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ABSTRACTS

SEVENTH NATIONAL CONGRESS OF PHARMACOLOGY

"PHARMACOLOGY: FROM EXPERIMENT TO CLINICAL PRACTICE"

MEDICAL UNIVERSITY – PLEVEN

17-19 OCTOBER 2014

Under the aegis of
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Dimitar Stoykov, Mayor of Pleven

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DESIGN OF BIOLOGICALLY ACTIVE SUBSTANCES

ORAL PRESENTATION

COBALTABISDICARBOLLIDES WITH PROMISING ANTITUMOR AND ANTIMICROBIAL PROPERTIES

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Summary
The aim of the present study was to evaluate the influence of newly synthesized cobaltabisdicarbollides (H[1], Na[3], Na[4], Na_3[5] Na[6], Na[7]) on viability and proliferation of tumor and non-tumor cells as well as their antimicrobial effects.
Human (A439, MCF-7, HeLa, Hep-2, K562), rat (LSR-SF-SR), mouse (P3U1) and chicken (LSCC-SF-Mc29) tumor and bovine (MDBK) non-tumor permanent cell lines were used as experimental models in our study. The cytotoxic effects of the compounds were examined by MTT test, trypan blue dye exclusion technique, Comet assay, colony-forming method, double staining with acridine orange and propidium iodide. The antimicrobial activity was tested by the classic agar-diffusion method of Bauer-Kirby and determination of the minimum inhibitory concentrations on a broad range of Gram-positive and Gram-negative pathogenic bacterial strains (isolated either from animals or humans as well as control strains) and on *Candida spp.*
The results obtained revealed that: i) cobaltabisdicarbollides Na[7], Na[6] and Na[3] express higher cytotoxic/cytostatic activities than Na[4], Na_3[5] and H[1]; ii) One of the most promising antibacterial agents - Na[4], has also been demonstrated to possess comparatively low toxicity in cultured non-tumor bovine MDBK kidney cells; iii) Chicken hepatoma LSCC-SF-Mc29 cells that express v-myc oncogene were found to be the most sensitive to the inhibitory properties of the tested compounds.
The examined cobaltabisdicarbollides decrease to a different extent viability and proliferation of the treated cells and some of them were proved to possess promissing antimicrobial properties.
Key words: cobaltabisdicarbollides, tumor cell lines, cytotoxic/cytostatic activity, antimicrobial effect

CYTOTOXIC AND ANTIPROLIFERATIVE EFFECTS OF METAL COMPLEXES IN MULTIDRUG RESISTANT CELLS

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Summary
The aim of our study was to evaluate the effect of Zn(II), Cu (I, II), Co(II), Ni(II), Fe(II, III) complexes with various ligands (bile acids, non-steroidal anti-inflammatory drugs, mixed ligands) in multidrug resistant cells.
The following human cell cultures were used as experimental models – A431 (squamous cell carcinoma) and its clones expressing MDR, MRP or ABCG2 multidrug resistance genes; NCI-H1650 (non-small cell lung cancer) and its clones G7 and C11 that are resistant to tyrosin kinase inhibitor Gefitinib. The investigations were performed by MTT test, neutral red uptake cytotoxicity assay, FACS, double staining with acridine orange and propidium iodide, colony forming method.
The compounds examined suppress to a different extent the in vitro growth of the treated cells in a time- and concentration- dependent manner. The mixed ligand Cu(II) complex $Cu_2(BAMP)(dipy)Cl_4$ [BAMP = N,N'-bis(4-antipyrylmethyl)-piperazine; dipy = 2,2-dipyridyl] was found to be the most pronounced cytotoxic and cytostatic agent active also against drug-resistant cells.
Searching for new cytotoxic agents in multidrug resistant cells is of particular importance. Experiments are underway to clarify the structure-activity relationship as well as the mechanisms of action of metal complexes in multidrug resistant cells.
Key words: metal complexes, tumor cell lines, drug resistance, cytotoxic/cytostatic activity

POSTERS

TOXICITY AND PHARMACOLOGICAL ACTIVITY OF TWO NEWLY SYNTHESIZED NEUROPEPTIDES ANALOGUES OF TYR-MIF-1 AND NOCICEPTINE

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Summary
Two newly synthesized neuropeptides (Pajpanova et al, 2013) P1 (analogue of Tyr-MIF-1) and P2 (analogue of Nociceptine) are object of this research.
The aim of the research wais to study their basic pharmacological and toxicological effects on laboratory ICR male mice.
Some basic toxicological characteristics of the two compounds applied in several doses (5, 10, 20, 50 mg/kg b.wt. i.p) was studied. Their influence on the nociception (according to Acetic acid test) was estimated in effective doses (4, 8, 16 mg/kg b.wt. i.p). Statistic was performed according ANOVA analysis.
The two compounds demonstrated toxic effects on the central nervous system (CNS) in a dose of 50 mg/kg, which disappeared until 48 hour. There was no mortality after the acute treatment – both on 48th hour (acute toxicity) and on the 5th day after administration (prolonged toxicity). There were not found pathological changes in the internal organs of treated animals. The two compounds demonstrated different effects on the nociception- P_1 increases it and P_2 has significant antinociceptive effect (over 25%). Established analgesic effect of P2 is dose dependent and probably is specific. Probably it is related to chemical similarity of P2 to nociceptine and maybe is result of possible interaction with opioid receptors in CNS.
Key words: neuropeptides, Tyr-MIF-1, nociceptine's analogue, nociception

EFFECTS OF NEW NEUROPEPTIDES WITH SHORT CHAINS ON COGNITIVE FUNCTIONS OF MICE

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Summary
New active molecules synthesized on the basis of well-known active peptides demonstrated pharmacological activity in previous studies (Pajpanova et al, 2013). Two of them - P1 (analogue of Tyr- MIF – 1) and P2 (analogue of Nociceptine) are object of the present study.
Purpose of the study was to evaluate the effect of P1 andP2 on cognitive function and their interaction with barbiturates in Albino ICR male mice.
Cognitive functions were evaluated using: step through test (for learning and memory), Rot-a-rod test (for muscular coordination) and Holeboard test (for exploratory activity). Interaction of the two compounds with hexobarbital (100 mg/kg i.p.) as duration of sleeping time in minutes was tested. Statistic was performed according ANOVA analysis.
P1 increased the exploratory activity and has significant improving effect on learning and memory. Neuromuscular coordination was not influence by two compounds.
The two compounds significantly shorten duration of hexobarbital sleeping time (P1 by 40% and P2 by 50%). The mechanism is still unknown. It is possible to be a result of functional antagonism between neuropeptides and hexobarbital on the level of CNS and receptors interactions. But it is not excluded the possible ability of neuropeptides to accelerate the elimination of HB.
The two newly synthesized neuropeptides are biological active substances with significant improving effect on the cognitive functions. P1 и P2 shorten significantly HB sleeping time. Future research will clarify the mechanisms underlying of established activities.
Key words: new neuropeptides, cognition, hexobarbital sleeping time

PHARMACOLOGY OF MALIGNANT DISEASES

POSTERS

TWO DIFFERENT MODELS OF INSULIN RESISTANCE IN RATS WITH A DIFFERENT IMPACT ON N-METHYL-N-NITROSUREA-INDUCED CANCEROGENESIS

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Summary
Some cancer diseases are though to be linked with an insulin resistance. Such is a case with prostate cancer which is with an increased prevalence in obesity and insulin resistance. In rats the state of insulin resistance could be create easily with a diet manipulation i.e. overfeeding with fructose or with fats.
Our aim was to compare two models of prostate cancers in hyperinsulinemic rats, treated with fructose or with high-fat diet.
Male rats (mean weight 230 +/- 15 g) were randomly assigned to (1) fructose-feed group (10% fructose solution instead of tape water) (n=20), (2) high-fat diet group (nuts instead of standard laboratory show) (n=20) and (3) a control group (n=20). After an adaptation period (1 week), prostate cancerogenesis was aimed to be induced with N-methyl-N-nitrosurea and testosterone for 20 days. Rats were observed until the end of their life or until the 13th month. Incidences of prostate cancers were recorded. Body weigh, insulin and glucose were also measured.
From the control group 15 rats developed prostate cancer, from the fats overfeed group – 18 ($p < 0.05$) and from fructose-fed group – only 9 ($p < 0.05$). Body weight, insulin and glucose of the

control group was significantly low than in the two treated groups ($p < 0.05$). Body weight, insulin and glucose did not differed significantly between treated groups.
Fructose and fats overfeeding impact in a different way prostate cancerogenesis in rats. It can not be explained by high insulin or glucose per se. Additional researches is needed in order to explain mechanisms of those different models of insulin resistance on prostate cancerogenesis.
Key words: prostate cancerogenesis, insulin resistance, N-methyl-N-nitrosurea, testosterone

METAL COMPLEXES OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS AS PUTATIVE ANTICANCER AGENTS

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Summary
The aim of our study was to evaluate the influence of meloxicam, isoxicam and their metal (ZnII, CuII, CoII, NiII) complexes on viability and proliferation of cultured human and animal tumor cells.
The investigations were performed using methods with various cellular/molecular targets and mechanism(s) of action such as MTT test, neutral red uptake, crystal violet staining, double staining with acridine orange and propidium iodide, FACS, Comet assay, colony-forming test. Permanent human (8 MGBA, HeLa, MCF-7), rat (LSR-SF-SR) and chicken (LSCC-SF-Mc29) cell lines were used as model systems in our studiy.
The results obtained reveal that the compounds investigated decrease in a time- and concentration- dependent manner viability and proliferation of the treated cells; metal complexes are more active cytotoxic and cytostatic agents as compared to the ligands alone; virus-transformed tumor cells (hepatoma LSCC-SF-Mc29 and sarcoma LSR-SF-SR) express higher sensitivity to the antitumor activity of the compounds.
Metal (ZnII, CuII, CoII, NiII) complexes of non-steroidal anti-inflammatory drugs meloxicam and isoxicam possess antitumor activity in vitro and merit further investigations.
Key words: non-steroidal anti-inflammatory agents, metal complexes, tumor cell lines, cytotoxic/cytostatic activity

PHARMACOLOGY OF THE BEHAVIOR

ORAL PRESENTATIONS

FINDING A MODEL FOR COMORBIDITY BETWEEN ATTENTION DEFICIT HYPERACTIVITY DISORDER AND EPILEPSY

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Summary
Attention deficit hyperactivity disorder (ADHD) can coexist with epilepsy and its prevalence among patients with epilepsy is higher than in the general population. Bi-directional relationships are suggested to underlie the predisposition of patients with ADHD to epilepsy and vice versa. However, mechanisms of psychiatric comorbidity in temporal lobe epilepsy (TLE) are

not very well characterized. Validation of relevant animal models for these conditions can help understanding the mechanism underlying behavioral disturbance in epilepsy.
We investigated whether spontaneously hypertensive rats (SHRs), commonly used as a model of ATHD, can be used for studying mechanisms of comorbidity of ADHD and epilepsy.
Kainate (KA) model of TLE was used to provoke onset of spontaneous recurrent seizures (SRSs) in SHRs, which were continuously video and EEG-recorded. A battery of behavioral tests was applied during chronic phase to test motor activity, anxiety level, depressive-like behavior and spatial memory. Neuronal damage and monoamine levels in vulnerable brain areas were analysed.
SHRs are characterized with typical behavioral symptoms such as hyperactivity, an attention deficit and impulsiveness, deficient sustained attention and decreased monoamine functioning. Naïve SHRs demonstrated abnormal electro-physiological and behavioral characteristics as well as monoamine deficit in the frontal cortex and the hippocampus, which were also evident in epileptic rats. In addition, SHRs shows higher seizure susceptibility in models of temporal lobe epilepsy and disturbed circadian rhythms.
Taken together, SHRs afford a system for reproducing and studying mechanisms of comorbidity between epilepsy and ADHD and is suggested as a screening method for mechanism-driven therapeutic approaches.
Key words: attention deficit hyperactivity; kainate model of temporal lobe epilepsy; epilepsy; spontaneously hypertensive rats

QUANTITATIVE MONITORING OF PSYCHOMOTOR ACTIVITY DURING PHARMACOLOGICAL TREATMENT OF DEPRESSIVE EPISODES

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Summary
Psychomotor disturbances are among the core clinical symptoms of endogenous depression. They reflect the underlying pathophysiology and the neurobiological effects of pharmacological treatment of the depressive episode. The objective recording and quantitative assessment of the psychomotor activity during such treatment could serve as an objective measurement of the therapeutic response.
We used a novel objective and quantitative approach to study the dynamic changes of psychomotor activity in patients with endogenous depression during pharmacological treatment of their current depressive episode.
A group of 88 depressive patients was compared with a group of 30 healthy controls. Psychomotor activity was objectively recorded and measured by computerized ultrasonograhic cranio-corpo-graphy (CCG). Both groups were examined twice. The examination of the patients was at the first day of their hospitalization and the day before their discharge from the hospital. The examination of the controls was with similar intervals but without hospitalization and treatment.
There was no significant difference in psychomotor activity of the controls between their first and second CCG-recording ($p>0.05$). Psychomotor activity of the patients was slowed down at their first CCG-recording and was significantly speeded up ($p<0.05$) after pharmacological treatment during the hospitalization. The normalization of psychomotor activity was related to the level of clinical improvement.
Objective recording and quantitative assessment of psychomotor activity of patients with endogenous depression during the pharmacological treatment of their current episode by computerized ultrasonographic CCG could be a sensitive measure of their improvement and might be used for objective treatment monitoring.
Key words: psychomotor activity, cranio-corpo-graphy, depression, pharmacotherapy, treatment monitoring

EFFECTS OF PLANT POLYPHENOLICS IN DEPRESSION. OUR EXPERIENCE WITH ARONIA MELANOCARPA FRUIT JUICE

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Summary
Polyphenols are a group of naturally occurring phytochemicals which are present in high amounts in fruits, vegetables and natural products. They are characterized by the presence of multiple hydroxyl groups on aromatic rings. These compounds are divided into two main categories: flavonoids and nonflavonoids. In the last decade, animal studies have indicated that polyphenols are able to cross the blood-brain barrier and to localize within the brain tissues independently of their route of administration. Recent research has focused on traditional herbal medicines for antidepressant drug development. *Hypericum perforatum* is the only herbal alternative to classic synthetic antidepressants in the therapy of mild to moderate depression. *Aronia melanocarpa* fruit juice (AMFJ) is extremely rich in polyphenolic substances (6600-7000 mg/l as gallic acid equivalents). The main polyphenols in AMFJ are procyanidins, flavonoids mainly from the subclass of anthocyanins, and phenolic acids. In our experiments, AMFJ applied for 30 days to young/healthy Wistar rats demonstrated an antidepressant-like effect in the forced swim test. AMFJ showed an antidepressant-like effect in rat models of depressive-like behavior: i) female rats treated with ethanol for 14 days, ii) male rats exposed to constant light for 14 days, and iii) male rats exposed to social isolation in the course of 30 days. These effects of AMFJ could be attributed to its polyphenolic ingredients. Studies in animal models of depression have established that polyphenols reduce oxidative stress in the brain, modulate monoaminergic neurotransmission and reduce the non-adaptive responses to stress.

Key words: polyphenols, depression, *Aronia melanocarpa* fruit juice, rats

USE OF ULTRASONIC CRANIOCORPOGRAPHY FOR ASSESSMENT OF ALCOHOL WITHDRAWAL

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Summary
There is a need for objective methods of assessment of alcohol withdrawal syndrome (AWS) in humans. Our earlier reports suggest a positive relation between degree of disequilibrium and severity of withdrawal and that equilibrium improves after treatment. The purpose of the present study is to explore the use of a more precise method for objective monitoring of AWS.
Twenty five alcohol dependent patients and 23 healthy controls were successively tested twice with computerized ultrasonographic craniocorpography (Comp-US-CCG) in admission and after detoxification. Subjects executed the stepping test of Unterberger. The severity of AWS was assessed with the CIWA-Ar scale.
72% of patients had abnormal equlibriometric results during withdrawal and 32% - after detoxification. Patients decreased their lateral body sway and increased the number of steps. Degree of imbalance positively correlated with the severity of AWS. Patients had significantly larger lateral body sway than the controls in both measurements.
We conclude that alcohol dependent patients have objectively detectable impairments of dynamic equilibrium expressed by locomotor ataxia during AWS and after detoxification.
The recovery of ataxia can be explained by treatment with high doses of thiamine. Comp-US-CCG could be a method for monitoring of the AWS as the balance abnormalities reflect its severity and it can detect improvement at clinical level.

Key words: alcohol, withdrawal, equilibrium, thiamine, detoxification

EVALUATION OF EXPERIMENTAL TECHNIQUES IN THE PSYCHOPHARMACOLOGY OF BEHAVIOR

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Summary
The rapid evaluation of psychopharmacology is a corollary of both of its intrinsic functions - the pharmacotherapy of behavioral disorders, as well as, the study of brain function utilizing biochemical and behavioral methodologies.
During the past decade, systematic methodologies have been developed for the experimental analysis of behavioral alterations, e.g. mental illness in man. Historically, PSYCHOLOGY (which originally grew out of the study of the human mind) was separated from ETHOLOGY (the biological study of behavior) in terms of methods (psychopharmacological); It is important to realize that of quite different types of question can le asked about behavior using psychopharmacology: I. Proximate causation ("How does it work"); II. Development or Ontogeny; III. Function ("How is it for").
In conclusion it is evident that the problem about the pharmacological control of behavioral alterations is not easy and psychopharmacologists must work still more closely together with neurophysiologist, experimental and clinical pharmacologist.

EFFECT OF ELAGIC ACID ON THE COGNITIVE FUNCTIONS OF MICE

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Summary
Present study investigated the pharmacological effect of polyphenolic compound Ellagic Acid (EA) on male Albino mice after oral administration (500 mg/kg, 5 and 14 days). Our experiments indicated poor but rapid oral absorption of EA and it depends on its solubility using different solvents. In doses 25 mg/kg b. wt. and 50 mg/kg b. wt. EA demonstrated antioxidant activity and decrease lipid peroxidation in mouse blood serum and liver in condition of experimental oxidative stress. EA prolonged also the duration of hexobarbital narcosis (more than 3 times in comparison to control group). We suggest that EA has the potential to interact with drug metabolizing enzymes and especially with hepatic cyt P-450 dependent monooxygenases.
Our experiments established significant preventive effect of EA on the processes of learning and memory (step through test) and exploratory activity (Hole board test) in mice under condition of oxidative stress. We suggest that established improving effect of EA on the cognitive functions of mice is associated with its antioxidative and neuronal protective activity.
Key words: ellagic acid, toxicity, cognition, hexobarbital, antioxidant

DEPRESSION AND POSSIBILITIES OF CURING IT BY ACUPUNCTURE

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Summary
Psychotic disorders could be described as often met clinical cases. Depression has this special feature beside his frequency that about 60% of the

patients are not cured by specialist and often was left without treatment.
The cure by antidepressants is a long lasting, expensive process that now in Bulgaria is reimbursed by National Health Care Box Office. This fact is defining the high social significance of research that could lead to a new, quicker cure without addiction.
Our goal was to make a conclusion about acupunctures possibilities to work independently to drugs treatment.
Used methods were statistical research and evaluation of the publications about clinical results in using acupuncture compare to medical treatment in depression cases. The research includes our own clinical practice cases.
The results have been statistically processed and give evidence of good perspectives for using acupuncture method separately to drugs method.
Key words: depression, acupuncture and antidepressant drugs, antidepressant addiction

POSTERS

EFFECT OF SUBCHRONICALLY INTRACEREBROVENTRICULARLY INJECTED CB1 RECEPTOR LIGANDS ON LEARNING AND MEMORY IN RATS

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Summary
Given a modulatory role of the endogenous cannabinoid system role in cognitive processes we examined the effect of CB1 receptor agonist HU–210 and CB1 receptor antagonist SR 141716A injected i.c.v for 7 days on the learning and memory of male Wistar rats. Rats were tested in a passive avoidance test (step through) and in an active avoidance test (shuttle box). It was found that HU–210 (5 g/1µl) impaired the performance of rats in both tests (i.e. affected negatively learning and memory). The shuttle box test showed a decreased number of avoidances during the two training days and at the retention test (24 h after 2nd training day), while in the step through test a shortened latency time on the retention tests (3 hours and 24 hours after training) was observed as well as a decreased percentage of rats that have reached the learning criterion, as compared to the controls. The administration of SR 141716A (3 g/1µl) enhanced learning tested by both avoidance tasks (expressed as increased number of avoidances during the two training days and increased the percentage of rats that have reached the learning criterion). However, memory enhancing effects were displayed only in the shuttle box test, expressed as an increased number of avoidances during the retention test. These findings suggest that CB1 receptor ligands have a cognition-modulatory effects, i.e. HU 210 impairs learning and memory processes in active and passive avoidance tests, while SR 141716A positively effects learning processes mainly.
Key words: learning, memory, CB1 cannabinoid receptors, shuttle box, rat
Acknowledgements. This study was supported by Grant MU-Varna.

ANXIOLYTIC-LIKE EFFECT OF VIP MICROINJECTED INTO HIPPOCAMPAL CA1 AREA OF RATS WITH A MODEL OF DEPRESSION

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Summary
Aim of the present study was to examine the effects of vasoactive intestinal peptide (VIP) at a dose 10 and 100 ng, VIP antagonist VIP_{6-28} (10 ng) and the combination (VIP_{6-28}+ VIP) microinjected unilaterally into the hippocampal CA1 area on the anxiety in rats with olfactory bulbectomy (OBX) model of depression. The elevated plus-maze was used to evaluate the anxiety-like behavior of the rats. The experiments showed an increased level of anxiety in OBX rats as compared with the sham-operated controls. VIP (100 ng) and the combination infused unilaterally into the left or right CA1 area produced an anxiolytic-like effect in OBX rats, expressed by an increased number of entries into the open arms, time spent there and open/total number ratio. Both VIP (10 ng) and VIP_{6-28} did not affect anxiety. An unexpected finding in our study was that upon pretreatment with VIP_{6-28}, VIP (10 ng), injected unilaterally (left or right) exerted a potent anti-anxiety like effect (increased the number of open arm entries, open arm time and the ratio open/total number of entries). Our data point to a possible involvement of hippocampal VIP-ergic neurons in modulating emotional processes or adaptive responses to stressful stimuli in a rat model of depression.
Key words: vasoactive intestinal polypeptide, depression, hippocampus, anxiety, rat

ROLE OF NEUROPEPTIDES IN HIPPOCAMPAL HEMISPHERIC ASYMMETRY OF LEARNING AND MEMORY IN RATS

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Summary
The aim was to study the effects of neuropeptides angiotensin II (AT II), cholecystokinin-8 (CCK-8) and vasoactive intestinal peptide (VIP) microinjected unilaterally (left or right) into the hippocampal CA1 area on learning and memory (shuttle-box) of rats. It was found that ANG II (50 μg) facilitated learning and memory microinjected into the left CA1 area as compared to the controls and to the right-side. The combination (losartan100 μg + Ang II 50 μg) microinjected into the left CA1 area improved learning and memory. These findings suggest that Ang II infused on the background of the inhibited CA1 hippocampal AT1 receptors ameliorated the cognitive processes. CCK-8 (0.01 μg) exerted a favorable effect on learning and memory when injected into the left but not into the right hippocampal CA1 area. VIP (50 ng) microinjected into the left hippocampal CA1 area impaired learning and memory but there is no effect when applied into the right CA area.
In conclusion, the hippocampal lateralized learning and memory effect of AT II, CCK-8 and VIP depends on the hemisphere of injection. These findings suggest a differential hemispheric distribution of AT II, CCK-8 and VIP receptors mediating learning and memory processes or an interaction between brain neurotransmitters (serotonin, CCK, GABA, Ach), or a differential distribution of their receptors in the brain hemispheres.
Key words: angiotensin II, cholecystokinin-8, vasoactive intestinal polypeptide, learning and memory, shuttle box, rat

EFFECT OF LONG-TERM TREATMENT WITH LOSARTAN ON DIURNAL VARIATIONS OF DEPRESSIVE-LIKE BEHAVIOR IN SPONTANEOUSLY HYPERTENSIVE RATS

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Summary
The selective AT1 receptor antagonist losartan has been shown to decrease arterial blood pressure, to have neuroprotective and alleviating effect in acute seizure models.
AIM: The purpose of the present study was further to assess the efficacy of the treatment with the selective AT_1 receptor antagonist losartan during epileptogenesis on deleterious consequences of kainate (KA)-induced status epilepticus (SE) in spontaneously hypertensive rats (SHRs).
Systolic arterial blood pressure (ABP) was measured by the tail cuff method. SE was induced by KA and treatment with losartan (10 mg/kg/day, in drinking water for four weeks) started three hour later. A battery of behavioral tests (open field test, OF, elevated plus maze, EPM, forced swimming test, FST and sucrose preference test, SPT, radial arm maze) was applied during chronic epileptic phase. The spontaneous motor seizures (SMS) were detected by video and EEG monitoring (video -24 h/3-5 month after SE).
Losartan treatment increased the latency for onset of the first SMS and decreased frequency of seizures in SHRs. Losartan treatment failed to affect hyperactivity and demolished diurnal variations in epileptic rats. In naïve rats losartan treatment increased the preference to sucrose while it exerted diurnal variations in epileptic rats and less sucrose consumption during the light phase. Losartan decreased immobility time in epileptic rats without diurnal variations. Losartan treatment was unable to restore working memory deficit in epileptic rats.
Treatment with losartan during epileptogenesis accompanied with high blood pressure alleviated seizure activity and exerted some behavioral changes in a phase-dependent mode.
Key words: angiotensin AT1 receptor antagonist, kainate model, motor activity, anxiety, memory, spontaneous epileptic seizures

CHANGES IN LEARNING AND MEMORY IN DRUG-INDUCED AMNESIA WITH DIAZEPAM IN RATS TREATED WITH LACOSAMIDE

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Summary
Patients with epilepsy may have impaired cognitive abilities. It has been seen that not only epilepsy but antiepileptic drugs at therapeutic doses can also disturbs memory formation, retention and orientation. Benzodiazepines (Diazepam) are often used in experimental models of drug-induced amnesia, which affects mainly long-term memory.
The present study was undertaken to assess the effect of a new anticonvulsant – Lacosamide (LCS) on cognitive function in a drug-induced model of amnesia with Diazepam (DZP).
We trained 24 male adult rats, divided into three groups (n=8): first group was treated with saline p.o.; second – with saline p.o. and DZP 2.5 mg·kg i.p.; third – LCS 3mg·kg p.o. and DZP 2.5 mg·kg i.p. All rats were trained on apparatus for passive and active avoidances-Shuttle box. Learning sessions were performed for 5 days and on the 12th day memory retention was measured.
The controls increased significantly the number of avoidances on learning and memory sessions ($P<0.05$). The animals with model decreased significantly the number of avoidances and escapes during learning and memory sessions in comparison with the control animals. The group with LCS increased significantly the number of active and passive avoidances on the 5th day of the learning session and on memory retention compared with the group with drug-amnesia ($P<0.05$).
The used dose of LCS shows a tendency for a neuroprotective effect on cognitive function in DZP model of amnesia in rats.
Key words: Lacosamide, Diazepam, learning, memory, shuttle box

CONTINUOUS TREATMENT WITH PSYCHOTROPIC DRUGS OF FOUNDLINGS IN HOMES FOR DISADVANTAGED CHILDREN

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Summary
Attention to the care received in homes for disadvantaged children by foundlings is increasing during the last decade. The proper and correct usage of medication is an action that preserves their life, as neurological problems are commonly observed in foundlings. These conditions need prolonged medication and could be harmful if not supervised and cured correctly. Their correct treatment is a serious medico-social problem.
The aim of our study is to assess the effect of continuous medication of foundlings in Bulgaria. Observation of foundlings in Gomotartzi (district of Vidin, Bulgaria) and analysis of their medical treatment and documentation.
For the purpouse of our study 54 medical records were evaluated, including those of 33 children aged from 3 to 18 y.o. and 21 adults aged from 19 to 21 y.o. passing their childhood in Gomotarzi.
All of foundlings from Gomotarzi are with diagnosis of different grades of cognitive impairment and concomitant diseases as hydrocephalia, cerebral palsy, Down syndrome, epilepsy, spastic paresis, psychological disorders (aggression, auto-aggression). Neurotropic medications are used in those patients, as follows: one patient was on haloperidol, one – on thioridazine, six – on Sodium valproat, nine - on clonazepam, was discovered only one with a possibly drug caused extrapyramidal syndrome. Periodic diagnostic laboratory and EEG-tests were performed, as well as continues surveillance from the medical staff. Tardive dyskinesia was observed in the child on haloperidol after 1 year of treatment. Half of foundlings are with under nutritive state. Hospitalization because of aggression and autoaggression was done for 3 foundlings. Poor dental status was recorded for all 54 foundlings. 8 cases of bone fractures were recorded. Blindness is diagnosed for one of foundlings and 9 of the children are permanently in bed (can not walk).
Our study reveals that a lot of serious medical conditions are present in foundlings fledged in homes for disadvantaged children. Those children need constant supervision, examination, treatment and sanitary care so they can be properly raised. Often they need prolonged medication, which could be harmful if not supervised and cured correctly.
Key words: children, psychotropic drug, foundling

NOVEL L-VALINE PEPTIDOMIMETICS MODULATE COGNITION AND NEUROTRANSMISSION IN HIPPOCAMPUS OF SOCIALLY ISOLATED RODENTS

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Summary
Novel peptidomimetics M6 and P6, incorporating L-valine and nicotinic /isonicotinic acid were synthesized. Previous data demonstrated improvement of cognition in rodents, changing serotonin content in mouse brain and monoamine levels in rat hippocampus. Purpose of the study was to assess the effects of the isomeric M6 and P6 on cognition of experimental mice and rats

with syndrome of social isolation (SI), and upon neurotransmission in rat hippocampus.
Effective dose treatment (100 mg/kg, i.p., for 3 days) was administered to male socially isolated Wistar rats and Albino ICR mice. Behavioral tests for memory (Step-through and Hole-board), and radiometric tests for rat hippocampal cholinergic and serotonergic neurotransmission were used. Levels were compared to control animals. Student Fisher T-test and ANOVA were used to assess data.
Cognitive dysfunctions were confirmed in both mice and rats, accompanied by alterations in hippocampal 5-HT and acetylcholine release. The compounds modulated significantly long-term memory and exploratory behavior. Spatial memory and fear-conditioned memory decreased, as evident in Hole-board and Step-through tests. M6 and P6 altered significantly hippocampal serotonin release and uptake of SI animals compared to control groups of rats, and influenced acetylcholine release in hippocampus in SI rats bidirectionally. The variation in effects of the compounds may be due to positional isomery and physicochemical differences.
The tested peptidomimetics may be effective modulators of animal behavior and cognition due to possible affinity to hippocampal receptors. The isomers induce changes not just in serotonergic, but also in cholinergic neurotransmission and thus represent potential pharmacological agents.
Key words: peptidomimetics, cognition, hippocampus, serotonin and acetylcholine

NOVEL PEPTIDOMIMETIC AFFECTS COGNITION OF YOUNG SOCIALLY ISOLATED RATS

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Summary
Early life stress can be detrimental for mental health. Maternal deprivation (MD) with social isolation (SI) is a classical animal model of damaged cognition. A novel peptidomimetic M6, a derivative of L-valine, demonstrated significant neuroprotective effect on adult rodents in earlier studies. The purpose of this study was to test M6 for pharmacological modulation of cognition in young rats after MD.
Male and female Wistar rat pups were separated from their mothers on the 21th day. Five-week-hosting in isolated cages ensued (Valzelli method, modified by Petkov). Control animals after MD were collectively hosted - 6 rats in a cage. Half of the animals (grouped and isolated) received M6 daily 150 mg/kg (i.p.) for 3 days. Control rats (male and female) /N=6 animals for each group/ received only Oleum Helianthi. Cognitive parameters of the experimental animals were tested with Hole-board and Step-through tests 24 hours after last treatment. Data analysis used SPSS and ANOVA.
The isolated rats were more exploratory active than grouped animals. They also had better learning and memory 24 hours after treatment. Exploratory activity was lower in male than in female grouped rats. The effect of M6 was memory modulating and gender-dependent: memory improved in male grouped animals and not so much in isolated animals; memory improved in female isolated and not in grouped rats.
The peptidomimetic M6 is a pharmacologic agent that improves learning and memory in grouped young male rats and in young female rats with social isolation syndrome.
Key words: peptidomimetic, social isolation, memory modulation

GENDER DIFFERENCES IN THE BEHAVIORAL CHANGES INDUCED BY EXPERIMENTAL MODELS OF DIABETES MELLITUS TYPE 1 AND SPONTANEOUS HYPERTENSION IN RATS

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Summary
Diabetes mellitus (DM) is the largest metabolic disease in the world, which tends to steadily increase in people affected by it. Among heavy damage in renal, cardiovascular and visual systems, DM causes serious damage to the nervous system and related sensory and behavioral disorders. Existing evidence on gender differences and co-morbidity with hypertension in some damage caused by DM give us reason to suppose that the experimental rats of two strains - normotensive Wistar and spontaneously hypertensive (SHR) could exhibit sexual dimorphism in metabolic and behavioral abnormalities associated with the development of DM.

Male and female Wistar rats and SHRs (16 weeks old at the beginning of the study) were used. Experimental diabetes, type 1 (DM1) was induced by an intraperitoneal injection of streptozotocin at a dose of 65 mg/kg and confirmed 48 hours later by elevated plasma glucose level. Behavioral methods for the analysis of the effects of DM1 on motor activity and coordination, painful sensitivity, state of anxiety and depression and SMART video tracking system (Harvard Apparatus, US) were used.

Naive female rats were more active then male of both strains. DM1 decreased motor activity in Wistar rats of both genders, but did not change significantly the motor activity of SHRs. DM1 declined motor coordination in male Wistar rats. Untreated female SHRs showed a lower level of depression compared to male and only diabetic female Wistar rats developed depressive-like behavior in forced swimming test.

Taken together the results showed a gender dependence of diabetes-induced behavioral complications in normotensive and hypertensive rats.

Keywords: diabetes mellitus type1, gender, spontaneously hypertensive rats, motor activity, depression

MODULATION OF SEROTONIN LEVELS IN HIPPOCAMPUS OF SOCIALLY ISOLATED RATS BY NEW L-VALINE PEPTIDOMIMETICS

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Summary
Newly synthesized peptidomimetics on the basis of essential aminoacid L-valine (as positional isomers with codes M6 and P6) were studied. Previous data have shown that they can significantly improve cognitive functions in rodents. Social isolation of animals and humans often leads to aggressive behavior and cognitive deficits.

Purpose of the study was to evaluate the modulating effect of newly synthesized peptidomimetics on cognition deficit in aggressive rats and the accompanying changes in the Serotonin content in hippocampus.

Treatment of rats (aggressive and grouped) with the peptidomimetic compounds was for 3 days, 150 mg/kg b.w. i.p. The serotonin release and uptake in hippocampal tissue was measured by a radiolabelling method. Behavioral tests for learning and memory (step through) and exploratory behavior (Hole board) in rats were used on experimental model of aggression induced by social isolation (6 weeks).

The two compounds affect serotonin release and especially serotonin uptake in hippocampal tissue of animals (both in aggressive and in grouped). It was found out that the two isomeric peptidomimetics modulated memory functions (short-term, long-term memory and exploratory behavior).

The new compounds probably are effective modulators of aggressive behavior due to their possible affinity for serotonin receptors in hippocampus. Their influence on serotonin levels

in the hippocampus of aggressive animals deserves further studies and future development as potential pharmacological modulators.

Key words: aggression, cognition, peptidomimetics, L-valine, serotonin

EFFECT OF PINEALECTOMY ON ANXIETY AND DEPRESSIVE-LIKE BEHAVIOR IN WISTAR RATS

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Summary

Melatonin is involved in the control of circadian and seasonal rhythmicity, possesses potent antioxidant activity and exerts a neuroprotective and anticonvulsant effects. AIM: In present study, the regulatory role of endogenous melatonin on emotional behavior associated with anxiety and depressive responses was analyzed.

Wistar rats were sham-operated (sham) or pinealectomized (PIN). Behavioral tests for motor activity (open field, OF), anxiety (elevated plus maze, EPM, light-dark test, LDT) and depressive-like behavior (sucrose preference test, SPT, and forced swim test, FST) were executed three months after surgery. Brains were removed to explore the release of serotonin in the hippocampus.

Pinealectomized rats did not differ in motor activity in OF and EPT from sham rats. However, experimentally-induced melatonin deficit caused higher anxiety level in EPM and LDT. Pinealectomized rats were characterized by a depressive-like behavior and decreased release of 5-HT in the hippocampus compared with sham rats in SCT and FST.

Melatonin deficit in the pineal gland cause increased increased anxiety and depressive-like behavior, which is accompanied by a decrease of serotonin release in the hippocampus.

Key words: melatonin, pinealectomy, anxiety, depression, serotonin

BEHAVIORAL TESTS IN RAT MODELS OF METABOLIC SYNDROME

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Summary

In rats fed high fat (HF) and high fat-high fructose (HFHF) diet in order to develop a model of metabolic syndrome, psychomotor tests were carried out to assess the potential effect of diet manipulation on animal behavior.

The open field test was used to measure the general horizontal locomotion. In addition, the time spent in the central areas and the number of entries into the central squares was recorded as an inverse index of anxiety. The social interaction test was also used to measure the level of anxiety. The Porsolt test was used to estimate the depression index of the animals. The object recognition test was utilized to assess potential memory effects of the intervention.

Locomotion in open filed was not affected by diet manipulation. The time spent in center was significantly reduced in HFHF rats, a similar effect though non-significant, was also observed in HF rats. A trend towards reduction was also seen in the entries in center in both groups. The time spent in social interaction was significantly decreased in HF group. These results reveal a tendency to increase the anxiogenic state of animals subjected to diet manipulation. In the Porsolt test there was a non-significant reduction in the time of active swimming in both experimental groups. The object recognition test did not reveal memory changes in rats fed the diets used.

The present results show that diet manipulation aimed at metabolic syndrome increases the likelihood of developing anxiety and possibly depression in rats.

Key words: high fat diet, high fat high fructose diet, anxiety, depression, rats

RESEARCH OF THE BLOOD LEVELS OF HPA AXIS HORMONES IN RATS IN COLD STRESS MODEL, RECEIVED ACUPUNCTURE TREATMENT

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Summary
A lot of work has been done to understand mechanism of acupuncture. Anyway the mechanism of direct mechanism of acupuncture works is not clear.
Our goal was to create an analytical method for researching the blood level quantity of HPA axis hormones in rat model. The used analytical devices - LC/MS/MS (QExactive, Thormt. USA) gives possibilities to analyze whole number of hormones of the HPA axis in rats.
The substances Sigma Aldrich have been used as evidence.
The results shows statistically differences blood level of HPA axis hormones between rats in cold stress model, treated with acupuncture.
Key words: acupuncture, stress hormones level, LC/MS/MS analyzes

ELABORATION OF THE EXPERIMENTAL MODEL FOR RESEARCHING THE MECHANISM OF CURIBULE EFFECT OF ACUPUNCTURE IN DEPRESSION CASES

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Summary
Stress is one of the keys to understand brain organization and way of work. It was discovered biochemical changes in the blood level of hormones in crash driving survivors, soldiers in the battle field, sports man in marathon condition and others. To connect these facts with acupuncture we need of clear experimental model that will provide files about brain harmonization under stress conditions.
Our goal: by researching special literature to create a model for pharmacology experiment over rats. The usual rat stress model has been made using immobilization, cold, or light. Acupuncture as a methodic of stress relieving have been used not so often, and from literature have been found that a lot of experiments does not met the quality standards.
Special attention was given to publicized map of transcription of the acupoints of human system to small animal (rat) for making model more reliable. The experimental model includes a group for researching also sham acupuncture. The model need of special attention and discussing, because the before used models are connected with small number of hormones and this does not gives possibility to research real connection between hormones of HPA axis \Hypothalamic–pituitary–adrenal axis\. Our model includes all HPA hormones that have been releasing during the cold stress and it relieving by acupuncture. The analytical method is HPLC coupled with MS and gives possibility to define all hormones together.
Results – created model include defining of more hormones and gives new information about the mechanism of acupuncture stress relieving.
Key words: stress, acupuncture, experimental rat model, HPA hormones

PHARMACOECONOMICS

ORAL PRESENTATIONS

"STATUS QUO" OF PHARMACOECONOMICS IN BULGARIA

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Summary
The goal of the study is to analyze the scientific, regulatory and educational status of pharmacoeconomics in Bulgaria during 1990 – 2014 years.
Legislation analysis and desktop study were performed towards the published pharmacoeconomic articles and educational programs in Bulgaria. It was searched the Scopus and PubMed databases with key words pharmacoeconomics, cost-effectiveness, cost-utility, cost-benefit analyses. On total 120 articles were found. The education programs provided by different universities and organizations were also compared. Health care and pharmaceutical legislation were revised and regulations development was systematized in the respective field.
On total 120 articles were systematized. An increase with near 50 published articles since 2011 was established. Most of the article are cost of illness analysis (40%), followed by cost-effectiveness (20%); cost-minimization (10%); cost-utility and budget impact analyses. The educational programs are presenting basic methodologies and its practical application and are relatively synchronized for pharmacy students, while for medical ones there is a lack of in depth education. Professional organizations also perform educational courses highly appreciated by the regulators and general health care audience. Interest towards them arouse sharply after the regulatory inclusion of pharmacoeconomics for the purposes of pricing and reimbursement of medicines in Bulgaria.
In conclusion there is sufficient and well-grounded regulatory, educational and scientific basis for pharmacoeconomics in Bulgaria.

Key words: pharmacoeconomics, science, education, Bulgaria

BIOPHARMACEUTICALS IN THE TREATMENT OF THE RHEUMATOID ARTHRITIS

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Summary
The aim of this study was to describe the difference in the treatment of rheumatoid arthritis, analysis based on inquiry study with patients.
Together with the Association of patients with rheumatoid arthritis the study including 119 patients was conducted between April and September 2012. The questionaries' had the goal to gather basic information about the characteristics of the patients, their pharmacotherapy, frequency of physicians' visits, hospitalizations, and their health status self-evaluation. The direct health costs were calculated based on the insurance fund tariff.
Nineteen patients were excluded due to insufficient information. The female patients prevail significantly over the male patients with 78 to 22 and the average patients age was 54.78 years. More than half of the patients (58 patients) were unemployed. Twenty-five percent of the patients were treated with biopharmaceuticals. According to Bulgarian Positive Drug List the biologic drugs that can be prescribed and reimbursed from the national insurance found are: rituximab, certolizumab, golimumab, tocilizumab, etanercept and adalimumab. The biological therapy is the most expensive one 2961.03 BGN per patient/month.
The biological drugs are not fully reimbursed from the Bulgarian Health Insurance Found. More than half of the patients with biologic treatment reported moderate pain (17 patients), 4 patients reported mild or no pain and 4 patients reported severe pain. Only one patient reported that he is not taking his medicines regularly. Fifteen of the patients with biologics were diagnosed with rheumatoid arthritis more than

ten years ago and only three are diagnosed in the last five years.

Key words: pharmacotherapy, rheumatoid arthritis, cost of illness, pain, biopharmaceuticals

SOCIAL AND ECONOMIC BURDEN OF OSTEOPOROSIS IN BULGARIA

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Summary

Quality of life is an important measure for assessment of social and economic burden of chronic diseases. DALY is a standard measure for social burden of diseases providing information about the years of life with disability with particular disease.

The goal of this study is to analyze the social burden of osteoporosis in Bulgaria, using illustrative DALY example.

Retrospectively was performed documental analysis of disabilities caused due to osteoporosis. The burden of osteoporosis was then calculated using the standardized WHO approach adjusted to data for women life expectancy for Bulgaria. DALY = YLD+YLL, where YLD is year lived with disability and YLL is years life lost.

According to the literary data the total morbidity from osteoporosis for EU is 22 million women. For 3.5 million documented fractures 620 000 are hip fractures, 520 000 are spinal fractures, 560 000 are wrist fractures and 1 800 000 are others.

YLL were calculated as 4 for the Bulgarian women, considering only the shorter life expectancy for the local population. The results show that there is a difference in YLD according to the age group: 50-54 years – 0.0013; 55-59 – 0.0678; 60-64 – 0.112; 65-69 - 0.170; 70-74 – 0.260; 75-79 – 0.3673; 80-84 – 0.486; 85+ – 0.713. And the same is observed for disability weights varying in accordance to the type of the fracture as the most frequent are the hip and spinal fractures.

We can conclude that DALY for one woman with osteoporosis in Bulgaria is 5.906. The results confirm the social importance of osteoporosis and the EU data that osteoporotic fractures account for 2 million disability adjusted life years (DALYs) annually.

Key words: osteoporosis; DALY, social burden, economic burden

APPLICATION OF THE DATA FROM CLINICAL TRIALS IN PHARMACOECONOMIC ASSESSMENTS

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Summary

The aim of the study was to analyze the methods for the assessment of the data from randomized clinical trials for the efficacy of the medicinal therapies and their application in pharmaco-economic assessments. The methodology: the three main criteria for the assessment of clinical trial data were analyzed – the systematical bias, reliability and validity. The possible approaches to address important issues related to the selection of prospective data from clinical trials were discussed, as well as the choice of a therapeutic alternative for comparison, the intermediate against the final health results, the adapting of the data to the actual therapeutic practice and the selection of quantitative methods for the modeling of the final expenses and health results.

The pharmacoeconomic assessment is a comparison of the ratio cost/result between two medicinal alternatives. Therefore, the use of data from clinical trials of the "medicine – placebo" type is unacceptable for the economic assessments of the efficiency. The relevant data for pharmacoeconomic assessment compare the new medicinal therapy with the "gold standard" for the specific diagnosis in the current medical practice. It is permissible to use the secondary data from clinical trials in order to calculate comparatively the expense/result. The impact of the intermediate health results of the clinical trials on the final health results, such as morbidity and

mortality, is usually established by constructing models based on the secondary data from epidemiological studies. The data from clinical trials is characterized by high internal and low external validity. Therefore, the secondary data has to be adapted to the actual therapeutic practice. The choice of the quantitative methods for the modeling of the final health results has an important role for the accuracy of the pharmacoeconomic assessments.

The use of data on the efficacy of medicinal therapies from randomized clinical trials is in the basis of pharmacoeconomic evaluations. Prior to being used for the purposes of the economic analysis of medicinal therapies, the data has to be evaluated in terms of its systematic bias, reliability and validity.

Key words: pharmacoeconomic evaluation, final health results

QUALITY OF LIFE OF HCV PATIENTS IN BULGARIA

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Summary

Nine million European citizens suffer from HCV infection as higher prevalence is reported for Southern and Eastern Europe. In 2002 according to WHO data 86 000 HCV related deaths and 1.2 million DALYS are reported. DALY is a standard measure that gives disability adjusted life year important for calculation of social burden of the disease, mortality and risk factors.

The goal of the study is to analyze the social burden of HCV patients in Bulgaria, using the WHO approach for DALY.

It is a retrospective, documental analysis of HCV infection based on EU data and prospective DALY calculation for HCV patients in Bulgaria. National Institute for statistics and National Consensus for diagnostics, monitoring and treatment of chronic viral hepatitis in Bulgaria were reviewed for morbidity data. DALY= YLD+YLL, where YLD is year lived with disability and YLL is years life lost.

According to the literary data for WHO European region for 2002 about 95% of HCV related DALYs are calculated for preventable stages of the disease. 81% of DALYs are lost due to HCV related cirrhosis. 25% from the liver transplantations in 2004 are due to HCV infection. HCV is a reason for 200 104 YLDs. 191 537 (96%) from them are result from HCV related cirrhosis.

The results show that YLD is different for the different age groups and also for the disability rates for cirrhosis and hepatocellular carcinoma (HCC) and also differs from the frequency of incidences in the age groups. We calculate that DALY for a patient with cirrhosis and HCC due to HCV in Bulgaria is 9.44.

The results show that the HCV impact the quality of life of patients and that the infection is a social, economic and health problem in front of the EU healthcare professionals.

Key words: HCV; DALY, quality of life, social burden, economic burden

PRICING AND REIMBURSEMENT OF MEDICINAL PRODUCTS IN BULGARIA: IS IT TIME FOR RISK SHARING AGREEMENTS?

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Summary

The current publication focuses on some country-specific measures, part of the new legislation on the pricing and reimbursement of medicinal products in Bulgaria, aimed at ensuring greater affordability of medicinal products in the light of scarce financial resources and need for risk sharing agreements.

We analyzed the legislative changes in the pricing and reimbursement of medicinal products came into force in Bulgaria in 2011-2013.

In October 2011, possibility of negotiating of

discounts for some fully reimbursed medicinal products (products that are not referents for other INNs) by National Health Insurance Fund (NHIF) was introduced in the legislation. Later, in the summer of 2012, based on Ordinance 10, this measure was extended to all medicines, included in the Positive Drug List (PDL). From that moment until the end of 2013, only one negotiation procedure was performed and with a limited success. Out of approx. 650 medicinal products, 10% were discounted according to NHIF information after 2012. A new pricing and reimbursement regulator was set up in March 2013 – the National Council of Pricing and Reimbursement of Medicinal Products (NCPRMP). Price reviews performed by NCPRMP in 17 reference countries in every 6 months for the medicinal products included in PDL were introduced as another mechanism for price decrease. In 2013 the prices of 842 medicinal products were reduced and the reduction per pack varied from 0.1% to 57.26% which led to substantial reduction of treatment costs. However, 140 medicinal products were delisted from PDL in 2013, a process mostly initiated by marketing authorization holders or NHIF.

The legislative changes in the last 3 years resulted in price reductions of 842 medicinal products in the PDL in the range 0.1%-57.26%, which became more evidential with the establishment of NCPRMP. The delisting of large number of medicinal products might be a sign for a need for introduction of risk sharing agreements in order to balance between the scarcity of financial resources, availability of treatments and market strategies of marketing authorization holders. Further investigation is needed regarding the reasons for delistings, required by the holders.

Key words: pricing, reimbursement, medicinal products, pharmaceuticals, regulation, Bulgaria

POSTERS

REFERENCE VALUES VERSUS TIERED REIMBURSEMENT. WHICH IS THE BEST APPROACH IN DRUG POLICY?

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Summary

The aim of the study was to demonstrate a model of the annual cost of reimbursed medicines with the use of a combined approach to drug policy, including reference pricing and tiered reimbursement rates compared to the price of each drug group. The survey addresses five medicinal products for the treatment of cardiovascular diseases - lisinopril, bisoprolol, amlodipine, valsartan and indapamid.

The methodology includes comparison of the public and private expenditures on the five medications based on reference reimbursement values and tiered reimbursement rates. The model is built on the assumption that the choice of reimbursement approach does not affect drug prescriptions and drug consumption. The real statistics on sales of medicinal products in the first half of 2013, as well as the actual reference reimbursement values during the same period, have been used.

The model identified the following positive results from the implementation of tiered reimbursement levels: a decrease in the total cost of the five medicinal products (30%); lower deductibles (43%); motivation of pharmaceutical manufacturers to reduce reimbursement prices and approach the reference values; motivation of doctors and patients to use the recommended medicines with financial benefits for both parties – reduced fee and surcharge, respectively. The only negative in the differentiated reimbursement levels, identified in the model, is the increase in public spending.

The implementation of motivational mechanisms is a need for both patients and doctors, so that cost-effective drug therapies would be the preferred choice. Additionally, the introduction of a system to manage medical prescriptions would also be suitable. The mixture of motivational incentives to patients (lowering the cost of deductibles) and to doctors (fee for recipe) would lead to redistribution of prescription medicines.

Key words: pharmaceutical costs, reimbursement, management, approaches

PHARMACOECONOMICS AND DRUG POLICIES. RESULTS OF APPLICATION OF PRICE REBATES AND AGREEMENTS ON CONTROLLED ACCESS OF PATIENTS IN THE MANAGEMENT OF PUBLIC EXPENDITURES

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Summary
The aim of the study was to identify and evaluate economic performance of application of the policy to negotiate discounts on medicinal products and agreements on controlled early access for patients in Bulgaria.
The methodology involves comparison of the amounts of public spending on medicines in two periods - during the course of the analyzed drug policies (2007-June, 2009), and the period, in which negotiations on the price of medicines and programs for controlled access of patients was discontinued (July, 2009-2012).
After June 2009, the government of Bulgaria did not apply methods for controlling public expenditure on medicines, such as bargaining price concessions from manufacturers and implementation of agreements on controlled access of patients. This leads to an annual increase in the expenditures of the National Health Insurance Fund (NHIF) on drug products for home treatment on average by 17% for the period 2009-2012. This trend will continue in future periods since expenditure control only through price control by means of a reference system and the positive list of medicines is ineffective.
The implementation of combined drug policies in the form of negotiations on rebates with manufacturers and agreements on controlled access of patients and reference pricing is required.
Key words: drugs, prices, pricing, negotiating, National Health Insurance Fund

ADAPTATION OF DATA IN THE TRANSFER OF PHARMACOECONOMIC EVALUATIONS

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Summary
The aim of the study was to analyze the performance requirements and requirements for adapting data from pharmacoeconomic evaluations and to build a model, which includes formal requirements of the National Council on prices and reimbursement for presenting data and reporting the results of the adaptation of pharmacoeconomic evaluations in the transfer from other countries.
The practice of the Swedish Agency for Dental and Pharmaceutical Benefits (TLV) and the Dutch Health Insurance Board (CVZ) practice were analyzed. These are statutory bodies in both countries and are responsible for decisions on reimbursement of medicinal products. The choice of the two countries is dictated by their different ways of funding their health systems - through taxes or through health insurance contributions.
The analysis of such requirements in the Netherlands and Sweden shows that they cover largely the same factors that affect cost/result ratios, such as demographic and epidemiological factors for morbidity, various health resources and variations in clinical practice, incentives for health care institutions and professionals and differences in prices of medical services and medicines.
The established and systematic factors determining differences between countries require scientific interpretation and adaptation of data from pharmacoeconomic evaluations to the local health economic environment.
Key words: pharmacoeconomic evaluations, health and economic environment

IMPACT OF THE GENERIC COMPETITION ON REFERENCE PRICES OF CARDIOVASCULAR MEDICINES

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Summary
The goal of the study is to analyze the impact of introduction of new generic substances in the positive drug lists on the prices of reimbursed cardiovascular (CV) medicines.
It is a retrospective, observational and statistical analysis of the changes in reference prices of monoproducts acting on cardiovascular system for the period 2009-2014 years. On total 48 INN out of 12 ATC groups were included in the analysis. T-test analysis was applied towards the price changes.
Out of 48 INN 2 new molecules were introduced and for 28 products new generic products enter in the list during the period. For 91.7% of the CV medicines was observed price decrease and for the rest 8.3% increase was reported (doxasozine, propranolol, metoprolol, telmisartan). Within the therapeutic group with new generic molecules entrance the general rule is that the reference prices decrease. The latter is also observed when new dosage forms are introduced as is the case with the group of sartans, ACE inhibitors and anti-hyperlipidemic. The decrease is more than two fold in the newly emerging therapeutic groups of ACE inhibitors, AT angiotensin receptor blockers, and antihyperlipidemic. In the group of well-established cardiovascular glycosides and antiarrhythmic medicines prices remain stable. The prices decrease is more evident after 2013 probably as a result of the legislative changes introduced in the period. All price changes were found to be statistically significant.
This study confirms that the generic competition lead to significant price decrease, but other factors also play an important role in the process.
Key words: cardiovascular medicines; generic medicines policy, medicines prices, reference pricing

DRUG RELATED PROBLEMS – THE BENEFITS FROM APPLYING PHARMACEUTICAL CARE IN COMMUNITY PHARMACY PRACTICE

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Summary
The purpose of the study is to define the potential health benefits from medication follow up in community pharmacy as part of the service Pharmaceutical care.
A literature search was performed through Springer, Scopus, Medline, Google Scholar and other. Key terms used for the search were: drug related problems, pharmaceutical care, hospitalizations. The screening reduced the identified papers to 17 articles for the period from 1990 to 2013. Data were analyzed using thematic, interpretive and comparative analysis methods. The medication follow up is one of the main elements of Pharmaceutical care concept. It may be supportive in prevention of many possible drug related problems like treatment failure, inappropriate dose, clinical significant interactions, drug related hospitalizations etc.
Knowing the effects of medication follow up and implementation of this service in the pharmacy practice may lead to reducing negative drug related events, shortening of the hospitalization period and reducing the cost of health care at a higher efficiency. According to Gyllensten et al. the drug related problems were considered preventable in 24 to 45±15% of the patients with drug related morbidity.
Findings indicate that the medication follow up is essential part of Pharmaceutical care for reducing the drug related morbidity and minimizing expenditures for drug related morbidity and mortality.
Key words: pharmaceutical care, adverse drug reaction, community pharmacies, hospitalizations, health benefits

ECONOMIC ASSESSMENT OF PHARMACOLOGICAL AND NON-PHARMACOLOGICAL TREATMENT OF HODGKIN'S LYMPHOMA

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Summary
Hodgkin's lymphoma (HL) is a rare malignant disease of a lymphatic system which arises from Reed-Sternberg cells. The prevalence for Bulgaria is 2.4/100 000.

This publication is focused on the progress of pharmacological and non-pharmacological treatment of HL, especially in case of relapse or progress, and on the economic impact of each treatment option.

The analysis is from the payer's perspective and it is based on clinical trials, pharmacoeconomic analysis, literature data for therapeutic alternatives and corresponding dosage regimens and Bulgarian Positive Drug List. Cost analysis and budget impact analysis were applied. The costs are calculated for 175 Bulgarian patients with relapse or progress for period of 3 months.

There are several chemotherapy regimens, which could be combined with radiotherapy. The alternative in case of relapse regress is salvage therapy combined with autologous stem cell transplant (ASCT), followed by applying allogeneic stem cell transplantation (aloSCT). A new treatment option is antibody-drug conjugate Brentuximab vedotin. The costs for salvage therapy (average), alloSCT and Brentuximab are: 130 350.19, 12 040 000, 10 662 230 BGN, respectively. The highest price is for aloSCT followed by Brentuximab. The budget impact analysis shows that implementation of Brentuximab vedotin is associated with lower costs in comparison with alloSCT (the difference is 1 377 770 BGN), but highest – with salvage therapy for the period.

The therapy for HL is adequate and there is development despite of high price. The new drugs with innovative mechanism of action meet unmet medical needs for these patients with HL.

Key words: Hodgkin's lymphoma, Brentuximab vedotin, cost analysis, budget impact analysis

PHARMACOTHERAPY COST ANALYSIS OF *IN VITRO* FERTILIZATION – A CASE STUDY

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Summary
Current study analyses the cost of pharmacotherapy of *in vitro* fertilization during the period 2009-2013 in a specialized gynecology clinic in Sofia. It is a prospective, observational analysis of the pharmacotherapy and cost of in vitro fertilization. Bottom up approach for the cost analysis is used. During the period 2009 - 2013 was observed all women admitted to the clinic and collected information about the therapeutic protocols, prescribed medicines and doses, average length of therapy and its cost. Statistical analysis is applied towards the pharmacotherapy and cost data.

On average 136 (SD 21.92) women were admitted varying from 105 to 179 for 10.7 (SD 1.47) days. 11% were on long therapeutic COX protocol and all other on shorter. Therapeutic protocols include Follitropin alfa IU (prescribed to 103 women at average dose of 1171 IU (SD314.16)); Follitropin beta IU (prescribed to 299 women at average dose of 1634 IU (SD 423.5)); Urofollitropin 75IU amp (prescribed to 243 women at average dose of 21.3 IU (SD 7.37)); urFSH+urLH 75IU:75IU (prescribed at 354 women at average dose of 23.4 IU (SD 8.8)); cetrorelix amp 0.25mg prescribed at 264 women at average dose of 3.84 IU (SD 1.32); ganirelix amp 0.25mg prescribed at 299 women at average dose of 4.01 mg (SD1.32); Human chorion gonadotropin prescribed to 535 women at average dose of 6752.52 IU (SD 1216.23); Nafarelin mcg/ml prescribed at 8 women at dose of 17700 mcg (SD 10725); triptorelin acetat 0.1mg amp prescribed at 63 women at doses of 5.5 (SD 3.25) mg at 14 women and average dose

of 7.5 mg (SD 2.5); clomiphen citrate and letrozole for 15 women at average dose of 8 mg (SD 2.4). The average cost of pharmacotherapy is varying among the years with highest value of 1803.776 (SD 624.89) BGN in 2009.
In vitro fertilization is cost and resource consuming procedure having in mind the pharmacotherapy.
Key words: *in vitro* fertilization, pharmacotherapy, cost analysis.

NATIONAL HEALTH INSURANCE FUND COST ANALYSIS FOR RARE CANCER DISEASES AND ORPHAN MEDICINES

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Summary
The purpose of this study is to analyze the financing of pharmacotherapy for patients with rare cancer diseases in Bulgaria for the period 2011 - 2013 year.
It is a macroeconomic cost study of expenditures of National Health Insurance Fund for rare diseases. Data was collected by the National Health Insurance Fund to the pay for cancer drugs in Annex 1 of the PLD for the period 2011 to 2013 and on this background of cancer orphan drugs in diagnostic groups and trade names. These data were processed to calculate the proportion of funds as part of the overall budget of NHIF on diseases,on INN of medicinal products, on ATC groups to which they belong.
In 2011, due to a change in legislation, NHIF pays all rare diseases, which are 15 diagnoses, in 2012 were 16 and, in 2013 (till August) - 23, including the rare cancer drugs, which are 6 diagnoses. In record, National Health Insurance Fund reimbursed orphan drugs in 2013 on ATC code is as follows: Group A – 1, group B - 2, group C - 1, group G – 1, group J - 2, group L – 4, group V - 1. With the update of Annex 1 of the PDL on 01. 03. 2011, NHIF started to pay four cancer diagnoses - Malignant neoplasm of breast, endometrial, prostate and kidney for which the costs for the year was 11 386 965 BGN, which represents 2.41% of the total cost.
International non-proprietary names with the greatest weight in these four diagnoses are: Anastrozole - 22.66%, Letrozole - 20.88%, Goserelin - 18.62%, Bicalutamide - 15.16%. The costs of group L - Antineoplastic and immunomodulating drugs, have increased in 2011, compared to 2010 by 54.6 percent - from 37 013 480 BGN to 57 207 328 BGN, as a share of pharmacotherapy of cancer diseases is 18.9%, including rare cancer diseases.
In Bulgaria, the financing of the pharmacotherapy of rare diseases follows the general principles of financing of all medicines and this creates the risk of underfunding as of patients with rare cancer diseases and other citizens (patients), as both groups rely on funds, paid by NHIF.
Key words: rare diseases, orphan medicines, health care financing, access to pharmacotherapy

DRUGS POLITICS AND PHARMACOLOGY RESEARCH IN CHINA

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Summary
In last decade Chinese economics have been described as a world most rapidly development. In the press has been commented since 1990 years economic increase around 7%. In the area of Health care Chinese government manage to have better results with only 88 doll/per capita health expenditure compare to us with 421 doll/per capita.
One of the most important areas is the drugs politics. Chinese Health government have been used a special strategy that include best international practice and Chinese clinical experience.
Our goal is to find out how they could do drug politic so successful.
The method of research include analysis of the government documents and own clinical experience in Chinese hospitals.

The conclusions are:

- Regulation of the pharmacology research have been directed by special government organization
- Pharmacology researches have been made together with the leaded drugs companies
- China has been made a special low to protect researches especially in area of Traditional Chinese Medicine.

Key words: drugs politics, pharmacology research, Chinese innovation low

DRUG TOXICOLOGY

ORAL PRESENTETIONS

EPIGENETICS IN TOXICOLOGY

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Summary
Genetics, epigenetics and environment may together affect the susceptibility for toxic effects of various pharmacological substances. Epigenetic regulations, including changes in DNA methylation or acetylation leading to modifications in chromatin structure, are behind toxic alterations in various organs. Here we summarize our experience with pharmacological substances as well as alcohol in field of epigenetics. Strong evidence suggested that pharmacological changes can affect the DNA methylation and consequently gene expression. In addition, alcohol may cause epigenetic changes in POMC. Some antipsychotic drugs affect histones and cause epigenetic modulations in brain. Several genes in brain are targets for epigenetic changes and toxic effects of pharmacological substances. Our results and published data suggest that epigenetic plays a role in toxicity of various drugs.

Key words: epigenetics, drug toxicity, DNA methylation, DNA acetylation

FACTORS INFLUENCING METABOLISM AND TOXICITY

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Summary
Biotransformation can be a key determinant of drug toxicity and can be influenced by many factors., including *endogenous and exogenous.* A non-toxic parent molecule may be transformed by drug metabolizing enzymes to toxic metabolites (metabolic bioactivation). Conversely a toxic molecule may be transformed to non-toxic metabolites (detoxication). Our experimental studies included investigation on some drugs and prospective pharmacologically active substances from natural and synthetic origin with proved or supposed hepatic metabolism. Newly synthesized and plants isolated compounds, as well as approved drugs have been analyzed for their effects on liver metabolism and toxicity. Experimental models for liver injury have also been used. We performed our studies in different conditions tracing the influence of various factors - age, sex difference, pathological conditions using SHR (spontaneously hypertensive rats), rats in a state of stress, neurosis and dependence. *In vitro* and *in vivo* methods have been used to characterize the metabolizing activity at different levels – subcellular (microsomes and mitochondria), cellular (isolated hepatocytes) and whole animals. Since many drug interactions are a result of CYP-enzymes' inhibition or induction, we also assessed the influence of these processes on possible drug interactions.
Experimentally determined differences in drug metabolism related to age, sex and pathological conditions can affect the metabolism and toxicity of drugs which required monitoring in order to optimize the therapy and prevention of the health

risk.

Key words: drug metabolism, CYP-enzymes, inhibition, induction, bioactivation, detoxication

TOXICOLOGICAL IMPORTANCE OF IMMUNE SYSTEM/DRUG METABOLISM INTERACTIONS

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Summary

Numerous experimental and clinical data suggest that the major system (host defense) that protects humans from infectious organisms interacts with the principal system that affords protection from chemicals including drugs (drug metabolizing enzymes). The main event of this interaction is loss of cytochrome P450 content after stimulation of immune system.

Experimental evidences: In animal models, cytochrome P450 is depressed by various types of *infections* (bacterial, viral, and parasitic) or experimental *diseases*. The cytochrome P450 down-regulation is time, dose and immune stimulus dependent.

Clinical evidences: There are many documented examples of compromised drug metabolism in humans with impaired immune system (e.g., influenza, HIV, adenovirus). For example, in man, acute viral infections of the upper respiratory tract, bacterial pneumonia and BCG vaccination are able to reduce the clearance of theophylline by down-regulating multiple isoforms of the hepatic cytochrome P450. Theophylline plasma levels in bronchitis children increased during influenza epidemics. Quinine blood levels were increased during Plasmodium falciparum malaria infections.

Which CYP isoforms are more affected after immune stimulation: In humans CYP2A6, CYP2A7 and CYP2C19 were down-regulated in HBV- and/or HCV-infected livers compared with normal livers.

Mechanisms of cytochrome p450 depression by immunostimulation seems to imply the secretion of pro-inflammatory mediators like cytokines, interferones, NF-B by immune cells and bacterial endotoxins (LPS), which contribute for decreased CYP protein synthesis through transcriptional suppression and mRNA destabilization. Immunostimulants would also activate macrophages or Kupffer cells leading to the secretion of reactive oxygen species (ROS) and NO and ultimately to the loss of CYP mRNA.

Key words: immune system; drug metabolism; adverse drug reactions

IN VITRO/IN VIVO EXPERIMENTAL STUDY ON SOME METABOLIC INTERACTIONS WITH AMPHETAMINE – POSSIBLE MECHANISMS

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Summary

The objectives of this study were to investigate the influence of induction and inhibition on amphetamine hepatotoxicity *in vivo/in vitro* and to elucidate a possible involvement of CYP 3A and CYP 2D in the toxic mechanisms. The role of induction was traced in male Wistar rats, treated with nifedipine (5 $mg.kg^{-1}$, 5 days), a substrate and inducer of CYP3A and then hepatocytes isolated from those animals were exposed to amphetamine (100 $\mu mol\ L^{-1}$ for one hour) alone and along with amiodarone (14μM) - CYP 3A inhibitor and quinidine (75μM) - CYP2D inhibitor. The influence of nifedipine on amphetamine toxicity was judged by measuring MDA quantity and GSH levels, and the possible interactions between both compounds was evaluated by the activity of EMND, AH and cytochrome P450 quantity. Amphetamine induced increase MDA production and GSH depletion and led to significant decrease of P 450

quantity and EMND activity, without changing AH activity. In combination with nifedipine, however, EMND and AH activities were increased by 34% and 21%, versus controls. GSH levels and MDA quantity have not been influenced by nifedipine in this group. The *in vitro* part of the study, carried out in freshly isolated rat hepatocytes showed that nifedipine potentiated amphetamine cytotoxicity, judged by decreased cell viability, GSH levels, increased LDH activity and MDA production. Pre-incubation of nifedipine-treated hepatocytes with either amiodarone or quinidine significantly lowered amphetamine cytotoxicity. Using and inducer and inhibitor of CYP3A and CYP2D proved their involvement in amphetamine-mediated liver damage.

Key words: amphetamine toxicity, nifedipine, isolated hepatocytes, amiodarone, quinidine

IMPLEMENTATION OF SAFETY PHARMACOGENETICS IN PREEMPTIVE RISK-ASSESSMENT OF ADVERSE DRUG REACTIONS TO ANTIDEPRESSANTS – CURRENT STATE AND OUR EXPERIENCE WITH SELECTIVE SEROTONIN REUPTAKE INHIBITORS

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Summary

Depression represents the most costly brain disorder in Europe and the third leading cause of disability worldwide. About one in five people may receive an antidepressant at some point of their lives and as many as one third of patients do not respond to adequate treatment. To date, there is no reliable set of biomarkers for antidepressant selection and the initial treatment is still based on "trial and error" approach.

Despite the improved tolerability of newer agents, adverse drug reactions (ADRs) to antidepressants are still common and problematic. About 86% of patients on selective serotonin reuptake inhibitors (SSRIs) report at least one ADR (Hu et al.) and over 55% notify a high level of burden. Intolerable ADRs influence the patient compliance, affect the drug-related quality of life and may lead to antidepressant discontinuation, compromised achievement of remission and reduced prevention of relapse. Genetically based differences in patient profiles, along with environmental and personality factors, are considered as relevant to overall assessment of side effects and optimal antidepressant response. Genetic-guided therapy for cytochrome P 450 genes and older antidepressants has already been incorporated into clinical practice, but preemptive genotyping in regard to ADRs with newer drugs is still in its infancy.

We report current findings on safety pharmacogenetics of antidepressants and summarize the limitations and implementation challenges of genetic testing for prediction of ADRs to SSRIs. Results from our study on common genetic variants in important polymorphic genes and their influence on antidepressant switch and noncompliance with preceding treatment are discussed.

Key words: safety pharmacogenetics, antidepressants, SSRIs, adverse drug reactions

EFFECTS OF NALTREXONE ON METABOLIC FUNCTIONS

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Summary

Beta-endorphin is an endogenous opioid peptide and plays a role in regulation of various brain functions. It is an important mediator of food and drink intake. Endorphin production is associated with the metabolic syndrome. Published results demonstrated that the diet-induced obesity is associated with the changes of hypothalamic mu opioid receptors. We have been interested in the effects of alcohol on hypothalamic beta-endorphin. Our published data demonstrated that acute alcohol treatment of hypothalamic cells in

cultures increased the release of beta endorphin (Boyadjieva and Sarkar, 1994, 1995). In addition our studies also documented that the chronic alcohol intake decreased the hypothalamic beta-endorphin. Results from Guido M. et al. (2006) demonstrated that both opioid antagonists naloxone and naltrexone ameliorate the metabolic imbalance when it appears in the climacteric period, and mainly by increasing insulin clearance. We also examined the effects of naltrexone on ethanol-induced changes of beta-endorphin. In this study we determined the effects of naltrexone on alcohol-induced changes of metabolic parameters of male rats. Our data indicated that chronic treatment with naltrexone affects both beta-endorphin levels in hypothalamus and glucose metabolism in rats with a model of chronic alcoholism. Taken together, our results suggest that opioid receptors may modulate metabolic functions.

Key words: alcohol, beta-endorphin, metabolic functions

IN VITRO EFFECTS OF TWO GROUPS BENZIMIDAZOLES ON ISOLATED RAT HEPATOCYTES

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Summary
Two groups of benzimidazoles, with proved anti-helminthic activity, were investigated on rat hepatocytes, isolated by two-stepped collagenase perfusion. The functional-metabolic status of isolated hepatocytes was characterized by cell viability, lactatdehydrogenase (LDH) leakage in the medium, level of reduced glutathione (GSH) and lipid peroxidation, measured by the production of malondialdehyde (MDA).

The first group had anti-helminthic effect against *Syphacia obvelata* and *Trichinella spiralis*. Compared to Albendazole, KA-159 and KA-158 were the only one compounds from the first group that showed statistically significant lower cytotoxic effects on isolated rat hepatocytes.

The second group - derivatives of Thiabendazole, were active only against *Trichinella spiralis*. From this group only KA-112 had lower cytotoxicity on isolated rat hepatocytes, compared to Albendazole.

The difference in the hepatotoxicity of KA-158 and KA-159 from I-st group, KA-112 from II-nd group and Albendazole might be due to difference in their structure and metabolism.

Key words: isolated rat hepatocytes, benzimidazoles, cytotoxicity

CHANGES IN SOME LIVER BIOCHEMICAL PARAMETERS IN MALE AND FEMALE DIABETIC SPONTANEOUSLY HYPERTENSIVE RATS

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Summary
The metabolic syndrome is assumed to exist when three or more of the following risk factors are present: abdominal obesity, high triglycerides, low HDL levels, hypertension, hyperglycaemia, while fasting. The present study was conducted to assess the effect of hypertension and diabetes on some liver biochemical parameters in experimental model of streptozotocin-induced

diabetes in spontaneously hypertensive rats (SHR). Twenty four male and female SHRs, strain Okamoto-Aoki of uniform age were randomly divided into four groups (n=6). Group 1and 2 are non - diabetic control male and female SHR; Groups 3 and 4 are challenged with Streptozotocin (40 mg/kg i.p) rats. Blood pressure and fasting glucose levels were monitored once a day for two weeks. The animals with highest blood pressure and blood glucose levels were chosen for the experiment. Livers were collected for assessment of reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS), measured as malonedialdehide (MDA), and aniline hydroxylase (AH) activity, a marker enzyme for cytochrome P450 (CYP2E1). There was an increase in the MDA production and the activity of AH, and decrease in the concentration of GSH in diabetic SHR livers, compared with non-diabetic SHR livers. These results are more pronounced in male diabetic rats, compared to female. On the basis of these data we can conclude that the two pathological conditions – diabetes and hypertension, part of the metabolic syndrome interfere not only with the endogenous protective mechanisms but also with the metabolic activity of the liver.

Key words: SHR, diabetes, streptozotocin, metabolism, rats

POSTERS

XANTHATE METABOLISM BY DIFFERENT MONOOXYGENSES AND REACTIVE OXYGEN SPECIES

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Summary

Xanthates (alkyl derivatives of dithiocarbonic acid, ROCS2K) are well known metal ions chelating agents with variety of biological properties as antitumor and antiviral effects. Our previous studies have shown that xanthates were powerful and selective mechanism-based inactivators of some cytochrome P450 isozymes (2B6 and 2E1).Other P450's were either inhibited (1A1, 2D6, 2C9, 4A11) or not affected (3A2, 3A4). The primary side of P450 attack on xanthate molecule is the carbon atom. There are data that some reactive oxygen species are playing major role in the cytochrome P450 xanthates metabolism.

In this study, we are following the metabolism of xanthates (spectroscopically and by HPLC) by some other reactive oxygen dependent enzymatic and non-enzymatic systems like flavine monooxygenase (FMO3), horseradish peroxidase (HRP), hydroxyl radicals (Fe^{3+}/ascorbate/H_2O_2/with or without EDTA) and superoxide radicals (UV-degradation of riboflavine/methionine) producing systems.

FMO3 oxidized one of the sulfur atoms giving perxanthate (the same metabolite is producing by incubation of xanthates with H_2O_2). On the other hand, HRP-compound I (when the ratio enzyme/H_2O_2 is 1:1) oxidizes xanthate mainly to bisxanthate. Xanthates inhibited deoxyribose degradation in Haber Weis reaction mainly by scavenging the hydroxyl radicals concentration. Superoxide generated by the photooxidation of riboflavin, metabolized xanthates to unknown metabolites without oxidation of the sulfur atoms. The same was done by purified CYP 2B6.

The readiness of xanthate molecule to interact with different reactive oxygen species reflect in their potent antioxidant and scavenger activity, which could be on the background of their antiviral and anticancer activity.

Key words: xanthates, cytochrome p450, metabolism, reactive oxygen species

ACUTE INTOXICATIONS WITH NEUROLEPTICS AND ANTIDEPRESSANTS: SURVEY

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Summary
Taking into account the increasing use of antidepressants in the world, as well as the vital use of neuroleptics in patients with schizophrenia, we have examined and summarized the data obtained for intoxications with these drugs.
The data available related to intoxications with neuroleptics and antidepressants have been obtained from the Department of Toxicology at the Military Medical Academy - Varna, as well as from additional literature sources, mentioned below in the exposition. On the basis of these data we did our summaries concerning the frequency of the acute intoxications with the indicated medications.
Upon performing a retrospective analysis of the acute poisonings in the Varna region for a 5 year period (2006-2010), based on the adopted for treatment in the Department of Toxicology at the Military Medical Academy - Varna 4960 patients with acute intoxications and acute allergic reactions, was found that the drug intoxications are with a relative share of 18.3% and rank third as a cause of hospitalization.
According to the retrospective analysis, we concluded that is observed a decrease in the drug poisonings, particularly with neuroleptics and antidepressants, compared to data from the recent past, when they have occupied a leading position among the intoxications. It is believed that the reduction of the frequency of the drug poisonings is due to the regular allocation of medicines in the pharmacy network.
Key words: neuroleptics, antidepressants, intoxications, analysis

PPARγ AGONISTS AND LIVER STEATOSIS: MODE-OF-ACTION CHARACTERISATION AND *IN SILICO* STUDY

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Summary
Ligand-dependent activation of hepatic PPARγ has been proposed as a molecular initiating event (MIE) in a prosteatotic mode-of-action (MoA). In this study we aimed at (i) *in silico* study of the PPARγ-ligand interactions as important for elucidation of the MIE; (ii) evaluation of the quantitative evidence supporting key intermediate events within the MoA.
PPARγ-ligand complexes were extracted from Protein Data Bank. For the molecular modelling purposes MOE 2012.10 software was used. The following tools were applied: Protonate 3D, Ligand Interactions, Site Finder and Pharmacophore Analysis. MoA assessment was based on a literature search and followed the AOP/OECD principles.
118 PPARγ-ligand complexes were analyzed. The study outlined important features of the PPARγ ligand-binding pocket, as well as the main interactions with some of the most potent agonists. A pharmacophore model for the PPARγ full agonists was developed and the pharmacophoric features were discussed in relation to their role for the ligand binding to PPARγ. The performed assessment of the developed toxicity pathways outlined PPARγ target proteins relevant to the studied MoA.
The results can be used for: (i) safety evaluation of new compounds targeting PPARγ; (ii) rational design of compounds with controlled activity towards the PPARγ receptor.
Key words: PPARγ; liver steatosis; molecular initiating event (MIE); molecular modelling; pharmacophore
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RISKS OF ORAL METHOTREXATE ADMINISTRATION IN OUTPATIENTS

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Summary
Methotrexate (MTX) is a cytostatic agent used in oncology. Because of its immunosuppressive properties MTX is also used in autoimmune disorders. Low-dose MTX regimens in the treatment of rheumatoid arthritis and severe psoriasis are considered to be safe. However, pharmacovigilance centers warn of serious and even fatal incidents due to errors in oral MTX administration. The aim of this case series presentation is to identify the specific factors related to the development of adverse drug reactions (ADRs) induced by MTX.
Prospective pharmacovigilance study in the Clinic of Dermatology, University Hospital, Stara Zagora.
We report 3 cases of patients with psoriasis vulgaris in which severe haematological abnormalities associated with prevoius administration of MTX were detected during hospitalization. A 73-year old female with malaise, vomiting and oral ulcers who had taken approximately 120 mg MTX was found to have pancytopenia. A 59-year old male hospitalized for psoriatic erythroderma who had erononeously taken 10 mg MTX daily instead of weekly for 8 days, was diagnosed with bicytopenia and toxic hepatitis. An 88-year old male with psoriatic arthritis presented with aphthous stomatitis, erosive crusted lesions, ecchymoses and aplastic anemia 2 weeks after treatment with 12,5 mg MTX once weekly plus i.m. Movalis® followed by Diclophenac Duo®.
The main predisposing factors for the development of these ADRs were patient-related dosage errors and concomitant administration of NSAIDs. Safe use of oral MTX requires clear dosing instructions and strict patient compliance. Potential drug interactions of MTX with other drugs should also be considered.
Key words: methotrexate, adverse drug reactions, pancytopenia

TOXICOLOGICAL CHARACTERIZATION OF SILICA NANOMATERIALS AS DRUG DELIVERY SYSTEMS *IN VITRO*

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Summary
Nanotechnology in a short period has brought vital changes in many areas of health care. In the past two decades, the advancement in nanotechnology and material science has resulted in a large number of organic and inorganic nano-medicine platforms. Different nanomaterials, including silica nanoparticles (NPs), drug - polymer conjugates, liposomes, quantum dotes, carbon nanoparticles which exhibit many unique properties, offer a promising drug delivery platform to realize the potential of nanomedicine. Here we present the current state of the toxicological properties of mesoporous silica NPs both empty and loaded with model drugs *in vitro*.
Key words: silica nanoparticles, cytotoxicity, HepG2 cells, isolated hepatocytes.

TOXICOLOGICAL STUDY ON EFFECTS OF BENZODIAZEPINES AND ETHANOL ON REPRODUCTION OF MALE AND FEMALE RATS

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Summary
Alcohol and benzodiazepine consumption is increasing in the recent years. There is insufficient data for the early- and late-onset effects of their combined usage on fetal development.

The aim of our experiments was to study the effects of benzodiazepine and ethanol administration on reproduction and fetal development of pregnant Wistar rats.
The experimental animals were treated separately or in combination with ethanol, diazepam and medazepam. The number of early fetal deaths and reproductive parameters in male and female rats-generation of the treated animals were observed. We used a reproduction method published by Boyadjieva N, 1988.
Our results show that ethanol potentiated the toxic effects of diazepam and medazepam on fetal development. We observed high frequency of fetal lethality and decreased sexual perception in the generations of rats treated with the combination of ethanol and benzodiazepines. Diazepam and medazepam administrations decreased reproductive abilities in male generations and ethanol potentiated the adverse effects of benzodiazepines.
Our toxicological studies suggest that combined consumption of benzodiazepines and ethanol may cause both early- (fetal lethality) and late-onset (decreased reproductive abilities) adverse reactions on generations of pregnant rats treated with the substances.
Key word: ethanol, diazepam, medazepam reproduction, fetal development

DRUGS CLASSIFICATION DURING LACTATION PERIODS

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Summary
Classification of drugs used during lactation is a poorly explored field in present day pharmacology.
The purpose of this study is to provide practicing health care specialists with a stable and broad qualifications system as the base for making the right choice when administering drugs to beast-feeding patients.
The development of the present qualification scheme is based on logical and statistical methodological approaches keeping in mind the purpose of this study - to classify drugs registered in Bulgaria.
Initially obtained results lead to interesting conclusions, which should be used for further therapy refinement in patients of this high-risk therapeutic group.
Drugs are divided into 4 categories:
1. Considered safe for the infant.
2. Relatively safe for the infant.
3. Safe for the infant after the elimination of the drug out of the mother`s body.
4. Considered safe for the mother, but toxic for the infant.
Key words: drugs, lactation, pharmacotherapy

IMMUNOPHARMACOLOGY

ORAL PRESENTATIONS

VITAMIN D, AUTOIMMUNE DISORDERS AND INFERTILITY

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Summary
Studies on vitamin D and its prohormones are increasingly extending beyond the focus and functions related to calcium and bone metabolism. The discovery of the nuclear vitamin D receptor (VDR) in 1974 and its localization in promyelocytes, monocytes, lymphocytes, ovarian, and placental endothelial cells, dermal fibroblasts allow the study of the noncalcemic vitamin D-mediated effects, such as cell proliferation and differentiation and immuno-modulation. VDR is expressed in many cells of the immune system, including monocytes, macrophages, dendritic cells, NK lymphocytes, T and B lymphocytes. Its concentration is high, however, in immature thymocytes and CD8 lymphocytes, regardless of the activation status. Other experimental models, demonstrate the effects of vitamin D on the immune system, some of which are regulated to the differentiation and

activation of CD4 lymphocytes, stimulating the number and function of regulatory T lymphocytes (Treg), reduction of the production of Th1 cytokines (IF-γ, IL -2, IL-17, TNF-α), stimulation of Th2 lymphocytes and others. A number of studies have linked vitamin D deficiency with autoimmune diseases such as insulin-dependent diabetes mellitus, multiple sclerosis, rheumatoid arthritis, SLE, and others. This allows not only the prevention of these diseases by supplementation with vitamin D and its analogues, but also to include them in their therapy.
Gender differences in the expression of active metabolite of vitamin D – 1.25-D and nuclear VDR, their localization in the endometrium, and the associated prevalence of autoimmune diseases in women and pathogenic effects associated with infertility are discussed.
Key words: vitamin D deficiency, immunity, fertility

TOXIC EPIDERMAL NECROLYSIS – IMMUNE-MEDIATED, DRUG INDUCED SKIN DISEASE

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Summary
Toxic epidermal necrolysis (TEN) is a life-threatening, rapidly progressive skin condition associated with high mortality. The disease is characterized by generalized erythema, confluent macules, massive bullae formation, subsequent generalized epidermal sloughing, erosion of the mucous membrane, persistent fevers and systemic symptoms. Various etiologies have been proposed, but drugs have been chiefly characterized as the offending agents. The medicines most often associated with TEN are antiepileptics, sulphonamides, β-lactam antibiotics, non-steroidal anti-inflammatory drugs, allopurinol. The pathophysiology of TEN has not yet been fully elucidated. Massive accelerated apoptosis has been proposed as the main mechanism underlying keratinocytic death in TEN. Several pathways can lead to apoptosis. CD8-positive T cells and macrophages play an important role in the extensive epithelial necrosis and subepithelial detachment. Various proinflammatory cytokines including tumour necrosis factor-α may contribute to epidermal cell death, as well as to fever and malaise.
Case series: Hospitalization of patients with TEN takes place mostly in the Toxicology Clinic, "Pirogov". Over the period 1972 to 2014 in the Clinic were treated 180 patients with TEN. The age of the patients varied from 19 months to 80 years (30 children and 150 adults). The patients were seen by a multidisciplinary team and received intensive supportive care according to a standard protocol. The survival rate observed was 60% throughout the whole period.
TEN is a life-threatening severe disorder, requiring timely diagnosis and treatment. When TEN is suspected, immediate drug discontinuance and prompt replacement with other drugs must be evaluated.
Key words: toxic epidermal necrolysis, life-threatening condition

AGE-DEPENDENCY OF CYCLOSPORINE (CSA) CONCENTRATION DISTRIBUTIONS IN LIVER TRANSPLANT PATIENTS – A COMPARATIVE OBSERVATIONAL PILOT POPULATION PHARMACOKINETIC STUDY

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Summary
A comparative population pharmacokinetic analysis was performed on CsA concentrations, "as withdrawn" in clinical setting, from a learning group of liver transplant patients.

20 patients (8 adults and 12 children) who were on Neoral® post-ortothopic liver transplantation over 2004-2009 were studied. All patients received Neoral® twice daily orally at 08:00 AM and at 08:00 PM. Whole blood CsA concentrations were measured by FPIA (Abbott Diagnostics). C0 drug concentrations were recorded in morning (C0AM) and in evening before each dosing (C0PM) and C2 concentrations - in morning and in evening 2 hours post-dosing (C2AM and C2PM). A total of 323 CsA C0 in children group and 242 C0 in adult group and a set of 117 CsA C2 in children group and a set of 133 C2 in adult group were analyzed. Population pharmacokinetic analysis was performed with dose normalized drug concentrations. All drug concentration distributions were skewed to the right. The measures of central tendency showed statistically significant higher estimates for adult group. Normalized CsA C0 at Day 2 after liver transplantation in adult patients correlated significantly with measured Scr levels ($r=0.62$, $p=0.031$). The findings can't be explained by body weight between groups differences since normalized cyclosporine concentrations were used. We expect that age-related non-genomic factors, controlling cyclosporine metabolism and distribution, could be a plausible reason.

Key words: cyclosporine, liver transplantation, pharmacokinetic analysis

POSTERS

ORAL THERAPY WITH LYCOPENE CAN PREVENT HUMAN SPERM DNA DAMAGE

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Summary

Reactive oxygen species can cause sperm DNA and chromatin damage. The use of antioxidants during sperm processing has been shown to prevent sperm damage, including DNA damage. Lycopene is a lipophilic carotenoid (plant pigment) with antioxidant properties. The antioxidant activity of carotenoids is mostly catalytic and these antioxidants can effectively quench singlet oxygen and trap peroxyl radicals. Studies have shown that lycopene can protect sperm DNA from oxidative damage.

The aim of this study was to evaluate the effect of lycopene on oxidative stress-mediated sperm DNA damage and to examine the antioxidant properties of lycopene in male reproduction. We examined the effects of oral therapy with Lycopene capsules on human sperm DNA damage.

Design: Prospective study during clinical practice in Andrology research laboratory

Patients: Fifty one men, patients of IVF clinic.

Materials&Methods: Assessments of sperm DNA fragmentation index (DFI) by DNA integrity test before and after 12 week therapy with Lycopen capsules 3 mg.

Main Outcome Measure(s): Sperm percent DNA fragmentation index.

The mean percentage of DFI before treatment with Lycopen is 27.98% (SD±10.47). After 12 week the DFI is significantly lower: 20.60% (SD±6.11), $p<0.005$. We will report our data about the control group undergoing L-carnitine caps therapy.

The data suggest that oral treatment with Lycopen offers protection against oxidative DNA damage. Our results may suggest potential positive outcome of IVF procedure in cases of male fertility.

Key words: sperm DNA damage, antioxidant, reactive oxygen species, Lycopene

PHARMACOTHERAPY OF INFECTIOUS DISEASES

ORAL PRESENTATIONS

ANTIVIRAL COMBINATION APPROACH: A PERSPECTIVE TO COMBAT ENTEROVIRUS INFECTIONS

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Summary
Human enteroviruses distributed worldwide are causative agents of a broad spectrum of diseases with a total enormously high morbidity, including a series of severe illness, embracing pathology of CNS, heard, beta cells of pancreas, skeleton muscles, etc. and the common cold contributing to the development of chronic respiratory diseases, and the chronic obstructive pulmonary disease. The significantly high morbidity and mortality in children and in the high-risk populations (immunodeficiency, neonates) definitely formulate the chemotherapy as the main tool for the control of enteroviral infections. Nevertheless of great work in this field, at actual time clinically effective antivirals for use in the treatment of enteroviral infection do not exist. The main reason for this state is the development of drug resistance, based on the trialed till now monotherapy courses.
Our team introduced for the first time in the research for anti-enteroviral compounds the testing of the combination effect of selective inhibitors of enterovirus replication with different mode of action. Moreover, as a result of RNA sequences of Coxsackievirus B1 mutants susceptible and resistant to disoxaril, representative of the most potent anti-enterovirus compounds, VP1 ligands, the molecular basis of the drug-resistance, was determined.
A large-scale study of the combined effect of a series of anti-enteroviral agents resulted in a selection of a series of very effective *in vitro* double synergistic combinations. The most prospective attainment of our studies was the development of a novel scheme for combined application of anti-enteroviral substances in coxsackievirus B1 neuroinfection in newborn mice. It consists of a consecutive alternating, not simultaneous, administration of the substances in combination. A triple combination disoxaril-guanidine. HCl-oxoglaucine (DGO) showing good efficacy was selected. Its effectiveness is expressed in lengthening of the mean survival time and about 50% reduction of mortality rate in infected newborns as compared both to the placebo group, partner compounds used alone every day, and to the same combination applied simultaneously every day. Studies of the drug sensitivity of viral brain isolates from mice, treated with this combination and the combination partners indicate that virus isolates from the group treated with the alternating combination not only preserve, but even increase their sensitivity to the drugs. Obviously, the consecutive alternating administration of anti-enteroviral substances hinders the occurrence of drug- resistance in the course of the experimental enteroviral infections in mice. DGO combination following CAA course manifested high effectiveness also to two models of Coxsakievirus B3 infection (neurotropic and cardiotropic). The replacement of disoxaril by pleconaril (PGO) confirmed the perspectivity of this approach for the development of effective anti-enteroviral chemotherapy.

DOES MEFLOQUINE (LARIAM®) THERAPY IMPROVE THE PROGNOSIS OF HUMAN JC POLYOMAVIRUS-INDUCED PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY?

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Summary
Progressive multifocal leukoencephalopathy (PML) is usually a fatal, demyelinating disease caused by infection with JC virus (JCV) and the progressive destruction of oligodendrocytes in

multiple brain foci of susceptible individuals. Several compounds with anti-JCV activities have been investigated but only mefloquine, an antimalarial agent, has been reported to show sufficiently high effect on the viral replication following penetration into the central nervous system at efficacious concentrations. The current material presents some of the available published data, suggesting that the activity of mefloquine against JCV should be considered for treatment of patients with PML.

Keywords: progressive multifocal leukoencephalopathy; PML; human polyoma virus JC; JCV; Lariam®; mefloquine

EFFECT OF ANTIBIOTICS ON PEPT-1 MRNA EXPRESSION IN POULTRY

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Summary
Within the study, effects of three probiotic *Lactobacillus strains* and antibiotics on the expression of peptide transporter 1 (PepT1) in chickens treated with two different antibiotics (enrofloxacin and doxycycline) has been investigated. PepT 1 is involved in nutrient utilization as it facilitates the absorption of di- and tripeptides and some peptidomimetic drugs. Its expression can be influenced by numerous factors such as food deprivation, pathological conditions and drug administration.
24 Ross and 24 Duk one-day-old chicks were included in experiments with enrofloxacin and doxycycline, respectively and were allocated to following groups: Control group (without treatment); Group treated with probiotics via feed; Group treated with a combination of probiotics and antibiotic (10 mg/kg, via drinking water for five days); Group given antibiotics only. Samples from liver, duodenum and jejunum were collected. Body weight gain was registered. Expression levels of PepT-1 mRNA were determined by RT-PCR and were statistically evaluated by Mann-Whitney test.
PepT-1 mRNA was found in the liver and the intestines. Enrofloxacin, administered alone or in combination with probiotics, provoked a statistically significant up-regulation of PepT-1 mRNA levels in the measured organ sites. Similar changes were found in the duodenum of doxycycline treated chickens. In contrary, doxycycline treatment caused down-regulation of PepT-1 mRNA in the liver and the jejunum.
A significant increase of PepT1 mRNA in enrofloxacin treated animals may improve the utilization of dietary peptide and in turn improve body weight gain. Less prominent changes were observed in doxycycline treated birds which is in accordance with slower increase of body weight gain of these chickens.

Key words: antibiotics, chicken, PepT-1, probiotics

PHARMACOLOGY OF INFLAMMATION AND PAIN

ORAL PRESENTATION

INFLAMMATION AND ITS TREATMENT: A SURGEON'S POINT OF VIEW

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Summary
Inflammation as a pathophysiological phenomenon triggers in tissues and organs processes, which may require timely therapeutic and

surgical measures to be overcomed. The choice of therapeutic agent depends on the type of disease, the stage of development of the inflammation, as well as its local and systemic manifestations. The authors discuss the possibility of current anti-inflammatory treatment and prophylaxis for preventing or arresting the development of the inflammatory process. On the other hand progressive course of inflammation requires accurate diagnosis and comprehensive treatment. Development of surgical sepsis necessitates the inclusion of a variety of pharmacological agents that do not guarantee a positive outcome without fail. From a surgical point of view the timely treatment of inflammation and prevention the onset of the inflammatory cascade plays a crucial role.

Key words: inflammatory response, surgery

PAIN IN OSTEOPOROSIS

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Summary

Osteoporosis is a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture. Besides the risk of bone fractures osteoporosis has the potential to cause pain and disability. Some people with osteoporosis may struggle with chronic pain, including low back pain: pain derived from internal consequences of the osteoporotic state without injury, which has been reported to account for pain in 89% of menopausal osteoporosis patients (Scharla et al., 2006). The exact mechanism for that pain still remains unknown, but some studies have tried to clarify that.

In this study, we discuss the basic mechanism of bone-related pain, which is probably common for bone metastases and osteoporosis and therapeutics approaches also that share similar action mechanisms. Osteoporosis treatment against pain itself potentially includes the prevention of possible fracture-induced pain by increasing bone mass density, which each agent originally aims to acquire. Furthermore, each anti-osteoporosis agent has been reported to have its own specific pain-related active site. This study presents experimental data for the development of osteoporosis in rats, a spontaneous pain and theirs pharmacological modulation.

Key words: chronic pain, osteoporosis, experimental model, pharmacological modulation

ACTIVATED SPINAL GLIA AS A MARKER FOR NEUROPATHIC PAIN AND TARGET FOR THERAPY IN EXPERIMENTAL MODELS OF PERIPHERAL NEUROPATY

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Summary

Accumulating evidence suggests that glial cells in the spinal cord contribute to the development and maintenance of neuropathic pain which determines the demand for pharmacological agents that inhibit that inhibit their activation.

We used two rats models of neuropathic pain - chronic constriction injury (CCI) to the sciatic nerve and streptozotocin-induced (STZ) diabetic neuropathy to assess the role of spinal glial activation responses in producing pain behaviors. We studied the effect of single and repeated dose of pharmacological agents such as minocycline (50 mg/kg, 5-HT2A/2C receptor agonist (+/-)-2,5-dimethoxy-4-iodoamphetamine (DOI, 0.6, 1 mg/kg), and the 5-HT2A/2C antagonist ketanserin (0.5, 1mg/kg) on pain behavior and glial activation. Nociceptive thresholds were measured by plantar heat and von Fray filament tests to determine the development of neuropathic pain. Glial response was determined

by changes in cell morphology, cell density and intensity of immunoreactivity with specific activation markers (Iba1 and anti-glial fibrillary acidic protein (GFAP) for microglia and astrocytes, respectively).
CCI and STZ diabetic rats produced significant mechanical allodynia and thermal hyperalgesia which corresponded to the activation of spinal glia, more pronounced in CCI rats. Minocycline, DOI and ketanserin slightly modulated neuropathic pain behaviors, but strongly suppress microglial activation.
The current findings suggests that after development of neuropathic pain treatment with minocycline, DOI or ketanserin does not significantly alter neuropathic pain behaviors which is not correlated with glial responses in either model.
Key words: neuropathic pain, chronic constriction injury, streptozotocin-induced diabetes, spinal glia

EFFECTS OF ACUTE AND LONG-TERM CAFFEINE TREATMENT ON DEPRESSION-INDUCED CHANGES IN NOCICEPTION

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Summary
Chronic unpredictable stress (CUS) is considered as a valuable animal model to study a major depressive disorder.
There are sufficient data that CUS induced an antinociception in animal models of phasic pain. Adenosinergic system plays a key role in pain transmission at different brain areas interacting with its receptors and other neurotransmitters. Generally, activation of adenosine A1 and A2 receptors produces opposite effects on the nociception and stress-induced antinociception.
We studied the effects of caffeine as a non selective adenosine receptor antagonist caffeine on the CUS-induced changes in nociception via two models of phasic and tonic pain in rodent.
CUS procedure was used to induce depressive-like condition in male Wistar rats and ICR mice; phasic pain model (paw pressure test) and tonic pain model (writhing test). Caffeine was administered acutely at doses of 2, 20 and 40 mg/kg and long-term at a dose of 8 mg/kg/day, 4 weeks. Desipramine (10 mg/kg/day, 4 weeks) was used as a reference antidepressant.
Acute caffeine injection showed opposite effects in these two types of nociception – enhancing of CUS-induced antinociception in paw pressure test and abolishment of CUS-induced pronociception in writhing test. Chronic caffeine administration (8 mg/kg/day, 4 weeks) per se increased nociception and similarly to the antidepressant desipramine was able to abolish the CUS-induced antinociception in paw pressure test in rats. Different impact of caffeine on the CUS-induced changes in nociception may due to the duration of treatment and accompanied by this up- and down-regulation of adenosine receptors involved in phasic and tonic pain pathways.
Caffeine treatment is able to alleviate CUS-induced alterations in both phasic and tonic pain in rodents.
Key words: chronic unpredictable stress; caffeine; nociception; mice

EFFECT OF AGE AND RENAL INSUFFICIENCY ON THE PHARMACOKINETICS OF A NON-OPIOIDE ANALGESIC FLUPIRTINE

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Summary
Advanced age is associated with changes in most factors that determine the pharmacokinetic behaviour of medications and their metabolites' leading to increased risk of ADR's. Flupirtine is a

centrally acting, non-narcotic analgesic, with a dual mode of action: analgesic and muscle tone normalising, provided as a modified release formulation (Katadolon® S) with dual PK-input over time.
The aim of the study was to investigate the effects of advanced age and renal dysfunction on the single- and repeated dose pharmacokinetics of flupirtine.
The study was carried out at the Department of Clinical pharmacology & Therapeutics, Sofia, on 4 groups of volunteers: Group (1) - young healthy subjects, Groups (2) and (3) - younger and older elderly healthy subjects, 60 to 69 years and 70 years of age, respectively, and Group (4)- patients with impaired renal function. All subjects received the same treatment: Day D01 and Day D03-D09: once daily 400 mg Katadolon® S in the morning. The 48h PK-profile of the first and last dose were investigated in all groups. Concentrations of flupirtine in plasma were determined by specific validated HPLC-method with fluorescence detection.
The pharmacokinetics of flupirtine is characterised by a large between-subject variability and obvious group difference: subjects with advanced age and renal dysfunction have higher exposure levels (C_{max}, C_{av}, C_0), compared to healthy volunteers. Treatment in such patients should be started at a lower than usual dose level with slow stepwise dose increases adjusted to the patient's needs and tolerability.
Key words: flupirtine, pharmacokinetics, advanced age, renal dysfunction

POSTERS

EXPERIMENTAL STUDY ON THE INVOLVEMENT OF 5-HT$_3$ RECEPTORS IN THE MECHANISM OF ANTI-INFLAMMATORY AND ANTIHYPERALGESIC EFFECT OF FLUOXETINE

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Summary
The aim of the present study is to determine the role of 5-HT$_3$ receptors in the mechanism of anti-inflammatory and antihyperalgesic action of antidepressant fluoxetine after single and repeated administration of the drug.
40 male Wistar rats were divded in five groups (n=8), treated for 14 days as follows: saline (control); diclofenac (positive control), fluoxetine 20 mg/kg bw, ondansetrone 0,5 mg/kg bw (5-HT$_3$ antagonist) and fluoxetine + ondansetrone. Inflammation was induced with intraplantar injection of 1% solution of carrageenan before treatment. Anti-inflammatory effect was measured by reduction of carrageenan-induced paw edema on the 2nd, 3rd, 4th and 24th hour. Nociceptive tests (Randall & Selitto) which employ mechanical (paw pressure) stimul were used. To evaluate the analgesic effect was used decrease in latency to withdraw the inflammed paw.
Single and repeated administration of fluoxetine showed significant anti-inflammatory and antihyperalgesic effect when compared with the control ($p<0.05$). After repeated treatment and in the first four hours, after single administration ondansetrone did not change significantly the anti-inflammatory effect of fluoxetine. At 24 hours in single dose treated animals the combination did not differ statistically when compared with the control. After acute and prolonged treatment the group that received fluoxetine + ondansetrone showed a statistically significant increase in paw pressure to withdraw the hind paw compared with that treated with fluoxetine alone ($p< 0.05$).
Fluoxetine has anti-inflammatory and antihyperalgesic effect in carrageenan model of inflammation which is mediated through the action of serotonine on 5-HT$_3$ receptors.
Key words: fluoxetine, carrageenan, inflammation, antihyperalgesia, 5-HT$_3$ receptors

INFLUENCE OF ROSUVASTATIN ON ACUTE INFLAMMATION AND TNF-A LEVELS IN RATS

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Summary
Statins are among the most prescribed drugs in clinical practice. Recent evidence indicates that statins possess pleiotropic effects apart from their lipid-lowering activity.
The aim of the present study was to investigate the anti-inflammatory effect of rosuvastatin in a model of acute inflammation.
Male Wistar rats were used in the study and were divided into four groups. Animals were treated orally for 90 days with rosuvastatin 10 and 20 mg/kg bw. Control group was treated orally with saline 1 ml/100 g. At the day of the experiment a fourth group received orally indomethacin 9 mg/kg bw. After the 90-day period paw edema was induced by carrageenan injection and the footpad volume was measured at the 4th hour using plethysmometer. Blood sample was collected and plasma concentrations of the inflammatory cytokine tumor necrosis factor-α (TNF-α) were measured.
Indomethacin significantly reduced the extent of the paw edema at the 4th hour after the carrageenan injection compared to the saline treated group. Administration of rosuvastatin in both doses produced significant inhibition of the edema compared to the saline treated group. There was no significant difference in the plasma levels of TNF-α between the rosuvastatin-treated animals and rats, receiving saline.
The results from our study suggest that rosuvastatin exerts anti-inflammatory effect, concerning the acute local inflammation, but does not reduce TNF-α plasma concentrations.
Key words: statins, anti-inflammatory, TNF-α, carrageenan-induced paw edema

INFLUENCE OF NALOXONE AND JTC-801 IN THE ANALGESIC EFFECTS OF β²-TRYPTOPHAN MODIFIED HEXAPEPTIDE ANALOGS IN RATS

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Summary
The aim of the present study was to examine the effects of the JTC-801 (NOP-receptor antagonist) and naloxone (opioid antagonist) in the analgesic activity of newly synthesized β^2-tryptophan modified hexapeptide analogs.
The experiments were carried out on male Wistar rats (180-200g) kept under normal conditions at ambient room temperature (22°C). The effects on nociception were examined by the Randall-Selitto paw-pressure test. NOP-receptor agonist (Nociceptin(1-13)NH_2, at dose 10μg/kg), JTC-801 (0.5mg/kg), and naloxone (1mg/kg) were injected intraperitoneally. The newly synthesized β^2-tryptophan modified hexapeptide analogs were synthesized in Department of Organic Chemistry, University of Chemical Technologies and Metallurgy, Sofia, Bulgaria. The newly hexapeptide analogs were injected intraperitoneally at a dose of 10μg/kg. All the substances were after being dissolved in 0.9% NaCl solution. All procedures were approved by the Animal Care and Use Committee of the Medical University of Sofia.
The results showed that newly synthesized analogs significantly decreased nociception in comparison to the referent substance on the 10th min with a decrease in pain threshold on the 20th and 30th min.
Both JTC-801 injected 10 min before the peptides and naloxone injected 20 min before the peptides significantly decreased the analgesic effect of the investigated peptides.
In conclusion, naloxone and JTC-801 influenced the analgesic effects of the newly synthesized hexapeptide analogs.
Key words: JTC-801, naloxone, hexapeptide analogs, nociception
Acknowledgments. The study was supported by Grant DTK 02/61 of the National Research Fund, Sofia, Bulgaria.

EFFECTS OF ENDOGENOUS CANNABINOIDS AFTER 1 HOUR OF IMMOBILIZATION: INVOLVEMENT OF THE OPIOID COMPONENT OF STRESS-INDUCED ANALGESIA

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Summary
The aim of the present study was to establish the opioid component of stress-induced analgesia (SIA) involvement in the effects of endogenous cannabinoids after 1 hour of immobilization.
Immobilization stress was obtained by placing male Wistar rats (120-150 g) in adapted plastic tubes for 1 hour. At the end of immobilization the animals were intraperitoneally (i.p.) injected with CB1-receptors` agonist (anandamide, 1mg/kg) and the Tyr-MIF-1 family`s peptides (1mg/kg). Opioid receptor antagonist (naloxone, 1mg/kg, i.p.) was applied 20 min before anandamide and the peptides. Pain perception was estimated by Paw pressure and Hot plate test.
Estimation of pain thresholds showed that naloxone applied after 1 hour of immobilization and before anandamide and MIF-1 led to a statistically significant increase during the whole investigated period. Similar results were obtained also after Tyr-MIF-1 and Tyr-W-MIF-1, but only on the 20th min of the experiment. On the 10th min a significant decrease in pain threshold was observed for Tyr-MIF-1, Tyr-W-MIF-1, and also for Tyr-K-MIF-1.
Estimation of HP-latency showed results similar to the described ones – a significant elongation for MIF-1 for the whole time of investigation and a decrease for Tyr-MIF-1 and Tyr-W-MIF-1on the 20th min.
In conclusion we can assume that the opioid component of SIA takes part in endogenous cannabinoids` effects after 1 h of immobilization.
Key words: endogenous cannabinoids, opioids, stress-induced analgesia, paw pressure test, hot plate test

ANALGESIC EFFECTS OF SOME NEWLY SYNTHESIZED PYRROLE DERIVATIVES IN RATS

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Summary
The aim of our experiment was to examine the analgesic properties of some newly synthesized pyrrole derivatives with structure similarities to tricyclic COX-2 inhibitors.
Nociception was determined in male Wistar rats (180-200g) by the Randall-Selitto paw-pressure test. Hyperalgesia and oedema were provoked by subcutaneous carrageenan injection (100 μg/paw) in the foot pad of the right hind paw. Contralateral paw received the same volume (0.1 ml) of saline. The new pyrrole derivatives were synthesized in the University of Chemical Technology and Metallurgy of Sofia, and were intraperitoneally administrated in doses of 10, 20 and 40 mg/kg. Their analgesic activity was compared to Celecoxib and Metamizol.
Our results showed that pyrrole derivatives based on tricyclic COX-2 inhibitors significantly increased the pain threshold as compared to Celecoxib and Metamizol. The effect, more pronounced in the inflamed paw, was dose-dependent. The results were represented as mean ± SEM.
Key words: nonsteroidal anti-inflamamatory drugs (NSAID), analgesia, pyrrole derivatives, nociception

EFFECTS OF NALOXONE AND SECOND MESSENGERS ON TYR-CAV-MIF-1 AND TYR-CIT-MIF-1 ANALGESIA AFTER THREE MODELS OF STRESS

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Summary
The aim of the present study was to examine whether naloxone and methylene blue were involved in the nociceptive effects of Tyr-Cav-MIF-1 and Tyr-Cit-MIF-1 after three models of stress.
Nociception was measured in male Wistar rats (180-220g) by paw-pressure test. Tyr-Cav-MIF-1 and Tyr-Cit-MIF-1 were synthesized in the BAS - Institute of Molecular Biology from Academic E. Golovinsky and prof. T. Pajpanova. Peptides (both at a dose 1 mg/kg), naloxone (1 mg/kg) and methylene blue (500 g/paw) were dissolved in saline and were injected intraperitoneally. Immobilization stress was provoked by one-hour immobilization of the animals in apposite plastic cylinders. Cold or hot stress were provoked by placing the animals in cold (4 Cº) or hot (38 Cº) chambers for one hour. All procedures were approved by the Animal Care and Use Committee of the Medical University of Sofia.
The results showed that naloxone decreased immobilization-, cold- and hot-stress induced analgesia after Tyr-Cav-MIF-1 and Tyr-Cit-MIF-1, which means that the opioid system was involved in the effects. The analgesic effects of both the peptides were also influenced by MB. The more pronounced effect was observed for Tyr-Cav-MIF-1.
Our conclusions are that the analgesic effects of Tyr-Cit-MIF-1 and Tyr-Cav-MIF-1 after one-hour of IS, CS and HS are mediated by second messengers with the participation of the opioid system.
Key words: peptides, stress, nociception, naloxone, methylene blue
Acknowledgments. This work was supported by grant from the Bulgarian National Scientific Research Foundation VU-L-04/05.

BONE HISTOMORPHOMETRY AS DETERMINING METHOD FOR EVALUATION OF EXPERIMENTAL OSTEOPOROSIS

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Summary
Postmenopausal osteoporosis is the most common bone disease in the developed world, which leads to loss of regulatory control of the process of remodeling. Bone remodeling is the key process in bone structural reorganization, and its alterations lead to changes in bone mechanical strength and increase risk of fracture.
We analyzed morphological characteristics of healthy, osteoporotic and pharmacologically treated osteoporotic rats. Female Wistar rats were divided into the following groups: sham-operated control (Co), ovariectomized (OVX), ovariectomized supplemented with *Apium* extract (equal to 2.4 mg/kg Quercetin; OVX+A) and Genistein (2.5 mg/kg) supplemented (OVX+G) groups for 8 weeks; 6 month after ovariectomy. At the end of the experiments, the femur and tibia of all rats were removed after death and prepared for histological examination (stained with haematoxylin and eosin). Cortical thickness and trabecular thickness of proximal tibial metaphysis were measured. Additionally, the bone density, reflecting the proportion of osteoid (i.e. porosity) proximally and distally from the metaphyseal cartilage was evaluated. Our study revealed significant intergroup (Co vs. OVX) differences in morphology and osteocyte lacunae indicating different remodeling patterns. The degree of change was most pronounced in OVX rats. The thickness of the trabeculae was reduced and they were often interrupted forming large open spaces with bone marrow between them. Treatment with apium and Genistein demonstrated altered, but similar normal bone structure. There were no statistically significant changes between the two treatment groups.
The data derived from osteons (basic structural units of the cortical bone) in different skeletal conditions can be employed as an accurate method for verifying changes and development of experimental osteoporosis.
Key words: Postmenopausal osteoporosis, treatment, cortical and trabecular thickness

PHARMACOLOGY OF EYE DISEASES

ORAL PRESENTATIONS

NEW DRUG FOR INTRAVITEAL APPLICATION FOR DISEASES OF THE POSTERIOR SEGMENT

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Summary
Intravitreal application of medications allows for a more effective, targeted delivery of the drug substances into the posterior segment of the eye while avoiding systemic toxic effects. New medications, suitable for intravitreal therapy of a wider scope of retina disorders, especially associated with increased VEGF release are now available.
The most common eye disorders with increasing frequency, especially with the growth of aging population are age-related macular degeneration (AMD) and retinal vein occlusion (RVO). AMD and venous disorders are associated with increased risk of injury, mortality, necessity for supportive care, impaired quality of life and exacerbated co-morbidities. VEGF is a key regulator of both physiological and pathological angiogenesis. VEGF has also been associated with vascular permeability and the formation of edema. Numerous approaches have been developed to block VEGF and VEGF signaling. In clinical practice we have gathered experience with the application of different anti-VEGF drugs, resulting in preserving and even improvement of visual function.
Eylea® (Aflibercept) is a new drug for intraocular application for the treatment of neovascular (wet) age-related macular degeneration (wetAMD) and central retinal vein occlusion (CRVO). The treatment is also known as VEGF Trap-Eye, and is approved at a recommended dose of 2 mg every four weeks for the first twelve weeks, followed by 2 mg dose every two months.
Aflibertsept is a fusion protein of key domains from human VEGF Receptors 1 and 2 with human IgG Fc. It contains all human AA sequences. Aflibercept has high affinity and binds VEGF more tightly than native receptors or monoclonal Ab. It blocks all VEGF-A isoforms and Placental Growth Factor (PlGF).
Results of scientific studies comparing Eylea (Aflibercept) with Lucentis® (Ranibizumab) do not show clinically significant differences.
We have applied Eylea® (Aflibercept) in two groups of patients: one group of treatment-naive patients, and a group of patients who have been previously treated with Lucentis® and Avastin®.
Eylea® (Aflibercept) has demonstrated efficacy in a proactive, treat-and-extend dosing approach to treatment-naive patients with wet AMD and provides ophthalmologists with an additional therapeutic options for patients with wet AMD/CRVO. It also provides visual benefit to patients who have received combined treatment.

OCULAR SURFACE DISORDERS IN PATIENTS TREATED WITH BAK CONTAINING ANTIGLAUCOMA DROPS

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Summary
Glaucoma is a chronic disease, which requires long-term medical therapy, often involving multiple ophthalmic medications. Many glaucoma patients present with an ocular surface disease related to their therapy, particularly with the use of IOP lowering medications containing the preservative benzalconium chloride (BAK). This directly affects their quality of life and compliance rate of glaucoma treatment.
It is very important to diagnose the pre-existing ocular surface disease before starting application of such medications. Both glaucoma and dry eye syndrome occur (DES) in older populations so it is common to observe an overlap between these two conditions: DES prevalent in glaucoma patients and vice versa. Glaucoma is a lifelong chronic condition and it is often concomitant with DES. Ocular tolerability to anti-glaucoma drugs influences both quality of life and adherence.

Insufficient tolerability may lead to discontinuation of glaucoma treatment and a lack of compliance, so the consequences are potentially very serious.

We demonstrate several typical cases of patients with dry eye disease and glaucoma who have stopped their medical treatment because of ocular surface severe side effects. In this group all patients received glaucoma medications containing benzalkonium chloride (BAK) and had history of DES which was not diagnosed and treated. They presented at the clinic with complaints of irritation, pain, photosensitivity and foreign body sensation. The patients underwent full eye examination including Shirmer test, TBUT, visual field test and OCT of RNFL. It is demonstrated that eye drops containing BAK alter the conjunctival and corneal surface, with increased conjunctival inflammation and activation of macrophages, lymphocytes and mass cells, and injury of corneal epithelium with metaplasia. This has been demonstrated in studies with impressive cytology of ocular surface. Other studies found that preserved anti-glaucomatous eye drops showed much higher readings for blepharitis and hyperemia.

We stopped all BAK containing medications and the patients were given preservative-free antiglaucoma eye drops. We demonstrated that BAK containing glaucoma agents had led to severe ocular surface disease in all patients. Corneal staining with fluorescein demonstrated epithelial erosions, causing progressive intolerance. This lead to poor compliance to the antiglaucoma therapy resulting in deterioration of intraocular pressure control and progression of the disease. Switching the medications to preservative-free agents was effective and well tolerated since all patients complaints diminished.

BAK toxicity is a rapid cumulative dose-related and chronic phenomenon. It is also neurotoxic. It is demonstrated that discontinuing BAK exposure to the cornea allows corneal nerves with disrupted axonal function to recover structurally and lead to improved corneal function.

Conclusion: The use of glaucoma medications containing BAK is associated with a number of ocular symptoms including OSD and dry eye syndrome. This leads to a marked decrease in the compliance and a reduction in the quality of patient care and control of glaucoma. Conditions such as dry eye syndrome, ocular allergy, meibomian gland dysfunction should be carefully evaluated before prescribing anti glaucoma treatment. In such cases preservative-free drops should be considered first option. This would lead not only to an enhancement of the quality of life of patients, but also to improvements in glaucoma control.

ANTIGLAUCOMA TREATMENT WITH BETABLOCKERS IN PATIENTS WITH CARDIOVASCULAR DISEASES

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Summary

Primary open angle glaucomas (POAG) are chronic progressive optic neuropathies, leading to characteristic morphological changes at the optic nerve head and retinal nerve fiber layer, with typical visual field loss in the absence of other ocular diseases or congenital anomalies. They are the second leading cause of irreversible blindness in Europe and the world. Early diagnosis and correct treatment are main premises for prevention from blindness.The prevalence of glaucoma increases with age (most often after the age of 50 years) accompanied often with cardiovascular and other diseases. Treatment with betablockers (BB) is wide used in those patients. Irrespective of the kind of treatment – local in glaucoma or systemic in cardiovascular diseases it is with efficacy in both diseases but in what degree? There is an interaction, but is it between the diseases or the drugs used for their treatment? Influence of BB on blood pressure, heart rate, heart insufficiency and mortality, as well as efficacy, local and systemic adverse events of different BB, interactions with other drugs and the necessity the ophthalmologists and patients to be acquainted with them is being discussed in order to prevent them and use the most benefit with minimal risk therapy.

Key words: glaucoma, cardiovascular diseases, betablockers, efficacy, adverse events, drug interactions

RECEPTORS AND GLIA: IMUNOCYTOCHEMICAL STUDY OF LOW VERTEBRATE RETINA

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Summary
Neurons and glia are the two types of cells which are present in central nervous system (CNS). They originate from a common source, the stem cells of the embryonic neural plate. Neurons are the main communicating cells in CNS. Gial cells were thought until recently a mechanical and metabolic support of the neurons only. However, the last studies show that glia has enormous significance for a wide range of variable neuronal functions.

Retina, which is an extracranial part of CNS, is a useful biological model for studying the interactions between neurons and glia. Main glial cells here are the Müller cells which are large cells splitting the whole thickness of the retina. We studied, using the indirect immuno-fluorescency, the distribution of different kinds of membrane receptors in retinal glia of frog and turtle retinas. More than 20 primary antibodies directed to all subunits of the NMDA receptor and to the ionotropic purine receptors P2X1-7, as well as to Vimentin, a marker of Müller glial cells, were applied.

The results obtained showed that all subunits of the NMDA receptor, as well as all seven purinoreceptors P2X1-7 were well represented in frog and turtle retinas. The NMDA receptors were distributed both in neuronal and glial retinal structures. However, the glial NMDA receptors differed from neuronal ones in their subunits composition. Purine receptors P2X1 -7 were predominantly expressed in the Müller cells.

The significance of the glutamate NMDA receptor and the ionotropic purine receptors for the neuron-glia interactions is discussed.

Key words: NMDA, purines, retinal Müller cells, frog, turtle

DOPAMINE AND SEROTONIN IN FROG AND TURTLE RETINA: AN IMMUNOFLUORESCENT STUDY

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Summary
Dopamine and serotonin are monoamines belonging to the group of the low-molecular weigh neurotransmitters. In the central nervous system (CNS) they are widely used being involved in variable functions as motor control, reward and punishment, mood etc. In retina, which may be regarded as a natural biological model of the CNS, the dopamine and serotonin functions are not entirely clear. That is why the aim of the present work was to study their distribution in the retinas of frog *Rana ridibunda* and freshwater turtle *Emys orbicularis*, which possess mixed and predominantly cone type of retina respectively.

All procedures with a frog and a turtle were in accordance with the Bulgarian law for scientific experiments. The animals were deeply anesthetized with halothane and decapitated. The eyes were dissected, and the posterior eyecups with retinas were immediately immersed in 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer for 15–30 min. After fixation, the retinas were dissected from the eyecups and cryoprotected in graded sucrose solutions (10%, 20%, and 30% w/v). Cryostat sections were cut at 14 μm and stored at −20 °C. Antibodies directed to the dopamine- and serotonin transporters were applied, using the indirect immunofluorescent method. The results obtained showed that both dopamine and serotonin were well expressed in frog and turtle retinas.

Dopamine transporter antibody caused staining of great number of amacrine cells' perikaria located very close to the border of inner nuclear layer (INL) and inner plexiform layer (IPL). In addition, in the more distal part of INL single perikaria, most probably dopaminergic interplexiform cells, were also stained. Both plexiform layers: the outer (OPL) and inner (IPL), showed dopamine transporter immuno-reactivity as well.

Serotonin transporter antibody also caused well expressed staining in both plexiform layers of the retinas. The final endings of the serotoninergic amacrine cells were revealed in the IPL. The OPL staining might be due to the synapses made by the putative serotoninergic interplexiform and/or horizontal cells. Well expressed staining of the glial Müller cells in turtle retina was also evident. The participation of the dopamine- and serotoninergic neurons in the complex retinal networks of frog and turtle retinas is discussed.

Key words: dopamine, serotonin, retina, frog, turtle

PHYTOPHARMACOLOGY

ORAL PRESENTATIONS

DEPARTMENT OF PHARMACOLOGY, PHARMACOTHERAPY AND TOXICOLOGY IN FACULTY OF PHARMACY- SOFIA- IN THE YEARS

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Summary

The Department of Pharmacology was founded 40 years ago in July 1973. The first head of the department was Prof. D. Staneva. The teaching course of pharmacology and toxicology started September 1973 with full and part-time pharmacy students. Prof. Ts. Stoichev was the toxicology lecturer. In 1997 toxicology became an independent subject due to new European requirements. In 2001 the subject "Basis of internal medicine" was transformed as "Pharmacotherapy".

Today the department conducts training three disciplines in Bulgarian and English languages. In addition, there are 3 subjects that are thought during specializations of under-graduate students and 5 elective courses.

For the last 40 years, over 5000 under-graduate students, many post-graduate students, 25 PhD students in doctoral programs of pharmacology and toxicology and more than 150 diploma thesis students have been trained in the department. Most of the diploma thesis students were in collaboration with leading European universities. Over these years of experience many textbooks have been published in Bulgarian and English language.

The department's staff has exceptionally high publication activity alone or in collaboration with others departments in faculty especially in foreign journals with impact factor in different modern molecular pharmacology and toxicology topics. There were studied more than thousand original compounds from synthetic and plant origin, synthesized or isolated in faculty.

The department "Pharmacology, pharmacotherapy & toxicology" is one of the basic structures of Faculty of Pharmacy essentially contributing to contemporary student education and is highly appreciated in all accreditations of the Faculty of Pharmacy.

Key words: Department of Pharmacology, Pharmacotherapy and Toxicology, Faculty of Pharmacy-Sofia

PHARMACOLOGICAL AND TOXICOLOGICAL INVESTIGATIONS OF NEWLY GALANTAMINE PEPTIDE ESTERS AND PYMADINE HYBRIDE COMPOUNDS

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Summary

Galantamine hydrobromide (GAL) is a reversible acetylcholinesterase inhibitor, with properties to increase the concentration of acetylcholine in several brain structures. It is

widely used for the treatment of different neurodegenerative disorders, including dementia of Alzheimer's type . The aim of this study is to determine the influence of newly synthesized Galantamine esters, containing peptide fragments in 6th position of Galantamine molecule: 3,4-dichlorophenyl-Ala-Leu-Gly-Galantamine, 3,4-dichlorophenyl-Ala-Val-Gly-Galantamine and hybride compound with pymadine after per oral administration in different doses on: 1) locomotor activity in mice; 2) cognitive processes in experimental model of learning and memory in male rats. The determination of the locomotor activity of mice was carried out in Ugo Basile Biological Research (Milan, Italy) apparatus, which give an account of locomotor activity of animals on the base of capacitive method. The dynamic of learning and memory was examined by two – way avoidance learning method in "shuttle box" e (Italy).

After per oral administration on mice, the locomotor activity is increased, in comparison with control group, as follows: 3,4-dichlorophenyl-Ala-Leu-Gly-Galantamine in a dose of 6 mg/kg induced an increase of locomotion from 50 to 110 min from starting of the experiment. Both tested doses of 3,4-dichlorophenyl-Ala-Val-Gly-Galantamine and 2 mg.kg 3,4-dichlorophenyl-Ala-Leu-Gly-Galantamine did not influence the locomotor activity. The ester GAL-LEU and hybride compound improves the dynamics of learning 1/5 day in dose 3 mg/kg and memory for the learned 12/5 day. It can be considered, that the effect is CNS connected, because the low increase of the locomotor activity is not significant.

Key words: galantamine hydrobromide, avoidance learning, Alzheimer's disease, peptide esters

IN VITRO EFFECTS OF FLAVONOIDES, ISOLATED FROM *BUPLEURUM FLAVUM*, ON DIFFERENT MODELS OF TOXICITY

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Summary

Bupleurum L. (Aspiaceae) species are used as herbal remedy in Chinese traditional medicine. *In vitro* hepatoprotective activity of *B. flavum* flavonoid mixture (BFF) and the isolated from it biologically active compounds rutin and narcissin, were evaluated on carbon tetrachloride (CCl_4) and *tert*-butyl hydroperoxide (t-BuOOH) toxicity models.

Freshly isolated rat hepatocytes were incubated with the BFF, rutin and narcissin at a concentration of 1 mg mL^{-1}. Compared to the positive control silymarin and to the effect of rutin and narcissin, the BFF showed the most prominent hepatoprotective activity judged by the preservation of cell viability, GSH levels, reduction of MDA production and LDH activity. The mixture was most active in *t*-BuOOH-induced injury model as compared with CCl_4 toxicity ($p < 0.001$).

In BFF, synergism of rutin and narcissin could be responsible for the more pronounced protective effect detected in both models of toxicity.

Key words: isolated rat hepatocytes, *Bupleurum flavum*, cytoprotection, antioxidant activity

THE METHODIC OF PHYTOTHERAPY IN TRADITIONAL CHINESE MEDICINES

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Summary

The big difference between Chinese and others ethnomedicines in using herbs is the way of use. Our goal is to compare clinical practice of using herbs in China with Bulgarian and take prepositions how to develop it.

Used method was analytical research of curing process, including taking diagnosis, prescriptions of herbs; make the form of drugs, way of monitoring cure process, prescriptions changes during the curing process, and traceability of whole process. Special places of the analysis have been given to program of education in Traditional Chinese Medicines Universities and clinical practice.
This was made on the basis of programs and practice in Nanjing Pharmaceutical University and Nanjing University of the Traditional Chinese Medicine, also our own experience in Chinese Hospitals and Pharmacies.
The special attention has been made in explanation of the special features in the procedures of curing process and receipt changes and the ex tempore elaboration of medicines.
The analysis of these pharmacology phenomena gives one more possibility for clinical practice, but for the implementation it is needed some changes in Health low in Bulgaria.
Key words: Chinese herbal medicine, phytopharmacology, medical education

POSTERS

ETHANOL EXTRACT FROM THE HEARTWOOD OF *COTINUS COGGYGRIA*, AND ITS MAJOR BIOACTIVE PHYTOCHEMICAL CONSTITUENTS FUSTIN AND SULFURETIN MODULATE INDOMETHACIN-INDUCED GASTRIC ULCEROGENESIS IN RATS

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Summary
The Smoke tree (*Cotinus coggygria*) is a medicinal plant that is traditionally used for its antiseptic properties. There are few reports about the internal usage of extracts from this species against gastric ulcer. The aim of this study was to define the polyphenol content of ethanol extract from *C.coggygria* heartwood (EECCW) and to explore its possible effect on the gastric oxidative status in experimental model of indomethacin-induced ulcerogenesis in rats.
Forty percentage EECCW were analyzed by HPLC-MS. EECCW was applied by oral gavage (volume: 10 ml/kg) as a pretreatment 3 days before a single intragastric administration of indomethacin (dose: 100 mg/kg). Gastric erosions were evaluated morphometrically and histopathologically. Malondialdehyde (MDA) in blood serum and stomach was measured as a biochemical marker of lipid peroxidation. Gastric necrosis was also evaluated by alkaline phosphatase (ALP) and uric acid (UA) assays.
The results from HPLC-MS analyses showed that fustin and sulfuretin were the major components of the EECCW (C_{fustin}=52.27 mg/L, $C_{sulfuretin}$=14.20 mg/L). Histopathological studies demonstrated that EECCW induced a reduction of the depth and severity of indomethacin-induced mucosal lesions. EECCW reduced the elevated by indomethacin gastric MDA, ALP and UA levels.
Indomethacin-induced gastric mucosal damage was accompanied by oxidative stress. EECCW-pretreatment alleviated the gastric lesions, and prevented the indomethacin-induced elevation of gastric ALP and UA. It could be suggested that the gastroprotective effect of EICCW was due to the antioxidant properties of fustin and sulfuretin as evidenced by the decreased gastric MDA levels.
Key words: *Cotinus coggygria*, flavonoids, HPLC-MS, antioxidants, gastric ulcer, rats

RESURRECTION PLANTS (REVIEW)

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Summary
Resurrection plants are able to survive extreme drought and then to recover their functions after rehydratation. The aim of this review is to compare chemical composition and biological effects of different resurrection species like *Haberlea rhodopensis* (HR), *Ramonda serbica*, *Ramonda nathalie*, *Ramonda myconii*, *Boea hygrometrica*, *Anastatica hierochuntica* (AH), *Myrothamnus flabellifolia* (MF).
Collecting and analyzing literature data. According to their chemical composition, all resurrection plants have high level of carbohydrates – sucrose, raffinose, galactinol, which protect macromolecules from free radicals and oxidative stress during dehydratation. They also contain specific secondary metabolites like myconosid in HR, anastatin A and B in AH and 3,4,5-tri-O-galloylquinic acid in MF. The mechanisms of desiccation tolerance in resurrection plants include morphological changes, proteins and carbohydrates accumulation, signal transduction. A comparison between the pharmacological effects of the resurrection species shows that they have high potential to be used in therapy: anti-aging effect (HR), inhibition of reverse transcriptase in HIV-1 (MF), hepatoprotection and inhibition of melatogenesis in murine melanoma cells (AH).
Regarding to their medical application, a further more profound experimental work on the pharmacology of these plants is necessary.
Key words: *Haberlea rhodopensis*, *Ramonda serbica*, *Ramonda nathalie*, *Anastatica hierochuntica*, *Myrothamnus fabellifolia*

ANTIDEPRESSANT-LIKE EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE APPLIED SUBCHRONICALLY TO RATS

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Summary
Polyphenols are bioactive substances found in many plants. *Aronia melanocarpa* fruits are one of the richest sources of polyphenols, amongst them proanthocyanidins, flavonoids and phenolic acids. Some of the polyphenols are reported to cross the blood-brain barrier and thus they can act centrally. The aim of the present study was to investigate the effect of *Aronia melanocarpa* fruit juice (AMFJ) on depressive-like behavior in subchronically treated (21 and 30 days) male Wistar rats utilizing the forced swim test (FST). AMFJ was applied orally through an orogastric cannula once daily at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg for periods of 21 and 30 days to the respective experimental groups. The FST was carried out after the treatment periods. The increased immobility time served as a measure of the depressive-like behavior. Applied for 21 days, AMFJ at the doses of 2.5 ml/kg and 10 ml/kg decreased significantly ($p < 0.05$) the immobility time in the FST compared with the control group. In rats treated with AMFJ for 30 days, the immobility time was dose-dependently decreased and at the dose of 10 ml/kg it was significantly lower ($p < 0.05$) than the control one. The decreased immobility time in the FST suggests an antidepressant-like effect of AMFJ in rats which could be due to its polyphenolic constituents.
Key words: *Aronia melanocarpa*, forced swim test, behavior, antidepressant, rats

ANXIOLYTIC-LILE EFFECT OF *ARONIA MELANOCARPA* FRUIT JUICE APPLIED SUBCHRONICALLY TO RATS

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Summary
The main biologically active constituents of *Aronia melanocarpa* fruit juice (AMFJ) are polyphenolics, mainly proanthocyanidins, flavonoids and phenolic acids. The aim of the present study was to investigate the effects of *Aronia melanocarpa* fruit juice (AMFJ) on anxiety in subchronically treated (21 and 30 days) male Wistar rats utilizing the social interaction test. AMFJ was applied orally through an orogastric cannula once daily at doses of 2.5 ml/kg, 5 ml/kg and 10 ml/kg for periods of 21 and 30 days to the respective experimental groups. The social interaction test was carried out at the end of the treatment periods on the 21st and 30th day, respectively. The time of social interaction between the test partners was used as a measure of anxiety. The longer time for social contacts showed lower degree of anxiety. In rats treated with AMFJ for 21 days, the social interaction time between the test partners increased dose-dependently and at the dose of 10 ml/kg it was significantly higher ($p<0.05$) than the control time. Applied for 30 days, AMFJ did not increase the time of social interaction between the rats which might be attributed to the fact that at such duration of treatment AMFJ could decrease the general locomotor activity of the animals. The findings from the present study suggest an anxiolytic-like effect of AMFJ in rats which could be due to its polyphenolic ingredients.

Key words: *Aronia melanocarpa*, social interaction test, behavior, anxiolytic, rats

EFFECTS OF FERULIC ACID ON LEARNING AND MEMORY IN RATS

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Summary
Ferulic acid (FA) is a phenolic acid found in foods such as coffee, apples, rice, oats and wheat. Up to now, FA has been studied predominantly for its antioxidant activity. In recent years there has been an increased interest in the effects of polyphenolic phytochemicals on the functions of the central nervous system. The aim of present study was to investigate the effect of FA on learning and memory in male Wistar rats using the one-way passive avoidance task (step through) and the two-way active avoidance task (shuttle box). FA was administrated orally (20 mg/kg as a 10 ml/kg solution) to different groups of rats for 7, 14, 21 and 30 days. Control groups were respectively treated with saline (10 ml/kg). At the end of each experimental period, the step through and the shuttle box tasks were performed on different animal groups. Administered for 7 and 14 days, FA had no significant effects on rat behavior in both tasks. After 21 and 30 days of administration, in the step through task FA significantly prolonged the latency time during the retention tests on the 3rd and 24th hour and increased the percentage of rats reaching the learning criterion (remaining in the illuminated compartment for at least 180 sec). After 21 and 30 days of administration, in the shuttle box task FA significantly increased the number of avoidances on the 1st and 2nd training days as well as at the retention test (24 h after the 2nd training session). The results showed that FA improved learning

and memory processes in young/healthy rats.
Key words: ferulic acid, learning, memory, rats

EFFECTS OF FERULIC ACID ON EXPLORATORY BEHAVIOR AND LOCOMOTOR ACTIVITY IN RATS

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Summary
Ferulic acid (FA) is the most abundant hydroxycinnamic acid in the plant world. It is found in cereals (brown rice, whole wheat and oats) as well as in coffee, apples, peanuts, oranges and pineapples. After the discovery that polyphenolic substances penetrate through the blood-brain barrier there has been an increased interest in their effects on the functions of the central nervous system. The aim of the present study was to investigate the effects of FA on exploratory behavior and locomotor activity in male Wistar rats. FA was administrated orally (20 mg/kg as a 10 ml/kg solution) to different animal groups for 7, 14, 21 and 30 days. Comparisons were made with controls respectively treated with saline (10 ml/kg). At the end of each experimental period, the changes in exploratory behavior and locomotor activity were recorded in an Opto Varimex apparatus (Columbus Instruments, USA). The number of horizontal and vertical movements recorded every minute for the first 5 min served as a measure of exploratory activity and habituation to the new environment. The total number of movements during the first 5 min and during the whole 10-min period of observation was used as a measure of locomotor activity. It was found that FA at all doses for all treatment periods did not significantly affect exploratory behavior and locomotor activity of rats compared to the controls. It also did not disturb habituation. As habituation is considered as an elementary form of learning, the present study suggested that FA did not disturb the learning and memory processes in rats.
Key words: ferulic acid, exploratory behavior, locomotor activity, rats

THE USES OF THE AROMATIC OILS IN CURING PROCESS OF THE BONES-JOINTS DESEASES

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Summary
The use of aromatic oils is quite exotic in clinical practice. Mostly they are part of SPA procedures, which is a disadvantage of our rehabilitation system.
Our goal is to report our positive results in several clinical cases with bones-joint diseases. In all of those clinical cases have been used combine of acupuncture and aromatic oils.
The results are analyzed statistically for comparing the time and longitudes of effect.
The conclusions are in two main directions:

- The use of aromatic oil reduces time needed to improve the patient. The use of aromatic oils is still an art, high dependently of medical skills of the person in charge.
- It is needed to be created a methodic of aromatic oil use and put the curable process in more scientific basement and make the results more predictable.

Key words: aromatic oil use in medical practice, arthritis, rheumatic arthritis, bones and joint trauma

PHARMACOLOGY OF ENDOCRINE DISEASES

ORAL PRESENTATIONS

MEDICAL TREATMENT OF POLYCYSTIC OVARY SYNDROME

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Summary
Polycystic ovary syndrome (PCOS) is one the most common endocrine disorders affecting about 5-14% of women of reproductive age and leading cause of infertility. The global medical importance of the disease is based on the fact that according to the new concepts PCOS is considered as typical representative of metabolic syndrome with insulin resistance and compensatory hyperinsulinaemia being the underlying mechanism. PCOS is associated with higher prevalence of visceral obesity, unfavorable ratio between atherogenic and antiatherogenic adipocytokines, impaired glucose tolerance/diabetes mellitus type 2, dyslipidaemia, and hypertension. At present lack of clear etiological cause of PCOS requires symptomatic treatment. The main goals include induction of ovulation, treatment of hyperandrogenism, and management of metabolic syndrome. Clomiphene citrate (CC) is the most effective first-line medication for anovulation. The traditional treatment sequence CC followed by FSH in failure to conceive is highly effective. Low-dose combined oral contraceptives (OCs) and antiandrogens are widely used potent drugs as a first-line treatment in PCOS patients presenting with hyperandrogenism. Combination of antiandrogens with progesterone-based OCs having antiandrogen effects results in potent synergistic suppression of androgen levels via different mechanisms of action. Together with lifestyle changes, insulin-sensitizing drugs are useful for managing metabolic disturbances and reducing the risk of diabetes and cardiovascular events. Metformin may be useful in infertile PCOS women resistant to clomiphene citrate. More individual therapeutic approaches should be developed, based on initial patient characteristics, to improve outcomes and decrease complication rates.
Key words: PCOS, clomiphene citrate, oral contraceptives, antiandrogens, insulin-sensitizing drugs

REPLACEMENT THERAPY IN ADULTS WITH GROWTH HORMONE DEFICIENCY

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Summary
The physiological role of growth hormone (GH) in adult life, after reaching the final height, has attracted increased interest in the last decades. The effects of GH replacement were first reported in 1989 and since then, studies have demonstrated sustained impact on substrate metabolism. The latter has provided sufficient basis for the administration of somatotropin in adult patients with growth hormone deficiency (GHD) as a part of the conventional substitution therapy in hypopituitarism.
GH induces profound effects on protein, fat, and energy metabolism; improves quality of life, psychological well-being and physical capacity, body composition, bone mineral density and some surrogate markers of cardiovascular risk. Mean substitution doses in adults are much lower than in pediatric practice and should be titrated according to serum levels of the insulin-like growth factor 1 (IGF-1).
Recently, the most disputable questions have been the optimal duration of the replacement and the appropriateness of GH administration in elderly patients (>60-70 years of age). In adolescents and young patients, however, there are irrefutable benefits from substitution therapy especially on body composition and the acquisition of peak bone mass which occurs years after the completion of linear growth.

Adverse events associated with replacement therapy are mainly secondary to GH-induced antinatriuresis and the correction of the water depletion. In general they are mild to moderate, arise within the first months of treatment and subside spontaneously or with dose-reduction. The potential mitogenic effects of somatotropin have raised considerable concerns. Surveillance studies, however, have not demonstrated an increased risk of malignancy in the adults who received GH.

Diagnosis and treatment of hyposomatotropism in adults is a routine practice in the Clinical Centre of Endocrinology in the last few years. The focus is on the continuation of replacement therapy in childhood-onset GHD persisting into adult life, with the close collaboration of pediatric departments.

Key words: growth hormone deficiency, GH replacement therapy

PHARMACOGENETICS AND ITS POSSIBLE ROLE IN THA TREATING TYPE TWO DIABETES MELLITUS AND OBESITY

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Summary

Achievements in genetics over the past years have confirmed that a number of genes are involved in the genesis of a type two diabetes mellitus (T2DM) and obesity. It is assumed that some of these genes may have an influence on the effect of the medicaments applied. Oral hypoglycaemic agents are best studied in the field of pharmacogenetics. Metformin is not metabolised and its exact mechanism of action is still the subject of intense research. It is known so far that a number of cationic transporters mediate its action. Variations in their pharmacokinetic genes can affect the respond of metformin. Pharmacodynamics of sulphonylureas affect two genes - ABCC8 (SUR1) and TCF7L2, which regulate the functional activity of potassium channels in the insulin-producing cells of the pancreas and the influence on insulin secretion. Therapeutic response to thiazolidinediones can be altered by mutations in the genes PPARG and ADIPOQ (adiponectin) involved in lipid metabolism. Despite the great hope that pharmacogenetics will make genotype-dependent therapy customization possible, hypoglycaemic treatment still cannot be tailored to individual genetic variations. Future breakthroughs shall be made in multifactorial metabolic diseases such as obesity and T2DM and new therapeutic advances are necessary in the light of pharmacogenetics considering the fluency of genetic variations and the therapeutic response.

Keywords: pharmacogenetic, diabetes mellitus type II, obesity, oral hypoglycaemic agents

EFFECTS OF VITAMIN K ON RATS FED HIGH-FAT HIGH-FRUCTOSE DIET

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Summary

This study was undertaken to test the hypothesis that osteocalcin – a bone derived vitamin K dependent protein, plays an important role in carbohydrate metabolism and disorders.

In rats fed high-fat high-fructose (HFHF) diet to produce a metabolic syndrome, vitamin K1 and K2 were administered orally throughout the 12 weeks period of diet manipulation. Insulin tolerance test was performed at the end of the study. Behavioral tests for locomotion, anxiety, depression and memory were also carried out. Body weight and food/liquids consumption were measured weekly. The animals were sacrificed after overnight fasting. Retroperitoneal fat pads and livers were weighed. Pancreatic and liver

tissues were examined histologically. Serum lipids, markers of oxidative stress, as well as insulin and osteocalcin (carboxylated – cOC and uncarboxylated – ucOC) were measured.
UcOC was reduced in all HFHF fed rats relative to controls and cOC tended to rise. These changes paralleled the high levels of triglycerides and cholesterol and the increased fat index in the diet manipulated rats, as well as the pathomorphological features of liver steatosis and pancreatic lipomatosis. The insulin levels were elevated. Vitamin K1, however, enhanced insulin sensitivity at the 90th min in the insulin-tolerant test, probably due to the more pronounced antioxidant effect. Both vitamin forms also showed antidepressant-like effect.
In conclusion, diet-induced metabolic syndrome is associated with low ucOC levels; vitamin K treatment is either indifferent or further aggravates most of its features. The present findings are in agreement with the proposed function of ucOC as a regulator of glucose homeostasis.
Key words: rats, high-fat high fructose diet, metabolic syndrome, osteocalcin, vitamin K

POSTERS

OUR EXPERIENCE IN TREATMENT OF VULVOVAGINAL CANDIDIASIS IN GIRLS WITH TYPE 1 DIABETES

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Summary
The impaired immune response and the glucosuria in girls with type 1 diabetes (T1D) are considered as risk factors for developing of vulvovaginal candidiasis (VVC). The most candidal infections are due to *Candida albicans*, but the proportion of non-albicans VVC has grown lately. Non-albicans species are resistant to conventional antifungal therapy and often induce recurrent vulvovaginitis.
The aim of this study was to present our experience in treatment of VVC among girls with T1D.
There were researched 45 girls with T1D at age of 11.9±1.1 , hospitalized during one year (2013) in the Endocrine Unit, Department of Pediatrics in University Hospital-Pleven. The following methods were used: genital inspection; microbiological culture of genital discharge; antifungal treatment – local (clotrimazole) or oral (single dose fluconazole-3mg/kg).
Clinical symptoms of VVC were presented in 20 (44.5%) from the girls with poor long-term metabolic control – Hb_{A1c} 11.1±1.0%. Among them positive cultures were found in 16 (35.6%) girls: 5 (31.3%) with bacterial and 11 (68.7%) with yeast etiology. *Candida albicans* was proved in 8 (72.7%) and *Candida tropicalis* – in 3 (27.3%) patients. Treatment in children under 6 years were only locally with clotrimazole cream. A single dose fluconazole (3mg/kg) was given orally to older girls. Clinical symptoms of VVC were cured in 8 (73%) of the treated patients, and the other 4 (27%) were presented with recurrent infections (mostly non-albicans).
Most of the girls with VVC and T1D respond positive to conventional antifungal therapy. But the treatment of recurrent yeast infections, caused by non-albicans species remained a problem and requires further researches.
Key words: vulvovaginal candidiasis, type 1 diabetes, girls, treatment

THE CHALLENGES IN THERAPY OF BRAIN OEDEMA IN CHILDREN WITH DIABETIC KETOACIDOSIS

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Summary
Diabetic ketoacidosis (DKA) is the most common cause of diabetes-related death in

children. Most *often* death occurs as a result of cerebral oedema (CO). No protocol for DKA has been shown to eliminate the risk of cerebral oedema developing.
The aim of the study was to present two clinical cases of children with CO complicating treatment of initial and recurrent DKA. To emphasize on the factors promoting the CO developing and on the challenges in its therapeutic strategies.
Case 1: A 12 years old girl developing CO before the start of the initial DKA treatment. Clinical presentation–leading signs were severe state of metabolic acidosis, unconsciousness, dehydration and shock. Recovering within 36 hours.
Case 2: A 8 years old girl with recurrent DKA and CO developing in the first 4 hours after starting rehydration. Complications: acute respiratory and heart failure (Lung oedema) and renal failure. Full recovering within 24 hours.
CO is an ever-present risk of DKA, which can be minimized by careful attention to rate of rehydration, choice of fluids used and attention to electrolyte management.
Careful monitoring is essential and treatment should be carried out only in centers with specialized nursing, medical and laboratory facilities.
Key words: cerebral oedema, diabetic ketoacidosis, treatment, children

MEDICAL TREATMENT OF CUSHING'S SYNDROM

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Summary
Cushing's syndrome (CS) is a rare but serious disease, with serious implications for the duration and quality of life of patients. It is characterized by significant morbidity and mortality. The main method of treatment is surgical operation, in case of failure or when patients are not suitable for surgical intervention is necessary to apply other methods of treatment. Medical treatment of CS has a limited role due to poor efficacy or poor tolerability. In recent years, making significant progress in drug treatment associated with the identification of new targets for drug response, both at the pituitary level (eg somatostatin analogues, dopamine receptors and epidermal growth factor, etc.) and at the level of the adrenal cortex (eg. ectopic-expressed receptors in ACTH-independent forms of CS). In this report, are considered old, well-known options for medical treatment of SC, and new opportunities for drug therapy.
Key words: Cushing's syndrome (CS), medical treatment

DOPAMINE AGONISTS AND THE RISK OF CARDIAC VALVE FIBROSIS IN PATIENTS WITH PROLACTINOMAS: A CROSS-SECTIONAL STUDY IN A SINGLE TERTIARY REFERRAL CENTRE

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Summary
Dopamine agonists (DAs) represent the first-line agents for the management of prolactinomas. Several cross-sectional studies have demonstrated a significantly increased risk of valvular fibrosis in Parkinson's patients treated with the ergot-derived DAs pergolide and cabergoline. Despite extensive research over the past decade, the risk of valvulopathy in prolactinoma patients on longterm cabergoline treatment has not been well-defined. Evidence-based information about the second most-prescribed DA in these patients, bromocriptine, is extremely limited.
The aim of this cross-sectional case-control study was to assess the prevalence and the risk of valvular lesions among patients on long-term bromocriptine or cabergoline therapy.
A transthoracic echocardiographic evaluation

was performed in 334 subjects enrolled into 4 groups: 103 cabergoline treated, 55 bromocriptine treated, 74 naïve patients and 102 controls. Clinically relevant valve regurgitations were equally prevalent in all investigated groups whereas subclinical valve fibrosis was significantly more frequent in both bromocriptine- and cabergoline treated patients (40% vs. 43.6% vs. 21.6% vs. 23.5%; p= 0.004). The odds ratios [OR] for developing valvular fibrosis were 2.27 [95% CI 1.17 to 4.41; p=0.016] for cabergoline and 2.66 [95% CI 1.22 to 5.78; p=0.014] for bromocriptine groups compared to non exposed to dopamine agonists subjects.

Long-term treatment with cabergoline and bromocriptine seems not to be associated with an increased risk of clinically significant valve disease but possible subclinical lesions should be expected. An echocardiographic examination is recommended at the start and periodically during therapy with DAs acting as full or partial agonists of 5-HT2B receptors (cabergoline, bromocriptine).

Key words: bromocriptine, cabergoline, prolactinomas, valvular lesions

THE EFFECTS OF LONG-TERM BENFOTIAMINE SUPPLEMENTATION ON STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHY

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Summary

Spinal glial activation is now considered an important component in the development and maintenance of allodynia and hyperalgesia in various models of chronic pain. There is evidence that benfotiamine, lipid-soluble derivate of vitamin B1 is useful in the treatment of symptomatic diabetic peripheral neuropathy.

The aim of the study was to investigate the effect of benfotiamine in streptozotocin-induced diabetic neuropathy and spinal glial activation.

Male Wistar rats were injected with freshly prepared Streptozotocin (70 mg/kg STZ, i.p.) and after confirming diabetes (glucose concentration >15 mmol/L) divided in groups as follows: healthy controls (Co); diabetics (D); rats receiving a daily dose of 100 mg/kg benfotiamine after the day they were injected with STZ; rats receiving 100 mg/kg benfotiamine per day starting 21 days after induction of diabetes. Changes in pain threshold were measured by paw pressure (PPT), plantar heat and von Frey hair tests. Spinal microglial and astrocyte activation was evaluated using Iba-1 and GFAP immunoreactivity respectively.

STZ-diabetic rats displayed significant tactile allodynia and thermal hyperalgesia compared with control rats. Long treatment with benfotiamine modulates allodynia and alleviates mechanical hyperalgesia (PPT). Quantification of cell markers Iba-1 for microglia and GFAP for astrocytes revealed extensive activation of microglia in the dorsal horn of diabetic rats, whereas a slight increase in astrocytes activation could be observed. Benfotiamine treatment did not significantly alter the immunoreactivity of microglia, but reduces the number of cells (mm^2) ($p < 0.05$).

Our findings suggest that benfotiamine (100 mg/day) exhibits no independent analgesic effect, but alleviates peripheral neuropathy and modulated spinal glia activation.

Key words: diabetic neuropathy, allodynia, hyperalgesia, spinal glia

Acknowledgements. This investigation was supported by Medical University Sofia (Grant 20/2013).

RAT MODELS OF METABOLIC SYNDROME

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Summary
Metabolic syndrome (MS) is a disorder comprising central obesity, dyslipidemia, insulin resistance, raised blood pressure.
The aim of the present study was to develop rat models of MS.
Male Wistar rats were divided in 3 groups: a control group (C) receiving regular rat chow diet, a high-fat (HF) group receiving lard enriched rat chow and a high-fat high-fructose (HFHF) group receiving lard and fructose enriched rat chow. HF and HFHF groups received also 10% fructose in the drinking water. The study lasted for 8 weeks. Body weights were measured weekly. At the end of the study insulin tolerance test (ITT) was performed. Liver and fat weight index were measured after sacrifice. Lipid biochemical parameters and insulin concentration in serum were determined. Liver triglycerides (TGs) were measured. The oxidative stress was assessed by thiobarbituric reactive substances (TBARs) in plasma and liver.
At the end of the study the animals did not differ in their body weights across the groups, but the fat index in both HF and HFHF groups was higher. Plasma TGs and cholesterol were raised in both groups. HDL-cholesterol was not changed but the ratio cholesterol/HDL-cholesterol was higher in HF and HFHF groups. Liver TGs were elevated in HFHF rats. ITT revealed insulin resistance in both experimental groups although serum insulin was elevated only in HFHF group. TBARs as a measure of lipid peroxidation were increased in both HF and HFHF groups.
Both experimental models display most of the signs of the MS.
Key words: metabolic syndrome, rats, high fat diet, high fat high fructose diet

MEDICAL TREATMENT OUTCOME OF ACROMEGALY IN A SINGLE TERTIARY CENTER

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Summary
Acromegaly is a rare disease caused by pituitary adenoma in approximately 95% of the patients. Trans-sphenoidal adenomectomy is a first-line treatment approach. After unsuccessful surgery medical treatment is recommended. Another available therapy is irradiation, however left as a third-line option.
Objective: To describe biochemical outcome in regards to different medical options in patients with acromegaly.
It was a retrospective analysis of patients treated with Dopamine agonists (DA) (Bromocriptine or Cabergoline), Somatostatin analogs (SSA) (octreotide, Sandostatin®LAR) or growth hormone receptor antagonist (GHRA) (pegvisomant) by the end of 2012 yr in our clinics. The study period extends to over 40 years. Patients with at least one follow-up and available GH and/or IGF-1 values were included in the analysis.
Bromocriptine adjuvant to surgery was applied in 133 patients with achievement of disease control in 18.8%. Remission rate in non-irradiated patients was 16.3% (18/110). Cabergoline adjuvant to surgery was applied in 70 patients with achievement of disease control in 31.4%. Remission rate in non-irradiated patients was 18.2% (8/44). Adjuvant Sandostatin® LAR led to remission in 27(38.6%) out of 70 treated patients. Combination therapy with SSA and DA resulted in disease control in 5 (25%) out of 20 patients. GHRA, alone or in combination with SSA and/or DA was applied in 13 patients, eight (61.5%) of which achieved normalization of IGF-1 values.
The efficiency of SSAs and pegvisomant could be further improved by appropriate dose adjustment. DAs could be a good cheap alternative in carefully selected patients.
Key words: acromegaly, bromocriptine, cabergoline), somatostatin analogs, growth hormone receptor antagonists

EFFECTS OF CLOZAPINE AND THIORIDAZINE ON PITUITARY THYROID FUNCTIONS

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Summary
Clozapine and thioridazine are drugs with a neurotherapeutic activity, which are used for a long treatment of psychiatric conditions. We have previously published effects of these medications on endocrine functions (Boyadjieva N., 1988).
The aim of this study was to determine the influence of clozapine and thioridazine on TSH, T3 and T4 levels of euthyroid rats exposed or not to cold stress.
The experiments were performed with three-month old male Wistar rats treated p.o. with clozapine or thioridazine for a period of 30 days. The rats were housed in six groups – control group, groups exposed or not to cold stress and treated with the medications. At the end of the period the rats were decapitated and TSH, T4 and T3 plasma and serum levels were measured with a radioimmunoassay.
Our results demonstrate that cold stress increased TSH plasma levels in the experimental animals. Furthermore, chronic treatment with clozapine decreased TSH levels in rats exposed to cold stress, while treatment with thioridazine increased TSH levels.
Our data suggest that chronic treatment with neuroleptics may influence pituitary thyroid functions.
Key words: clozapine, thioridazine TSH, T3, T4

PHARMACOLOGY OF SKIN DISEASES

ORAL PRESENTATIONS

MODERN STRATEGY IN TREATMENT OF PSORIASIS

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Summary
Psoriasis is an immune-mediated chronic disease and affects 1-3% of world human population. There is an algorithm for psoriasis care, based on different clinical stages of the disease, enlists local products, systemic medication and phototherapy. The new treatment with biological molecules (monoclonal antibodies and fusion proteins against immune T-cells or proinflammatory cytokines) requires depth knowledge of mode of action, pharmacokinetics and pharmacodynamics because of eventual serious adverse events. It's well known that psoriasis is not only disorder of skin and joints, but a systemic inflammatory autoimmune illness associated with others inflammatory dermatological and internal alterations. The high co-morbidity prevalence in psoriatic patients is an important medical and social problem of today. These facts require exact interdisciplinary approaches concerning adequate therapy and prophylaxis of potentially negative conditions some of them life-dangerous.
Key words: new therapy, psoriasis

CURRENT TENDENCIES IN THE TREATMENT OF ATOPIC DERMATITIS

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Summary
Atopic dermatitis (AD) is a common, chronic, relapsing, inflammatory skin disease that primarily affects young children. Around 50% of them develop symptoms within their first year of life. Around 75% with childhood onset of the disease have a spontaneous remission before adolescence, whereas the remaining 25% continue to have eczema into adulthood. The aetiology of atopic dermatitis is unknown, but filaggrin mutations in patients with atopic dermatitis were recently discovered. Atopic dermatitis is not always easily manageable and every physician should be familiar with the fundamental aspects of treatment. Barrier therapy continues to play an important role without evidence supporting use of one emollient over another. Herbs are widely used in the treatment of atopic dermatitis in Eastern Asian countries, and certain herbs regarded have anti-inflammatory properties by suppressing Th2 cell response. Studies on the topical treatment of atopic dermatitis largely supported the recommended use of topical corticosteroids and topical calcineurin inhibitors. In addition to topical treatment, severe acute or chronic AD often requires systemic immunosuppressant drugs or phototherapy. Short-term tapered treatment with oral corticosteroids is recommended for acute flares of severe, widespread atopic dermatitis. As *Staphylococcus* infections often trigger such flares, oral antibiotics should be prescribed simultaneously. Due to the risk of side effects, continuing treatment with oral corticosteroids is not recommended but a second immuno-suppressant drug, for example azathioprine, methotrexate, or cyclosporine A, for very severe, chronic, relapsing atopic dermatitis is introduced. Cyclosporine is used for control of severe atopic dermatitis in children. Although traditionally recommended at a dose of 5 mg/kg/day for 6 months, a longer duration of treatment may be necessary to bring into a remission. Dupilumab, a fully human monoclonal antibody that blocks interleukin-4 and interleukin-13, has shown efficacy in patients with atopic dermatitis.The blockade by dupilumab of these key drivers of type 2 helper T-cell (Th2)-mediated inflammation could help for marked and rapid improvement in all the evaluated measures of atopic dermatitis disease activity. Widespread eczema benefits from treatment with UV light. Narrowband UVB light is particularly suitable for treating adults with recalcitrant eczema. Broadband UVA light and a combination of UVA light and the photosensitizing drug *psoralene (PUVA)* can also be used to treat severe recalcitrant eczema. Oral antihistamines are recommended for itching but have no effect on the activity of AD. Nonsedating antihistamines should be used, but when night-time itching interferes with sleep, sedating antihistamines are recommended. In conclusion this abstract gives an overview of the treatment of atopic dermatitis.
Key words: atopic dermatitis, topical therapy, systemic immunosuppressant therapy, phototherapy

PHARMACOLOGY OF NEURODEGENERATIVE DISEASES

ORAL PRESENTATIONS

CLINICAL USE OF CANNABINOIDS

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Summary
The endocannabinoids are endogen ligands of the cannabinode receptors. The most important are two derivatives of the arachidonic acid – anandamide and 2-arachidonylglycerol. They are connected with the cognition, memory, appetite control, emesis, anxiety, motor behavior, sensory, autonomic and neuroendocrine responses. The endocannabinoid system participates in the pathophysiology of some neurological and psychiatric diseases like multiple sclerosis, Huntington's disease, Parkinson disease, depression, schizophrenia and drug addiction. The cannabinoid receptors (CB) are two classes. The CB_1 receptors are expressed mainly in the central nervous system, and CB_2 – in the immune cells. The suppression of the pain transmission is one of the main biological effects of endocannabinoids. The cannabinoid receptors take part in the descending noradrenergic control

of the nociception, mediated by the neurotransmitters noradrenaline and serotonin. The endocannabinoid system takes part in different neuroprotective functions also. The neuroprotective effect is realized through CB_1 receptor inhibition of the excitotoxicity and CB_1 receptor mediated inhibition of neuroinflammation. The endocannabinoid system participates in the control of muscle tone. The experimental studies revealed reduction of spasticity through CB_1 receptors. The most important active components of cannabis are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) that exert the effect through the cannabinoid receptors of the endocannabinoid system. Nabiximols (Sativex®) is a drug, that is a mixture of 1:1 delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), extracted from cloned Cannabis sativa and dissolved in ethanol. It is used as oral spray for treatment of spasticity in multiple sclerosis patients.

Key words: cannabinods, *Cannabis sativa,* cannabinode receptors, Nabiximols (Sativex®)

NEUROTRANSMITTER SYSTEMS INVOLVED IN INCONTINENCE/CONTINENCE OF COLO-RECTO-ANAL REGION

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Summary
Because of high medical and social impact the nerve-mediated diseases of large intestine are object of a great variety of studies. This summary presents experimental observations demonstrating neurotransmitter systems involved in continence/incontinence events of colo-recto-anal region using electrical stimulation, mechanographic recording technique and rat isolated intestinal segment-preparations as test models. The electrically-induced local motor responses of longitudinal and circular layers increased from the colon to the anal region demonstrating higher contractile potency than relaxation ability in the distal direction. The locally-applied electrical stimulation elicited synchronous orally directed ascending reflex motor responses and anally directed descending reflex motor responses, thus indicating that locally-induced nerve excitation propagated via intrinsic ascending and descending nerve pathways. In colon, the ascending cholinergic by nature motor responses were more pronounced and the motor responses of longitudinal muscle were more expressed than those of circular muscle thus presenting an essential role of the ascending reflex pathways and longitudinal muscle in the coordinated colon motility. The activation-dependent descending reflex motor responses of the anal canal involved electrical stimulation-displayed cholinergic and tachykininergic and distension-manifested nitrergic reflex pathways. Cholinergic system is more expressed in distal rectum underlying its contractile activity, while nitric oxide-dependent transmission(s) control the relaxation capacity of the internal anal sphincter and anal canal. These data suggest that a damage, as a nerve degeneration, of excitatory long cholinergic (in colon-anorectum) and inhibitory locality-dependent short nitrergic (in anal region) reflex pathways is a precondition for colo-recto-anal incontinence/continence.

Key words: large intestine motility, incontinence, continence

POSTERS

ASSESSMENT OF SUBSTANTIA NIGRA ECHOGENICITY IN PATIENTS WITH EARLY PARKINSON'S DISEASE

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Summary
Early diagnosis of Parkinson's disease is of

immense importance. Transcranial ultrasound indicates characteristic of echogenicity of the substantia nigra in patients with Parkinson's disease.

Transcranial sonography is a new, noninvasive ultrasound technique that characteristic echogenicity of the substantia nigra in patients with Parkinson's disease. Transcranial sonography was performed trough the temporal acoustic bone window according to a standard approach using a 2.5 MHz transducer.

We examined 12 patients with early Parkinson's disease and 10 age matched healthy controls by transcranial ultrasound. The results showed that in 8 severely early affected Parkinson's disease patients, the echogenicity of the substantia nigra was distinctly increased.

In conclusion, an abnormal increased echogenic size (hyperechogenicity) of the substantia nigra was established in patients with early Parkinson's disease. Transcranial sonography is a new, noninvasive ultrasound technique that has demonstrated an increased echogenicity of the substantia nigra in patients with Parkinson's disease, whereas in most healthy individuals, the substantia nigra is normal echogenicity by transcranial sonography.

Key words: Parkinson's disease, transcranial sonography, substantia nigra

Acknowledgments. The work was supported by Grant 29/2013 from the Medical Science Council of the Medical University of Sofia, Bulgaria.

THE EFFECTS OF LONG-TERM BENFOTIAMINE SUPPLEMENTATION ON STREPTOZOTOCIN-INDUCED DIABETIC NEUROPATHY

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Summary

Spinal glial activation is now considered an important component in the development and maintenance of allodynia and hyperalgesia in various models of chronic pain. There is evidence that benfotiamine, lipid-soluble derivate of vitamin B1 is useful in the treatment of symptomatic diabetic peripheral neuropathy.

The aim of the study was to investigate the effect of benfotiamine in streptozotocin-induced diabetic neuropathy and spinal glial activation.

Male Wistar rats were injected with freshly prepared Streptozotocin (70 mg/kg STZ, i.p.) and after confirming diabetes (glucose concentration >15 mmol/L) divided in groups as follows: healthy controls (Co); diabetics (D); rats receiving a daily dose of 100 mg/kg benfotiamine after the day they were injected with STZ; rats receiving 100 mg/kg benfotiamine per day starting 21 days after induction of diabetes. Changes in pain threshold were measured by paw pressure (PPT), plantar heat and *von Frey hair tests*. Spinal microglial and astrocyte activation was evaluated using Iba-1 and GFAP immunoreactivity respectively.

STZ-diabetic rats displayed significant tactile allodynia and thermal hyperalgesia compared with control rats. Long treatment with benfotiamine modulates allodynia and alleviates mechanical hyperalgesia (PPT). Quantification of cell markers Iba-1 for microglia and GFAP for astrocytes revealed extensive activation of microglia in the dorsal horn of diabetic rats, whereas a slight increase in astrocytes activation could be observed. Benfotiamine treatment did not significantly alter the immunoreactivity of microglia, but reduces the number of cells (mm^2) ($p < 0.5$).

Our findings suggest that benfotiamine (100 mg/day) exhibits no independent analgesic effect, but alleviates peripheral neuropathy and modulated spinal glia activation.

Key words: diabetic neuropathy, allodynia, hyperalgesia, spinal glia

Acknowledgements. This investigation was supported by Medical University Sofia (Grant 20/2013).

MALIGNANT NEUROLEPTIC SYNDROME – HISTORY, DIFFERENTIAL DIAGNOSIS, TREATMENT AND PROGNOSIS

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Summary
The history of discovery of the malignant neuroleptic syndrome (MNS) is an object of important scientific interest. Pathophysiological investigations concerning the MNS reveal lower levels of the dopamine, caused by D2-receptors blockade in the hypothalamus and nigrostriar system or dysfunction of the dopamine receptors. The blockade of transmission in the hypothalamus provides thermo disregulation and peripheral effects of the neuroleptics – the liberation of Ca^{2+} from the endoplasmatic reticulum. The aim of this study was to research and to follow up history, differential diagnose, treatment and prognosis of the MNS. The article describes the typical clinical symptoms and the biochemical constellations, compatible with MNS. The differential diagnosis, treatment and prognosis are discussed.
The analysis of the received results revealed benign prognosis, if the treatment starts to admit of no delay. The effects of the neuroleptics are not depending on the dose.
Key words: MNS symptoms, FALTER – syndrome, serotonin syndrome (SS), toxic encephalopathy, malignant hyperthermia

VARIA

ORAL PRESENTATIONS

PROFESSOR DIMITAR PASKOV – A SCIENTIST AND CITIZEN

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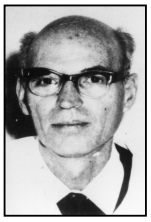
Summary
Prof. Dimitar Paskov (1914–1986) is the most renowned continuer of the work of prof. P. Nikolov. As a young doctor, he took part in the first stage of the Patriotic War (1944–1945). After the war, he was a research associate in the Bulgarian Academy of Science and then became head of the Department of Pharmacology at the Medical Faculty in Sofia (1962–1977). Prof. D. Paskov was on a specialization course in Saint Petersburg tutored by the world-renowned Russian pharmacologist S. V. Anichkov.



Prof. Paskov introduced a large number of experimental methods and created the foundations of a prospering scientific school of Bulgarian pharmacologists. He was the scientific advisor of 12 postgarduated students who successfully defended their PhD theses. He enriched the country with a number of original medicines: *Nivalin®, Dibazol®, Pymadin®, Tabex®, Nivalin P®, INHA-17®, Depreton®, Verbascan®, Aminton®, Dimex®*, etc. He investigated the therapeutic activity of a number of plants: Verbascum nobile et pseudonobile, Reseda luteola, Erysimum repandum, Betula alba, Leonurus cardiaca, Cytisus laburnum, Scrophularia canina, Calendulla officinalis. His unique pharmacological investigations on galantamine (Nivalin®) made Bulgaria famous among European pharmacologists and physicians. His monograph on Nivalin® was translated and published in Italy. Together with prof. D. Peichev he wrote a textbook in pharmacology for medical students, which underwent several editions. After his retirement

in 1977 he was the head of the research group at the Scientific Chemical Pharmaceutical Institute in Sofia.

THE PHENOMENON FUNCTIONAL BRAIN ASYMMETRY

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Summary
From historical perspective the problem "left brain"/"right brain" is reiterating every time new tools for studying and handling brain activity have been introduced in medical practice. The seminal work of Roger W. Sperry in the 1960s on patients who had undergone split-brain surgery, revealed, after testing of each disconnected hemisphere, that the left is to be specialized for language and the right for emotional and nonverbal functions and won Roger W. Sperry the Nobel Prize for Physiology and Medicine in 1981. Recently, molecular and functional imaging techniques have emerged as powerful tools to revealing new evidences for lateralization of human cerebral function. The introduction of new "omics" techniques in medicine has deepend our understanding about genetic, epigenetic and neuronal mechanisms underlying the brain symmetry/asymmetry and some variations in brain asymmetry have been identified. Moreover, an attempt was made to explain congenital defects with differently expressed brain asymmetry variations. Targeted studies indicate that there is an association between cerebral lateralization and neuro-chemical asymmetry in particular brain structures. It is noteworthy that several similarities between the human data and those previously reported in rats have been recorded, suggesting studies in the rat may reveal mechanisms and functions of brain asymmetry that are relevant to man. Some physiological factors can influence brain asymmetry. PET data indicate that asymmetry in dopamine D2/3 receptors of caudate nucleus is lost with age. Furthermore, age-associated reduction of asymmetry in human central auditory function are documented by MRI and may at least in part underlie the speech perception difficulties/ presbycusis experienced in the elderly. In the last decade targeted studies have accumulated a strong body of evidence indicative for the role of hemispheric lateralization as pathophysiological factor for some neuro-psychiatric disorders and their responsiveness/non-responsiveness to medicine treatment. Yet, more intriguing issue is searching for association between brain asymmetry and susceptibility for developing adverse drug reactions to anxiolytics, antidepressants and antipsychotics.

CHRONOBIOLOGY, CHRONOPHARMACOLOGY AND CHRONOTHERAPEUTICS

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Summary
Chronobiology is a biomedical science devoted to the study of biological rhythms. Biological rhythms exist at any level of living organisms. Circadian (24-h) rhythms are the most commonly and widely studied biological rhythms. The circadian rhythms contribute to the known predictable-in-time patterns in the manifestations of diseases and responses of patients to diagnostic tests and medications. The influence of the time of day on drug efficacy and toxicity defines the chronopharmacological approach in dosing time for drug effects or recommending a best time for drug administration in populations with well-synchronized circadian rhythms. Thus rhythmicity in the pathophysiology of disease is used as a basis for chronotherapeutics - purposeful variation in time of the concentration of medicines in synchrony with biological rhythm determinants of disease activity to optimize treatment outcomes.
Key words: chronobiology, chronopharmacology, chronotherapeutics, circadian rhythms

STUDY ON PERCEPTION OF UNDERGRADUATE MEDICAL STUDENTS ON UTILIZATION OF FORENSIC TOXICOLOGICAL CASES IN THE PHARMACOLOGY CURRICULUM

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Summary
The amount of pharmacological information is instantly increasing challenging the education of medical students. There is no time and there are not enough resources to transmit the pharmacological knowledge as it was done in previous decades. The active involvement of students in resolution of real-life cases could be one attractive method to focus students' attention on safety use of drugs.
Aim of our study was to implement forensic toxicological cases in the pharmacology curriculum of undergraduated 3rd year medical students.
Forensic expertises, medical documentations and brainstorming together with forensic pathologists were used as an additional tool for discussing pharmacological data with voluntarily involved students. Likert-type scale was used in assessment of student's perception. Increasing interest especially in psychotropic drugs was remarked. Motivated students undertook their independent researches on methadone intoxication in children in Bulgaria, in overuse of psychotropic drugs in Institutions for neglected children in Bulgaria, in sudden death after neuroleptic uses, death due to Crohn disease with discussion on treatment modalities, endocannabinoide toxicity, Lyell syndrome, Clostridium difficile infections. For each of those topics small group of students (3-4) was assigned and a poster or oral presentations were prepared and discussed with the other students. The active involvement of students in forensic cases is an attractive way to bring the pharmacology education of undergraduated 3rd year medical students to the real life.
Key words: pharmacology education, forensic toxicology

WEB-BASED TRAINING IN PHARMACOLOGY

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Summary
The web-site www.medpharm-sofia.eu presents up-to-date, currently updated, easily accessible and free of charge information. It covers a wide variety of pharmacology-related fields and serves as a basis for web-based learning. Selected pharmacological and other medical information is added. The site comprises over 650 files and over 350 specially selected links, organized in 20 folders and 54 subfolders. The information is addressed to undergraduate students in master and bachelor programs, post graduates and doctoral students trained in Bulgarian and English at medical universities in Bulgaria. The learning materials are based on sources published in the country and abroad. They reflect and put together expertise based on 42-year teaching of medical pharmacology. The files are in doc and ppt format allowing for both reading and further developing the topics by the students. In addition to lectures, updated parts of textbooks and handbooks of pharmacology by lecturers from our University, there are presentations prepared by students. The site is visited by numerous users. Placement is allowed of new presentations, posters and scholarly essays prepared by students and teachers from the whole country, addressing specific pharmacological and/or pharmacotherapeutic issues. Contributors are expected to be creative and resourceful in using language and illustrations. One of the objectives set is to build intolerance to plagiarism and piracy in science, as well as aptitude for analyzing, summarizing and synthesizing. Another objective is of instructive and ethical nature: there are many mantras preceding lectures, as well as a special folder meant for reflection and rest.
Key words: web-based training, pharmacology

PROTECTIVE EFFECTS OF BENFOTIAMINE ON DIABETIC NEPHROPATHY IN STZ-INDUCED DIABETIC RATS

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Summary
Diabetic nephropathy is one of the most serious complications of diabetes. Hyperglycemia and proteinuria are among the main reasons causing structural abnormalities in a diabetic kidney. Benfotiamine, a lipid-soluble analogue of vitamin B1, is a potent anti-oxidant that is used as a supplement for the treatment of diabetic complications, but experimental data are limited.
The purpose of the present study was to examine whether benfotiamine affects nephropathy in diabetic rats.
Diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (STZ, 70 mg/kg) in male Wistar rats. At the beginning of the experimental period, rats were assigned randomly into four groups: healthy controls (Co); diabetics (D); rats receiving a daily dose of 100 mg/kg benfotiamine after the day they were injected with STZ; rats receiving 100 mg/kg benfotiamine per day starting 21 days after induction of diabetes.
Blood glucose and urinary protein excretion were determined. Histopathological and immuno-fluorescence analysis of eNOS in the renal cortex and medula was performed in the different experimental groups.
The rats (D) had well-developed signs of diabetes after 2 weeks of STZ administration: hyperglycaemia, glycosuria, increased water consumption, and body weight loss ($p<0.5$). Diabetic rats have significantly higher proteinuria compared with Co. Daily benfotiamine treatment appeared to protect the diabetic rats from massive body weight loss and reduced the protein excretion throughout the whole study. General morphology of glomerulus and tubulointerstitial diabetes related lesions were much improved after treatment with benfotiamine. The most expressed eNOS immunreactivity was detected in diabetic rats.
Our results demonstrate that benfotiamine treatment has a beneficial impact on diabetic nephropathy.
Key words: streptozotocin-induced diabetes, hyperglycaemia, proteiuria, eNOS
Acknowledgements. This investigation was supported by Medical University Sofia (Grant 20/2013).

EFFECTS OF GABAERGIC DRUGS ON HYPERTHERMIA IN EXPERIMENTAL SEROTONIN SYNDROME

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Summary
Serotonin syndrome is likely to be observed as a result of an overdose of serotoninergic drugs or interactions with the combined administration of two or more drugs that increase the intrasynaptic concentration of the serotonin (5-hydroxy-tryptamine). Aim of this study was to establish the effects of GABAergic drugs diazepam, sodium valproate, and vigabatrin on the hyperthermic reaction in experimental serotonin syndrome in rats.
The animal model of the serotonin syndrome was prepared by intraperitoneal administration of 5-hydroxy-L-tryptophan and clorgyline. Body temperature experiments were conducted at ambient temperature of 22±1°C. The body temperature of the animals was measured with thermistor probes (TX-8), and monitored on multichannel recorder Iso-Thermex 16. The thermistor probes were lubricated and inserted

rectally to a depth of 6 cm.
Pretreatment with diazepam (5 mg/kg i.p.), sodium valproate (300 mg/kg i.p.), and vigabatrin (300 mg/kg i.p.) decreased hyperthermia in an experimental model of the serotonin syndrome.
The results obtained in present study suggest involvement of interactions between GABAergic and serotoninergic systems in the processes of thermoregulation.
Key words: diazepam, sodium valproate, vigabatrin, serotonin syndrome, hyperthermia

LEPTIN AND GABA INTERACTIONS ON THERMOREGULATION OF RATS

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Summary
Leptin, the obese gene peptide, is involved in the regulation of feeding behavior and energy balance. In the last years the effects of leptin and its interaction with other neuromodulators or neurotransmitters is widely recognized and studied. The present study was set to determine the effects of leptin, $GABA_B$-agonist baclofen and $GABA_B$-antagonist CGP35348, applied separately or in combinations, on thermoregulation of male Wistar rats. The substances used for *in vivo* experiment were administered intraperitoneally (i.p.). The measurement of the body temperature was done via thermistor probes (TX8) and monitored on multichannel recorder Iso-Thermex16 (Columbus Instruments, USA). *In vitro* experiments were made on rat PO/AH neurones recorded extracellulary by conventional electrophysiological equipment, using brain slice preparations. The separate intraperitoneal injection of leptin as well as $GABA_B$-antagonist CGP35348 produced significant hyperthermia in rats while the $GABA_B$-agonist baclofen caused a decrease in the core body temperature. The probable synergy between the hyperthermic effects of leptin and $GABA_B$-antagonist was not occurred. In the opposite, effect of this combination was lower compared to the result of the separate administration. When leptin was applied just prior $GABA_B$-agonist baclofen neither one of their separate effects appeared. *In vivo* effects determined were in correlations with *in vitro* changes of firing rate and temperature sensitivity observed in PO/AH neurons. The data from this study provide a new point of view concerning the interactions of leptin and GABA. These results are step of understanding the complicate mechanisms involved in the realization of thermoregulation.
Key words: leptin, $GABA_B$-agonist and antagonist, firing rate, temperature sensitivity, PO/AH neurons

EFFECT OF TESTOSTERONE PROPIONATE ON LEUCO- AND THROMBOPOIESIS IN EXPERIMENTAL CONDITION

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Summary
The effect of testosterone propionate (TP) on leucopoiesis and thrombocytopoiesis is not well enough studied both in human and experimental conditions. The aim of this study was to study the dynamics in the levels of serum testosterone, leucocytes and platelets during replacement therapy with testosterone propionate in dose 4 and 8 mg/kg body mass (b.m.). Orchiectomized, sham operated and aged male rats were used, in a condition of acute and chronic treatment (15 days, 15 weeks). The levels of serum testosterone, leucocytes and platelets were prosecuted.
Orchiectomy significantly lowered serum testosterone in a 15 day experiment and not significant in the chronic. Supplementation with testosterone propionate significantly increased the level of testosterone at the higher dose. Stimulation of leucopoiesis was found after 15-

day administration of orchiectomized rats with androgen deficiency. Significant changes in platelet were observed only in acutely treated old rats in both doses tested ($p = 0.012$, $p = 0.002$).
TP stimulates leucopoiesis after a short treatment orchiectomized rats and transient increases platelet counts in aging old animals.
Key words: testosterone propionate, andropause, leucocytes, platelets, orchiectomy

EFFECT OF SOLAR SIMULATED UV RADIATION ON THE FREE RADICALS FORMATION IN HYPOTHYROID RAT LIVER

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Summary
Prolonged sun exposure (or solar simulated UV irradiation) leads to oxidative stress in many tissues, mainly via UV-induced depletion of the endogenous antioxidant defense and over-production of Reactive Oxygen Species. In hypothyroidism the data concerning oxidative stress in the literature are controversial. Therefore, the aim of our study was to investigate the influence of hypothyroidism and UV radiation on free radicals formation in rat liver. Hypothyroid model was developed in male Wistar-Albino rats (with average weight of approximately 135g) by continuous administration of 0.01% Propylthiouracil in drinking water for 5 weeks. Hypothyroid state was confirmed by significant reduction of free thyroxin at about 0.44 ng/l, compared with 18 ng/l in controls, respectively. The model of hypothyroidism was proved also by loss of both appetite and body weight of hypothyroid rats. During the 5-th week rats were irradiated with sunlight simulated UV (SSUV) lamp for 60 minutes, divided into 4 portions with respective breaks. The accumulation of free radicals was measured spectrophotometrically with MTT-formazan as a marker molecule. The results were presented as percentage of corresponding controls.
The present study indicated that separately, SSUV increased, while hypothyroidism decreased oxidative stress in liver tissue. In agreement with the literature, we found that free radicals in hypothyroid rat's livers were in about 3 times less that these in the control group. SSUV exposure of both norm thyroid and hypothyroid rats resulted in a relative increase of the free radicals in the livers, in comparison with the corresponding unexposed group. This effect was stronger in the hypothyroidism.
Key words: hypothyroidism, UV radiation, free radicals, liver

INTERACTION OF PROPYLTHIOURACIL WITH MODEL SYSTEMS GENERATING SUPEROXIDE RADICAL

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Summary
Propylthiouracil (PTU) is a drug against Grave's Disease. It is used for developing animal models of hypothyroidism too. The oxidative stress-induced tissue damages are among the adverse effects of PTU. The superoxide radical, O_2^-, is essential for the generation of Reactive Oxygen Species and other free radicals. The interaction of PTU with O_2^- was not well investigated.
In the present work, the free radicals formation in presence of PTU in model systems generating superoxide, were estimated. The O_2^- was generated by decomposition of KO_2, pyrogallol autooxidation, and transformation of Xanthine to uric acid over xanthine oxidase (XO). The free radicals formed were detected using luminol-dependent chemiluminescence, and spectrophotometric methods. The activity of XO was determined by estimating the formation of uric acid. In presence of KO_2 and pyrogallol

containing model systems, PTU did not interact with superoxide radicals. In the presence of the X/XO system, a PTU-concentration increase decreased the XO activity and simultaneously increased the free radicals formation. If PTU and XO were present in the solution, but xanthine was not introduced, again a PTU-concentration dependent formation of free radicals was detected, but significantly lower. The UV spectra of PTU in presence and in absence of XO revealed interaction of the PTU molecule with XO. It was proposed that, in the system X/XO some free radicals may be produced due to interaction of PTU with XO.

Key words: propylthiouracil, xanthine oxidase, superoxide radical, free radicals formation

ALTERATION OF NADPH-D ACTIVITY IN THE SKIN OF HYPOTHYROID AND SOLAR SIMULATED UV-IRRADIATED RATS

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Summary

Hypothyroid state and UV radiation are associated with alterations in NADPH-diaphorase (d) which is a marker for NO synthase (NOS) activity. In the literature, hypothyroidism shows heterogeneous tissue response and the data are controversial.

By using histochemistry, in the present study we investigated the effect of hypothyroidism and UV exposure on the NOS activity in skin elements. Hypothyroidism was induced by continuous administration of 0.01% Propyl-thiouracil in drinking water for 5 weeks. In the last seven days hypothyroid rats, as well as controls, were irradiated with solar simulated UV (SSUV) lamp for 60 minutes, divided into 4 portions with respective breaks. Hypothyroid status was confirmed by measuring of free thyroxin. Histochemical reaction was visualized as blue color over NADPH-d containing skin elements.

We found that hypothyroid state decreased NOS activity in dermal and hair follicle fibroblasts, and macrophages. Unlike it, UV radiation acted as a potent stimulator of NOS activity. The enzyme activity in the skin of hypothyroid rats after SSUV exposure also tended to be high, but significance was not reached compared to controls. Alterations in NO production may participate in skin manifestations of hypothyroidism.

Key words: hypothyroidism, skin, nitric oxide (NO), nitric oxide synthases (NOS), SSUV, NADPH-d

ALANYL-GLUTAMINE IS CAPABLE TO AMELIORATE THE ALTERED ANTIOXIDANT STATUS OF CRITICALLY ILL PATIENTS WITHOUT ANY DIRECT ANTIOXIDANT PROPERTIES

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Summary

Severe sepsis is a complex pathophysiological condition caused by excessive secretion of pro-inflammatory mediators and reactive oxygen species. Recent reports have indicated that the clinical use of medications enhancing the endogenous antioxidant defenses may improve the disease outcome.

In this investigation we studied the antioxidant

properties of Dipeptiven (N(2)-L-alanyl-L-glutamine) and determined its impact on the changes in plasma antioxidant capacity in patients with severe sepsis. We aimed to prove its potential beneficial effects on antioxidant status and clinical symptoms.
The study included twenty eight patients diagnosed with severe sepsis and admitted to the ICU at University Hospital "Alexandrovska"-Sofia. Apart from the laboratory tests and vital signs usually required for monitoring septic patients additional evaluation of the plasma antioxidant status has been done. The determination of the oxidative status was assessed by measuring their plasma antioxidant capacity using two methods - TBARS assay and ABTS test. The antioxidant properties of Dipeptiven have been tested in the same model systems and it didn't demonstrate antioxidant activity.
This indicates that Dipeptiven supplementation to the standard therapy couldn't ameliorate directly the antioxidant status. The obtained results indicated that the complex therapy has been shown to be more effective compared to standard treatment. In this group of patients we observed a strong positive correlation between plasma's total antioxidant status and disease's severity (especially in no survivors). This can be attributed to the impact of Dipeptiven on the initiation and promotion of the complexes multiple stage oxidative processes in critically ill patients.
Key words: oxidative stress, antioxidant capacity, critically ill patients, alanyl-glutamine

ADVANCED GLYCATION END-PRODUCTS: PHARMACOLOGICAL CONTROL

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Summary
Advanced glycation end-products (AGEs) are a heterogeneous group of molecules formed from the nonenzymatic reaction of aldose sugars with free amino groups of proteins for an extended period of time. A key characteristic of AGEs is their ability for crosslink formation between proteins, which alters their structure and function - in cellular matrix, basement membranes, and vessel-wall components. Some of the effects of the AGEs are mediated by specific cell-surface AGE-binding receptors(RAGE).The RAGE-AGE interaction activates multiple intracellular signaling pathways which increase the production of growth factors, inflammatory cytokines, and oxidative stress. AGEs play pivotal role in diabetic vascular complications, ageing, chronic kidney disease, neurodegenerative diseases.
The aim of present work is to review the existing data on AGEs pharmacological control.
Drug anti-AGEs therapy involves: 1) Good glycaemic control; 2) Oral adsorbents of diet-derived AGEs; 3) Inhibitors of AGEs formation – carbonyl-trapping agents, metal chelators, antioxidants (vitamins C,E, pyridoxamine); 4) Cross-link breakers – prevent or reverse collagen cross-linking; 5) RAGE antagonists – anti-RAGE antibodies, inhibitors of RAGE-induced signaling; 6) Existing drugs with anti-AGEs effects – inhibitors of renin-angiotensin system, statins, oral hypoglycaemics (metformin, pioglitazone), aldose reductase inhibitors, anti-inflammatory drugs. A variety of drugs have been developed to target the AGE-RAGE axis and its pathological effects. Anti-AGEs strategies in clinical and experimental medicine remain a very challenging research field.
Key words: AGEs, RAGE, AGEs inhibitors, cross-link breakers

PHARMACOKINETICS OF ZINC IN BROILER CHICKENS AFTER SINGLE INTRAINGLUVIAL ADMINISTRATION WITH ZINC ASPARTATE

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Summary
The pharmacokinetics of zinc was investigated in broiler chickens after single crop intubation of 50 mg/kg 5% zinc aspartate suspension in 2% carboxymethyl cellulose solution.
Blood serum zinc concentrations were assayed on a biochemical analyzer. The pharmacokinetics of zinc was done using two pharmacokinetic approaches – compartmental method and non-compartmental analysis using pharmacokinetic software (TopFit, v. 2.0.).
After the intraingluvial application, zinc was rapidly absorbed ($t_{1/2abs.}$=0.104±0.02 h) by the alimentary system of birds attaining C_{max} of 63.60±3.94 mol/ml by hour 0.77 (compartmental method) and C_{max}=69.27±4.35 mol/ml by hour 0.92h (non-compartmental method). It is characterized with a long biological half-life ($t_{1/2}$) of 13.82±1.63h (compartmental analysis) and 15.96±1.73h (non-compartmental analysis) and long mean residence times (MRT) 20.12±2.35h and 23.00±2.50h, respectively. The distribution in blood and extracellular fluid was good as seen from $Vd_{(area)}$values – 0.77±0.05 l/kg (compartmental analysis) and 0.65±0.05 l/kg (non-compartmental analysis).
Key words: pharmacokinetics, chickens, zinc aspartate

SIGNIFICANCE OF HACCP PLAN FOR FOOD STUFFS AND FOOD SUPPLEMENTS SAFETY

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Summary
The free market and the quickly changing social and economic circumstances lead to poor control of the foodstuffs' quality and safety. HACCP is a part of the systems for foodstuff's safety management.
The main task of the HACCP system in the food industry is to implement requirements for the manufacture of a safe product meeting the specified qualitative indexes – organoleptic, physicochemical and microbiological. This system follows up, controls and prevents the probabilities during the manufacture various biological, chemical and physical risks to appear.
Ever heavier problem in the present day is the production and consumption of genetically manipulated foods and organisms. Over 50 countries, including all EU member-states, as well as most of the countries in Asia require labeling of the genetically engineered foods.
Important part of the HACCP system is the presentation of objective and full information about the foodstuff on the label, which is improving the consumers' awareness. Thus, the consumers are able to make a conscious and free choice of foods and to lead healthy way of life.
Time and the world experience prove the efficiency of HACCP system and, therefore, it is internationally recognized as the most reliable system of guaranteeing the safety of the foodstuffs.
Key words: Hazard analysis and critical control point (HACCP system), safety foodstuff, genetically manipulated foods and organisms

OXIDATIVE STRESS MARKERS AND HEME-OXIGENASE-1 IN FATTY LIVER, INDUCED BY DIET HIGH IN FRUCTOSE

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Summary
The dramatic rise in prevalence of fatty liver disease, obesity and diabetes - features of metabolic syndrome in developed countries is associated with overconsumption of dietary fructose. The pathophysiological mechanisms associated with development of fatty liver disease appear to involve multiple cellular adaptations to the oxidative stress occurring when fatty acid metabolism is altered. We explored the expression of heme-oxigenase-1 (HO-1) and its relationship with oxidative stress biomarkers in rat models of diet-inducible fatty liver and effect of allopurinol.

Fatty liver was triggered in male rats with high fructose diet (HFD) (35% fructose in drinking water for 16 week) HFD +Allopurinol (150 g/ml in drinking water for 16 week) group and control group. HO-1expression, triglycerides (TG), uric acid (UA), malondialdehyde (MDA), glutathione (GSH) levels and histological studies were assayed in liver.

HFD rat featured microvesicular steatosis. In HFD+All group microvesicular steatosis was not found. In HFD rats the expression of HO-1 was significantly increased ($p<0.01$) but GSH levels was decreased. Allopurinol augmented the increase of hepatic HO-1 expression ($p<0.01$). MDA, UA and TG levels were elevated in HFD ($p<0.05$). Allopurinol prevented the decrease in GSH ($p<0.05$) and inhibited the elevation in MDA, TG and UA levels.

Fructose diet upregulates HO-1 expression, which correlates with the increased indicators of oxidative stress. Allopurinol shows hepato-protective effect and its protection likely exerted by increased expression antioxidant enzyme HO-1 to restrict the development of fatty liver.

Key words: fatty liver, heme-oxigenase-1, oxidative stress, allopurinol

THE POTENTIAL OF MELATONIN IN ORGAN PROTECTION IN THERMAL TRAUMA

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Summary
Melatonin is a versatile molecule, synthesized not only in the pineal gland, but also in many different organs. Melatonin plays an important physiologic role in sleep and circadian rhythm regulation, immunoregulation, antioxidant and mitochondrial-protective functions, reproductive control, and regulation of mood. Melatonin has also been reported as effective in combating various extremal conditions. In animal model and burn patients melatonin reportedly exerts beneficial effects to restrict damage of gastric mucosa and liver after experimental thermal injury. Melatonin exerts protection against oxidative stress and ameliorates oxidative/nitrosative damage by a variety of mechanisms. Some actions of melatonin as a potential supportive pharmacological agent in burn patients include its: role as a stimulation of the activities of a variety of antioxidative enzymes, prevention of apoptosis, elevation of anti-inflammatory cytokines reduction in pro-inflammatory cytokines, adhesion molecules, endothelial dysfunction and microcirculatory disorders. Melatonin can modulate inflammation by decreasing nuclear factor-kappa B (NF-κB) signalling and activation of antioxidant enzymes by increasing nuclear erythroid 2-related factor 2 (Nrf2) expression that might be responsible for its organ protection. These combined actions of melatonin, along with its low toxicity and its ability to penetrate all morphophysiologic membranes, could make it a ubiquitously acting and highly beneficial molecule in burn patients.

Key words: melatonin, inflammation, oxidative stress, thermal trauma, organ protection

EXPRESSION OF HEME OXYGENASE-1 AND OXIDATIVE STRESS IN BRAIN AND LUNGS AT HYPERBARIC HYPEROXIA IN RABBITS

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Summary
Hyperbaric hyperoxia (HHO) is a possible complication of hyperbaric oxygen therapy (HBOT)- medical use of oxygen at a level higher than atmospheric pressure. HBOT found use in the treatment of different diseases and conditions. Prolonged exposure to above-normal oxygen partial pressures, or shorter exposures to very high partial pressures, can cause oxygen toxicity and lead to oxidative damage. Purpose of the study was to examine the expression of heme oxygenase-1 (HO-1) and malondialdehyde (MDA) (oxygen stress marker) in animals placed at an hyperbaric hyperoxia.

The study was conducted with two groups of New Zealand rabbits. The first group of the animals was subjected to HHO. The exposure was for 35 minutes at 3,7 ata of pure oxygen in a hyperbaric chamber. The second (sham) group is standing in the chamber for 35 min in normal air. After the chamber exposure, were taken tissues from brain and lungs for studing the expression of HO-1 and levels of MDA. The obtained results were processed by variance analysis.

There is statistically reliable variance of immune histochemical expression of heme oxygenase (HO-1), which was elevated in the animals subjected to HHO. The levels of MDA points changes in the studied organs of the animals.

The results received during the examination point to up regulated changes of HO-1 and oxidative damages of studied organs in HHO and are good basis for further studies.

Key words: hyperbaric hyperoxia, heme oxygenase-1, oxidative stress

ASCENDING AND DESCENDING COLONIC MOTOR ACTIVITY – COORDINATION AND EFFICACY OF LONGITUDINAL AND CIRCULAR MUSCLES IN A RAT MODEL

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Summary
Partitioned three-compartment organ bath, electrical field stimulation (EFS 0.8 ms, 40 V, 2-5-10 Hz, 20 s) and mechanographic on-line recording techniques were used to evaluate spontaneous and electrically-elicited local, ascending and descending reflex motor responses of longitudinal and circular muscles in a rat colonic segments. The electrically-elicited local motor responses of longitudinal or circular muscle didn't differ by pattern in the proximal or the distal part of the colonic segments suggesting similarity of local nerve networks serving each one of both muscles in the proximal and distal rat colon. Electrical stimulation could co-activate excitatory reflex pathways serving coordinated ascending contractile motor responses of both muscles and descending reflex pathways underlying opposite motor activity, contraction in the longitudinal and relaxation in the circular muscle. The spontaneous and electrically-induced contractility of the longitudinal muscle and the contractile ascending responses of the proximal colon were more expressed suggesting higher contractile efficacy of the longitudinal muscle and ascending reflex-mediated contractile activity in the coordinated propulsive motility of colon.

Key words: ascending motor response, descending motor responses, local motor response, circular muscle, longitudinal muscle, rat colon

EFFECT OF SELENIUM SUPPLEMENTATION ON THE REMODELING OF THE VESSEL WALLS IN ADULT SPONTANEOUSLY HYPERTENSIVE RATS

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Summary
The aim of this study was to investigate the influence of selenium (Se) supplementation on the remodeling of vessel walls with age in spontaneously hypertensive rats (SHR).
We used 37 male SHR, divided into 4 groups depending on the period of applied diet with varying Se content: from 6 to 8 months of age (2 groups) and from 10 to 12 months of age (2 groups). The diets contain adequate Se (0.11 μg Se /g of food) and Se supplementation (0.25 μg Se /g of food).
The Se nutritional status was assessed by measuring Glutathione peroxidase-1 (GPx-1) activity in whole blood, using "Ransel" kit of "Randox Laboratories LTD". We determined antielastin antibodies (AE abs) serum level using method of indirect ELISA. The changes of morphology and thickness of thoracic and abdominal aorta, and coronary artery was established under a light microscope.
The results showed increased GPx-1 activity in whole blood in group 8 months old rats with Se supplementation (p=0,021), lower serum level of AE abs (p=0.0013) and lower thickness of left coronary artery (p=0.005) as compared to rats receiving adequate Se diet. Se supplementation performed from 10 to 12 months could not increase significantly GPx-1 activity in whole blood and could not affect the severe alterations of the vessel walls and elastin destruction in SHR. In conclusion, selenium supplementation in SHR used before the age of 8 months can slow down spreading of degenerative changes of vessel walls along arterial tree.
Key words: selenium, SHR, vessel walls, anti-elastin antibodies

ANTIHYPERTENSIVE EFFECT OF CORMAGNESIN AND ITS COMBINATION WITH FUROSEMIDE IN CONSCIOUS SHR

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Summary
Aim: Study of the antihypertensive effect of Cormagnesin (magnesium sulfas) and its combination with Furosemide on conscious spontaneously hypertensive rats (SHR) after intravenous infusion.
Experiments were carried out on 5 groups of conscious male SHR (n=6) employing procedures approved by the local ethic committee. Under short anesthesia rats were chronically instrumented for blood pressure measurement and intravenous (i.v.) drug administration. The arterial blood pressure (AP) was measured through the indirect tale method. Systolic (SAP), diastolic (DAP) AP and heart rate (HR) were determined 30, 60 and 120 minutes after i.v. infusion.
Groups and treatments: 1st – control group: 0,9 % NaCl; 2nd – Cormagnesin 20 mg/kg; 3rd – Cormagnesin 40 mg/kg; 4th – Furosemide 10 mg/kg; 5th – Cormagnesin 20 mg/kg and Furosemide 10 mg/kg. Cormagnesin and NaCl were applied as 30-minutes i.v. infusion – 0,5 ml/100 g; Furosemide was injected intraperitoneally (i.p.).
After i.v. infusion of 20 mg/kg Cormagnesin we obtained significant decrease of the AP. There

was not significant hypotensive effect after the i.v. infusion of 40 mg/kg Cormagnesin. The hypotensive effect of Furosemide was not significant but the decrease of the DAP was more pronounced. Between the antihypertensive effects of Cormagnesin and its combination with Furosemide there was no significant difference but the effect of the combination was prolonged.
Our results suggest that the antihypertensive effect of Cormagnesin in the applied doses is not dose-dependent. The concomitant administration of Furosemide prolongs but does not potentiate the antihypertensive effect of Cormagnesin in SHR.

Key words: magnesium sulfas, antihypertensive effect, SHR.
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ENDOTHELIN AND UROTENSIN II SYSTEMS - POSSIBILITIES FOR PHARMACOLOGICAL IMPACT IN ARTERIAL HYPERTENSION

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Summary
The endothelin system includes a group of endogenous peptides that have a strong and prolonged vasoconstrictor effect. So far identified three isoforms have been identified: ET-1, ET-2 and ET-3. The effects of endothelins are mediated by interaction with three types endothelin receptors: ETA, ETB (ETB1 and ETB2), and ETC. Most important of the three peptides is endothelin-1 (ET-1). Urotensin II (UII) is a peptide originally isolated from goby fish. UII is bound to a class of G protein-coupled receptor known as GPR14 or the urotensin receptor (UT). UII is the most potent endogenous vasoconstrictor discovered to date. It possesses physiological effects similar to those of ET-1. A number of mechanisms through which ET-1 and UII participate in the pathogenesis of arterial hypertension are discussed in the review. Possibilities for therapeutics impacts on both systems for treatment of arterial hypertension are also examined. Effects of ET-1 and UII receptor antagonists in patients with hypertension are subject to in-depth research area. Antagonists of ET-1 and UII can represent an alternative approach for treatment of arterial hypertension and other cardiovascular diseases in the near future.

Key words: endothelin-1 , urotensin II, arterial hypertension

RENIN-ANGIOTENSIN SYSTEM INHIBITORS AND OSTEOPOROSIS

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Summary
Recant evidences suggest that local bone renin-angiotensin system (RAS) can be involved in metabolic bone disorders such as osteoporosis. The conditions related to activation of the RAS as hypertension, chronic heart failure (CHF), diabetes mellitus are frequently associated with osteoporosis. Patients treated with angiotensun-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARB) show an increased bone mineral density (BMD) and reduced fracture risk.
This review evaluates the role of the RAS in pathogenesis of osteoporosis and the effect of its pharmacological blockade in prevention and treatment of osteoporosis.
The component of of the RAS – renin, ACE, angiotensin-II (AT-II) receptors are expressed in the skeleton. Receptors for AT-II are localized in both osteoblasts and osteoclasts. AT-II

significantly induces the expression of receptor activator of NF-kB ligand (RANKL) in osteoblasts, a marker for osteoclastic activation. It is hypothesized that AT-II alters the ratio of core-binding factor alpha (Cbfa)/RANKL expression subsequently leading to the increase of osteoclast number and causes the imbalance between bone formation and bone absorption. Experimental, clinical and epidemiological studies demonstrate that these effects are completely blocked or attenuated by ACE inhibitors and ARB.
The bone RAS might be a local regulating system for bone metabolism. Pharmacological blockade of the RAS may be of benefit for management of osteoporosis.
Key words: RAS, osteoporosis, ACE inhibitors, ARB, RANKL

EFFECTS OF ENDOGENOUS CARBON MONOXIDE ON THE CONTRACTILE ACTIVITY OF SEGMENTS FROM GUINEA PIG *A. BASILARIS*

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Summary
Basilar artery is one of the arteries that supplies the brain with oxygen-rich blood. Carbon monoxide (CO) is known to increase cerebral blood flow, but the effect of CO on the vascular tone of large cerebral arteries is uncertain. Arterial segments with a length of 1.8 - 2 mm were mounted and tested on wire miograf (model 410A, JP Trading, Denmark). The substrate of HO - hemin, applied on the background of pretreatment with Vit E, cause the contractile response in both intact preparations and in those with the endothelium removed. In low concentrations, hemin has no significant effect on the studied preparations. In a concentrations of 100 nM - 10 μM hemin induce lower tone, best expressed in the highest concentration applied. Unlike the single treatment with hemin, the cumulative loading of vessels as well as the control and the treated with $CoCl_2$ animals, in concentrations from 1nM - 10 μM proved counterproductive. The results indicate the opposite effect of endogenous carbon monoxide as compared to previously known. In a. basilaris there is an influence of the endothelium, especially in conditions of oxidative stress. Probably under the influence of endogenous carbon monoxide it produces substances modulating vascular tone. It is possible an interaction with NOS-system and modulation the release of NO from the vascular endothelium.
Key words: a. basilaris, carbon monoxide, hemin

ASCENDING REFLEX MOTOR ACTIVITY OF COLONIC LONGITUDINAL MUSCLE IN A RAT MODEL

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Summary
The specific role of the longitudinal muscle in reflex motor activity of rat colon and involvement of excitatory and inhibitory neurotransmitters in its implementation are not fully understood.
In the present study, we examined *in vitro* the electrically-induced motor activity of rat colonic longitudinal muscle based on the local neuronal circuitry and the ascending reflex pathways, using partitioned organ bath. To clarify the role of excitatory and inhibitory neurotransmissions in the reflex pathways, the motor activity was pharmacologically analyzed by cholinergic-, tachykininergic- and nitrergic-related drugs. Atropine (0.3 μM) added in the oral compartment of the bath considerably decreased the ascending contractions of the longitudinal muscle from the proximal part of colonic segments elicited by electrical stimulation applied at a frequency of 5 Hz (5.4±0.6 mN, n=10, $p<0.05$ vs. control ascending responses). During atropine treatment, spantide (0.1 μM) further significantly suppressed the ascending contractile motor responses (3.2±0.3 mN, n=8, $p<0.05$). In the

presence of atropine, L-NNA (0.5 mM) restored to a great extent the ascending contractions of the longitudinal muscle while the later contractions were strongly depressed after the addition of L-Arginine (0.5 mM).
Atropine inhibited the electrically-induced ascending contractile responses of the longitudinal muscle of colonic segments indicating the essential stimulatory role of the cholinergic system in the colonic contractility. The present experiments demonstrated the involvement of cholinergic, tachykininergic and nitrergic neurotransmission in the ascending motor responses of the colonic longitudinal muscle.
Key words: atropine, L-arginine, NG-nitro-L-arginine, rat colon, reflex motor activity, spantide

CEREBRAL DOMINANCE AND PSYCHOPATHOLOGY

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Summary
In most respects, the left and right sides of the brain are asymmetrical in terms of function. Many researchers have looked for asymmetries that might "index" cerebral dominance (CD). If an easily observable asymmetries were diagnostic of brain laterality, then cerebral dominance could be studied in "normal" samples and that could help diagnose mental illness. Some brain areas show strong lateralization, particularly areas that are involved in language and spatial cognition. Brain function lateralization is also evident in the phenomena of right- or left-handedness. The most studied asymmetries related to mental illnesses are hand preference and language lateralization and findings support the hypothesis of decreased lateralization being a vulnerability model for schizophrenia-spectrum features. The review presents the mechanisms by which the disturbance of normal lateralization could lead to mental illness. It comments on possible origin of psychosis and its connection with language lateralization and hand preference. The association between decreased cerebral dominance and psychosis is not restricted to schizophrenia, but has also been shown in patients with unipolar and bipolar affective psychosis and subjects with high genetic risk for psychosis and schizotypy.
Key words: cerebral dominance, language lateralization, hand preference, schizophrenia

DEPARTMENT OF PHARMACOLOGY IN THE 40-YEAR HISTORY OF MEDICAL UNIVERSITY – PLEVEN

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Summary
The department of pharmacology was founded in June 1976. The first head of the department was Assoc. Prof. Tzvetan Boyadziev. Since 1989, Assoc. Prof. Russi Marev has been head of the department. In 1995, after teaching Clinical Pharmacology was introduced, the department was renamed to "Experimental and Clinical Pharmacology". The main activity is teaching pharmacology and clinical pharmacology to medical students, nurses, midwifes, lab technicians, and PhD students. Today, the department provides training in these disciplines in both Bulgarian and English. Over the years, the teaching staff have participated in writing many textbooks and handbooks of pharmacology. The research activity of the staff in different fields of experimental and clinical pharmacology (drugs and physical activity, medical education, adverse drug reactions, pharmacogenetics, drug-induced lung toxicity, motor activity in colo-recto-anal region) has resulted in three PhD theses, and many publications in Bulgarian and foreign journals and participations in scientific events.
Key words: pharmacology, clinical pharmacology, department of pharmacology

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Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med.

2002;347(4):284-7.
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Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12];102(6):[about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

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ADDITIONALLY SUBMITTED ABSTRACTS

ZN/AU AND ZN/AG COMPLEXES WITH PROMISING ANTITUMOR ACTIVITY IN VITRO

Tanya D. Zhivkova[1], Abdulkadir M. Abudalleh[1], Lora V. Dyakova[2], Pavel Mitrenga[1,2], Milena Georgieva[3], George A. Miloshev[3], Daniela-Cristina Culita[4], Gabriela Marinescu[4], Luminita Patron[4], Radostina I. Alexanderova[1]

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Summary
The aim of the present study was to evaluate the in vitro antitumor activity of 11 complexes of Zn, Zn/Ag and Zn/Au with Salen or derivatives of 2,6-diformyl-p-cresol (ampy, aepy, dmen).
Permanent cell lines established from cancers with various origin (human, rat, chicken), hystologic type, etiology and drug sensitivity as well as non-tumor cell cultures were used as model systems in our investigations. Short-term (24-72h, with monolayer cultures) and long-term experiments (16-18 days, with 3D colonies) were performed by methods with different cellular/molecular targets and mechanism(s) of action such as MTT test, neutral red uptake cytotoxicity assay, crystal violet staining, trypan blue dye exclusion technique, double staining with acridine orange and propidium iodide, Comet assay, FACS, colony-forming method. Applied at concentrations of 0.05-200 μg/ml the compounds investigated inhibit proliferation and induce apoptosis in the treated cells in a time-and concentration-dependent manner. Zn-dmen-Au, Zn-ampy-Au, Zn-aepy-Ag and Zn-Salen-Au have been found to be the most promising cytotoxic and cytostatic agents – their activity is similar to or even better than those of the clinically used anticancer agents cisplatina, 5-fluorouracil an daunorubicin.
The examined compounds revealed promising anticancer potential. Additional investigations are required to clarify better their mechanism(s) of action and biological safety.
Key words: Metal complexes, tumor cell lines, cytotoxic/cytostatic activity, antitumor agents

NEUROINFLAMMATION - THE BASIS FOR A NEW THERAPEUTIC APPROACH IN EPILEPSY

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Summary
Recent findings in experimental models and in the clinical setting highlight the possibility that inflammatory processes in the brain contribute to the etiopathogenesis of seizures and to the establishmen of a chronic epileptic focus. Despite progress in pharmacological and surgical treatments of epilepsy, relatively little is known about the processes leading to the generation of individual seizures, and about the mechanisms whereby a healthy brain is rendered epileptic. Clinical and experimental studies have provided proof-of-concept evidence that brain inflammation is an important factor in epilepsy. In particular, high levels of proinflammatory cytokines such as IL-1 β, TNF-α and IL-6 have been shown to be overexpressed in experimental models of seizures in brain areas of seizure generation and propagation, prominently by glia and to a lesser extent by neurons. Cytokines have been shown to profoundly affect seizures in rodents; in particular, IL-1 β is endowed of proconvulsant activity in a large variety of

seizure models. Key mediators of this process of neuroinflammation include interleukin-1β, high-mobility group box protein 1 (HMGB1), and Toll-like receptor (TLR) signaling

In our work, we focus on the rapidly growing body of evidence that supports the involvement of inflammatory mediators. in both the origin of individual seizures and the epileptogenic process. We first describe aspects of brain inflammation and immunity. Subsequently, we discuss how seizures cause inflammation, and whether such inflammation, in turn, influences the occurrence and severity of seizures, and seizure-related neuronal death. Further investigations into the role of cytokines, and more broadly of inflammatory mediators, in epileptogenic tissue may add important insights into the mechanisms of ictogenesis and epileptogenesis. These findings should yield new molecular targets for the design of antiepileptic drugs, which might not only inhibit the symptoms of this disorder, but also prevent or abrogate disease pathogenesis.

Key words: anticonvulsant drugs, brain inflammation, epileptogenesis, microglia, TNF-alpha, seizures

COMPARATIVE STUDY ON NEONATAL EFFECTS OF LEVETIRACETAM, VALPROIC ACID AND DIAZEPAM ON BEHAVIORAL CHANGES AND BRAIN CYTOKINES IN NEONATAL KAINAT MODEL OF EPILEPSY

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Summary

Experimental evidence and clinical observations indicate that brain inflammation is an important factor in epilepsy. Inflammatory cytokines such as TNF-alfa have been shown to be overexpressed in experimental models of epilepsy. Data exists that cytokines profoundly affect seizures in rodents.

Present study aimed to compare the effect of levetiracetam (LEV), valproic acid (VPA) and diazepam (DZ) on the behavioral changes and level of hippocampal TNF-alfa and IL-1 beta in kainic acid-induced model of epilepsy in pups. Pups were divided into 5 groups of 5 animals each. In the first group, animals were given saline (i.p.) to serve as negative control. In the second group rats were injected i.p. with kainic acid (KA; 10mg/kg) to serve as positive control. In the other 3 groups LEV, VPA, and DZ were administered once daily between 10:00 and 11:00 a.m. for 3 successive days (LEV - 50 mg/kg, i.p., VPA - 50 mg/kg, i.p., DZ - 5 mg/kg, i.p). On the third day, 2h after the last LEV, VPA and DZ injections, kainic acid was applied i.p. (10 mg/kg). Following the last administration all animals were observed during 3h for assessment of convulsive behavior according to the rating scale of Racin. To measure the levels of cytokines (TNF-alfa and IL-1 beta) all rats were sacrificed 6 hours after the last injection they received. The levels of cytokines were determined by rat-specific sandwich-enzyme-linked immunosorbent aasay (ELISA).

No behavioral changes were observed in the first group. In the second group, rats treated with KA showed well pronounced seizure syndrome. In the other 3 groups there were differences in the behavioral responses, but the seizures were less severe than KA group. The average symptoms rating in KA group reached a value of 5 in Racin's scale. DZ group reached value of 1-2. VPA group reached value of 2. LEV group showed value of 3. Levels of TNF-alfa and IL-1 beta were significantly raised in rats treated with KA, as compared with the 3 other studied groups. Levels of TNF-alfa and IL-1 beta in LEV, VPA and DZ-groups were lower than group KA. The lowest level of cytokines was recorded in LEV group. Despite of lower cytokines levels, pups from these 3 groups also exhibited epileptiformic activity, but less severe than group KA.

Our data indicated that KA has a capacity to induce the levels of cytokines in early stages development of the model of epilepsy. LEV and VPA suppressed cytokines level in pup's hippocampus and this could be involved in their potentially antiseizure effects. These data suggested that LEV may inhibit seizures by decreasing pro-inflammatory cytokines in hippocampus and possessed neuroprotective and antiepileptogenic properties. Taken together these results and the lack of neurotoxicity, these data support ongoing interest in administration of

LEV for neonatal seizures. Working forward from animal models, and simultaneously backward from patients on the basis of successful intervention, could provide us with the best understanding of those epilepsies in which inflammatory mechanisms are most critical.

Key words: behavioral changes, epilepsy, kainic acid, seizures, TNF-alfa

KETOGENIC DIET: METABOLIC INFLUENCE ON BRAIN EXCITABILITY AND EPILEPSY

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Summary

Although the ketogenic diet (KD) has been widely accepted as a legitimate and successful therapy for epilepsy and other neurological disorders, its mechanism of action remains an enigma.Therapeutic options for management of medically refractory seizures in young children are limited. Despite the plethora of old and new anticonvulsant medications, up to one-third of children with epilepsy continue to have seizures. A link between metabolism and brain function is clear. Since ancient times,epileptic seizures were treated with fasting, and the therapeutic benefits of fasting on epilepsy were confirmed nearly100 years ago. Alternative treatments have included the vagus nerve stimulator and dietary approaches. The ketogenic diet, a high fat, low carbohydrate, adequate protein formulation, has been used since early in the twentieth century for seizure control in refractory epilepsy. Clinical studies have verified the effectiveness of the KD. In general, at least half of all patients treated with the KD will exhibit a 50% or greater reduction in seizure frequency. Despite this effectiveness, the mechanisms by which the diet works are still unknown.

In this work we described mechanisms of action of ketogenic diet. We summarized key insights published from experimental and clinical studies of KD, and focused particular attention on the role that ketone bodies, fatty acids, and limited glucose may play in seizure control. We described on-going work in these areas that is providing better understanding of metabolic influences on brain excitability and epilepsy. Metabolic changes likely related to the KD's action anticonvulsant properties include ketosis, reduced glucose and enhanced bioenergetics' reserves. Recent evidences indicate that several mechanisms may exist for ketogenic diet including disruption of glutamatergic synaptic transmission, inhibition of glycolysis, and activation of ATP-sensitive potassium channels. The fact that a fundamental modification in diet can have such profound, therapeutic effects on neurological disease underlines the importance of elucidating mechanisms of KD diet. Future studies will provide unique insights into how diet can affect the brain, both in health and disease, and likely provide the scientific basis for the development of potent new treatment strategies for the epilepsies.

Key words: animal models, epilepsy, metabolism, ketogenic diet, seizures.

EFFECT OF ORLISTAT ON GHRELIN LEVELS IN OBESE RATS

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Summary

Orlistat (Xenical®) is the only antiobesity drug on the pharmaceutical market in Europe. Orlistat is a lipase inhibitor, well-tolerated and has a safety profile. Ghrelin is a hormone secreted by the endocrine cells of the stomach and has strong orexogenic potency. Our hypothesis was that orlistat could alter the secretion of ghrelin from the stomach. Thus, the aim of our study was to investigate the effect of orlistat on ghrelin levels in the blood in obese rats. Wistar male rats (n=30) were used in the study. The rats started a 2-month diet-induced obesity period. They were fed with a chow diet (control group) and a high-fat diet plus chow food (experimental group). After this

period rats were randomized into three groups: 1 group – on a chow diet (control group), 2 group – on a high-fat diet, 3 group – on a high-fat diet plus orlistat (5 mg/kg/daily). The drug treatment lasted for one month. Then, rats were anaesthetized and sacrificed. Blood was taken for measurement of lipids and glucose in the blood, and for analyses of plasma ghrelin levels (fmol/ml) by ELISA method (LincoResearch, USA).

There was a significant increase in body weight in the high-fat group compared to the control group ($p<0.001$). Further, obese rats showed a significant increase in blood glucose ($p<0.001$), and an increase in triglycerides ($p=0.2$). The group treated with orlistat demonstrated a decrease in body weight, glucose and lipid levels in the blood. More interestingly, this group showed a significant decrease in plasma ghrelin levels ($p<0.01$) in comparison to the high-fat group not treated with the drug. Orlistat could have an inhibitory effect on ghrelin secretion. Further studies are needed to elucidate this novel finding.

Key words: diet-induced obesity, ghrelin, orlistat

PREVIEW PROJECT FOR PREVENTION OF TYPE 2 DIABETES IN OVERWEIGHT AND OBESE PATIENTS IN EUROPE AND AROUND THE WORLD

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Summary

Obesity prevalence has increased dramatically over the past decades in children and adolescents across the world. Prediabetes is a very common condition among obese adults. Little is known about the effect of diet and physical activity on glucose levels in subjects with prediabetes and obesity. Thereafter, the aim of the PREVIEW project is to determine the preventive impact of a diet (protein and glycemic index) and physical activity (moderate vs. high) on the incidence of type 2 diabetes (T2D) in a population at risk.

A total of 2 500 overweight and obese participants (from Denmark, Finland, Netherlands, UK, Spain, Bulgaria, Australia, and New Zealand) will be recruited. The age range is from 10 to 70 years. All adult participants are first treated by a low-calorie diet (LCD) for 8 weeks, with an aim to reach $\geqslant 8\%$ weight reduction. The eligible participants are then randomized into two different diet interventions and two exercise interventions for a total of 148 weeks. This period aims at preventing T2D by weight maintenance and by independent metabolic effects of diet and physical activity. The two intervention diets differ by protein content (high vs. moderate) and glycemic index (low vs. moderate). The participants are weighted and supervised by groups during the whole 3-year period of the project. At different time intervals blood and urine samples are taken for further analyses.

The main primary and secondary endpoints of the study are the incidence of T2D during 3 years according to diet and physical activity based on oral glucose tolerant test and $HbA1_c$. In addition, endpoints are changes in body weight, waist circumference, body fat mass, etc.

Key words: obesity, prediabetes, low-calorie diet

EFFECTS OF TOPIRAMATE ON FREE RADICALS IN OBESE RATS

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Summary

Topiramate is a well-known antiepileptic drug. Recent data show that topiramate leads to weight reduction in subjects treated with low dosage of the drug. The aim of our study was to determine the influence of topiramate on weight and

metabolism in female obese rats, and furthermore to investigate its action on the plasma free radicals. Wistar female rats were used in the study. Rats were randomly assigned to either control diet (chow food) or an experimental diet (high-fat diet) to induce experimental obesity. The experimental model of obesity was created and published by the authors. The nutritional period lasted for one month.Then, rats were divided again into two groups - control and experimental (obese treated with Topiramate - 50mg/kg p.o.) Topiramate was administered for two months. Body weight was weekly measured. At the end of the study rats were anaestezised and decapitated. Blood, liver, adipose tissue, brain were taken for further measurements. The concentration of free radicals in the plasma was determined by the method of MTT - formazan. Topiramate significantly decreased the body weight of the obese group compared to the control group. Secondly, topiramate reduced the plasma levels of free radicals

Our data suggest that topiramate has a potent beneficial effect on body weight and metabolism in rats. Moreover, the drug decreases the plasma free radicals which may influence the mechanisms for development of obesity.

Key words: MTT - formazan,obese rats, topiramate

NUTRITIONAL MODELS FOR CREATING INSULIN RESISTANCE

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Summary

Insulin resistance is a widely spread physiological condition characterized by impaired answer of the body's cells to the action of insulin. The beta cells of the pancreas produce insulin but the cells of the body are resistant to its activity leading to increased level of glucose in the plasma. Consequently, the pancreas secretes even more insulin leading to hyperinsulinemia. Finally, insulin resistance might lead to the development of type II diabetes. Different nutritional factors might lead to insulin resistance. We investigated the potential of refined palm oil or fructose to cause such impaired answer in adult female and young male Wistar rats. In one of the models for creating insulin resistance we used refined palm oil which was applied *per os* via gastric tube. In the other model we applied *ad libitum* fructose solutions in different concentrations for a different period of time. For assessing insulin resistance we performed the oral glucose tolerance test (OGTT). The results demonstrated a diabetic activity of the refined palm oil diet. On the other hand, the different fructose diets did not alter the OGTT and showed no diabetic activity.

As a conclusion, a diet rich in refined palm oil might be used to create a model of insulin resistance and its pharmacological treatment can be further investigated.

Key words: fructose diet, insulin resistance, refined palm oil diet