPP was almost double compared with the acute experiment. IST co-pretreatment with haloperidol reduced the number of m-CPP induced VCM to a greater extent than in the acute experiment. The results are compatible with the hypothesis of VCM being caused by up-regulation of 5-HT2C receptors by haloperidol, given that IST is capable of acting as an antagonist for these receptors.