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The 19th Conference about Laboratory Animals organized by the Czech Laboratory Animal Science Association (SVLZ) was held in Musov – Pasohlavky near Brno in the South Moravia, May 3 – 5, 2016 in hotel Termal Musov, in a nature conservation area Palava, on a main route Brno-Vienna. A total 101 of scientists, experts of veterinary administration, animal science or lawyers participated in the meeting. Most papers were devoted to welfare and laboratory animal protection, other presentations were reported new experimental methods or data from experiments both in animals or new alternative models. The amended regulations and laws at the care of laboratory animals were presented, too.

EFFECT OF GENETICALLY MODIFIED MAIZE ON HEALTH OF WISTAR RATS AFTER 90-DAY EXPOSURE

R. Aláčová, J. Ondrejková, K. Ambrušová, A. Kebis, J. Kovrižnych, M. Kuricová, A. Líšková, E. Rollerová, E. Szabová, J. Tulinská, D. Zeljenková
Slovak Medical University, Bratislava, Slovak Republic

The GMO Risk Assessment and Communication of Evidence (GRACE) project is financed by the European Commission within the 7th Framework Programme. The GRACE project programme provides comprehensive reviews of the evidence on the health, environmental and socio-economic impacts of GM plants considering both risks and possible benefits. The one of the aims of GRACE project is to improve human health risk assessment of GM plants. This project will also check whether extended feeding trials can improve risk assessments compared with the analytical and *in vitro* methods available today. The rat feeding trial was conducted by taking into account the EFSA Guidance on conducting repeated dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee 2011) and the OECD TG 408. The trials were performed in compliance with GLP. Dietary treatments represented following groups: 11 % GMO, 33 % GMO, near-isogenic control, conventional 1 and conventional 2. We present the main results of 90-day feeding trial with GM maize MON810 from the Monsanto company. The results obtained show that the MON810 maize at a level of up to 33 % in the diet did not induce adverse effects in male and female Wistar Han RCC rats concerning animal weights and food consumption after subchronic exposure. No signs of morbidity and mortality were observed throughout the 90-day feeding period, and the daily clinical observations did not reveal any signs of functional deficits. The ophthalmological analyses revealed individual alterations in all five experimental groups in the 1st week, whereas no alterations were visible in the 12th week of the feeding trial. The hematology parameters WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT and LYM were similar in the control, 11 % GMO and 33 % GMO groups of male and female rats. Changes in levels of biochemical parameters were not dose-dependent. The activities measured in the serum of 11 % GMO and 33 % GMO fed rats were in the same range as the historical data of control animals of the same strain, age and gender. During necropsy gross lesions were observed in two out of 16 male rats that have been fed the 11 % GMO. Similarly they were observed in the conventional 1 and the conventional 2 diet as well as in 3 out of 16. The subsequent histopathological analysis of all gross lesions revealed that a papillary carcinoma of the mammary gland had developed in one female rat fed the conventional 2 diet. The feeding trial performed in the frame of the GRACE project shows that the MON810 maize at a level of up to 33 % in the diet does not lead to subchronic toxicity in male and female Wistar Han RCC.

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ALCOHOL ADDICTION IN THE METHYLAZOXYMETHANOL MODEL OF SCHIZOPHRENIA IN MALE AND FEMALE RATS

Z. Babinská[1], J. Rudá-Kučerová[1], J. Nádeníček[1], T. Štark[1], V. Micale[2]
[1]Masarykova univerzita, Farmakologický ústav LF, Brno, [2]CEITEC – Středoevropský technologický institut, Brno, Czech Republic
babinska@med.muni.cz

Almost 50 % of schizophrenic patients suffer comorbid substance abuse with nicotine and alcohol being the most prevalent drugs. This comorbidity is associated with substantially higher burden of the disease as these patients have higher rate of hospitalizations, shorter life expectancy and higher suicide attempt rate. Comorbid alcohol abuse leads to worse response to antipsychotic treatment and deteriorates development of positive signs of schizophrenia. Furthermore, comorbid alcohol abuse significantly aggravates course and prognosis of schizophrenia in women (Schmidt *et al.* 2011). The majority of both clinical and pre-clinical studies is conducted on male subjects only, therefore the aim was to assess addictive behavior in a neurodevelopmental model of schizophrenia induced by prenatal methylazoxymethanol acetate (MAM) exposure in both sexes. Vehicle or MAM (dose of 22 mg/kg on gestational day 17) was administered intraperitoneally to the pregnant dams (Sprague-Dawley strain) and study was performed with their 9 weeks old offspring (20 females and 20 males; groups: F VEH, F MAM, M VEH, and M MAM). Drinking in the dark paradigm with a sucrose fading procedure (Samson *et al.* 1988) was used to assess daily intake of 20 % alcohol and relapse-like behavior after 2 weeks of forced abstinence. Alcohol intake was calculated as grams of pure alcohol per kg of body weight. Alcohol consumption was significantly higher in MAM treated females in comparison to MAM treated male rats. This finding suggests that the prenatal MAM exposure may increase vulnerability for alcohol drinking in female rats. During the relapse phase this phenomenon was still present and also sex difference in control animals has reached significance when control females consumed more alcohol than control males. To our knowledge, this is the first report on alcohol drinking behavior in the MAM model. Our study suggests that female sex and schizophrenia-like phenotype induced by the prenatal MAM exposure may possibly work synergistically to enhance alcohol consumption.

Schmidt LM, Hesse M, Lykke J: The impact of substance use disorders on the course of schizophrenia – a 15-year follow-up study Dual diagnosis over 15 years. *Schizophr Res*, 130, 228-233, 2011.
Samson HH, Pfeffer AO, Tolliver GA: Oral ethanol self-administration in rats: models of alcohol-seeking behavior. *Alcohol Clin Exp Res*, 12, 591-598, 1988.

This study was financed from the SoMoPro II programme. The research leading to this invention has acquired a financial grant from the People Programme (Marie Curie action) of the Seventh Framework Programme of EU according to the REA Grant Agreement No. 291782. Further co-financing was by the project of specific research at the Masaryk University (MUNI/A/1284/2015).

ELECTROPHYSIOLOGICAL ANALYSIS OF SENSITIZATION MECHANISMS IN VISCERAL NOCICEPTORS BY ADENOSINE

M. Brozmanová[1], L. Mazúrová[1], M. Tatár[1], M. Kollárik[1,2,3]
[1]Biomedical Center Martin and [2]Department of Pathophysiology, Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Martin, Slovakia, [3]The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Adenosine is an important pronociceptive and inflammatory mediator. Clinical studies suggest that endogenous adenosine can contribute to non-cardiac chest pain and mechanical hypersensitivity in the esophagus by activation of esophageal nociceptive pathways. Esophageal vagal nodose nociceptors, as we have previously found, express the adenosine A2A receptors. We hypothesized that the activation of A2A adenosine receptors can induce mechanical sensitization in esophageal C-fibers that is mediated by TRPA1 channel. The activity of vagal nodose afferent C-fibers was recorded extracellularly in the isolated guinea pig esophagus preparation *ex vivo*. We evaluated the effect of pharmacological activation and inhibition of A2A receptor and potential downstream effector ion channel TRPA1 on mechanical responsiveness of esophageal nodose C-fibers. The selective adenosine A2A receptor agonist CGS21680 induced reversible mechanical sensitization of the response to esophageal distention (10-60 mm Hg) in vagal nodose C-fibers. Without causing an overt activation, CGS21680 induced (2.4±0.3)-fold increase in the mechanical response (to 30 mm Hg) at the half maximally effective concentration (EC50) 3 nM. The selective A2A antagonist SCH58261 abolished this sensitization. The activator of adenylate cyclase forskolin (1-10 μM) caused a (1.9±0.3)-fold increase in mechanical response, which was comparable to that of GCS21680. The nonselective protein kinase inhibitor A H89 partially inhibited the GSC21680-induced mechanical sensitization. Finally, the selective TRPA1 receptor antagonists AP18 and HC030031 prevented mechanical sensitization of nodose C-fibers evoked by GSC21680. We show that the activation of adenosine A2A receptor induces mechanical sensitization of vagal nodose C-fibers. Our results indicate that the signaling may involve Gs-adenylate cyclase-PKA pathway and that the downstream effector ion channel is the TRPA1.

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USING ASL METHOD FOR MONITORING OF BRAIN PERFUSION CHANGES IN RAT MODELS OF SCHIZOPHRENIA

E. Drazanova[1,2], L. Grossova[2,3], L. Dvorakova[2,3], J. Pistovcakova[1], J. Ruda-Kucerova[1], V. Micale[5], R. Demlova[1], T. Kasparek[4], Z. Starcuk Jr.[2]
[1]Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [2]Institute of Scientific Instruments, ASCR, Brno, Czech Republic, [3]Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic, [4]Department of Psychiatry, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [5]Applied Neuroscience Research Group, CEITEC, Masaryk University, Brno, Czech Republic

Several animal models simulate schizophrenia pathologies. We used the poly I:C and MAM developmental animal models for our investigation. Using arterial spin labeling (ASL) MRI method we examined brain perfusion changes in both developmental models in rats. The developmental poly I:C model was induced by acute subcutaneous dose of poly I:C (8 mg/kg) to 10 pregnant Wistar rat dams in the 15th day of their pregnancy. Vehicle was administered to 10 controls in the same time period. The MAM model was generated by acute intraperitoneal dose of MAM (22 mg/kg) to 10 pregnant Sprague-Dawley rat dams in the 17th day of their pregnancy. Control animals were obtained in the same way. The adult offspring males were subjected to MRI scanning. MR imaging was performed in an animal 9.4T MR scanner (Bruker Biospin 94/30 USR by Bruker, Ettlingen, Germany) using a receive-only 2×2 array surface coil (400 ARR R.BR) and a volume transmitter coil. Anatomical images were obtained with a RARE sequence with TR 3500 ms, TE 36.0 ms, image matrix 256×256, slice thickness 1.25 mm, 2D FOV 50.0×20.4 mm, RARE factor 2. ASL was performed with a FAIR-RARE sequence with TR 10000 ms, TE 37.78 ms, image matrix 128×96, slice thickness 1.25 mm, 2D FOV 50.0×20.4 mm; from one axial slice through the brain a set of 15 magnitude images with TI of 30, 50, 100, 200, 300, 500, 700, 900, 1000, 1100, 1500, 1800, 2200, 2800, 3200 ms was used to calculate the tissue blood flow map. All ASL data were analyzed in ParaVision 5.1 (Bruker), ASL blood flow maps were analyzed in manually drawn brain ROIs by own Matlab R2010a code. MRI data were analyzed in STATISTICA (StatSoft Inc.) software by the Mann-Whitney U nonparametric test (boundary of significance set to $p<0.05$). Statistical evaluation of the ASL results in the circle of Willis, hippocampus and somatosensory cortex revealed significantly higher circle-of-Willis perfusion in the poly I:C prenatally exposed animals. In the MAM prenatally exposed animals, significantly higher perfusion in the circle of Willis and the somatosensory cortex, and significantly lower hippocampus perfusion were found. Iadecola (1998) reported that dopamine profoundly influences all segments of cerebral circulation, and dopamine dysregulation is a hallmark of schizophrenia. Our findings show region-specific brain perfusion changes which could reflect dopamine dysregulation in poly I:C and MAM exposed animals.

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RAT ANIMAL MODEL OF MYOCARDIAL CRYONECROSIS

L. Dvořáková[1], P. Scheer[2,3], R. Jiřík[1], E. Dražanová[1], J. Hložková[2,3], Z. Starčuk Jr.[1]
[1]Magnetic Resonance and Cryogenics, Institute of Scientific Instruments of the Czech Academy of Sciences, v. v. i., Brno, [2]Department of Human Pharmacology and Toxicology, Pharmaceutical Faculty, Veterinary and Pharmaceutical University Brno, [3]International Clinical Research Center, St. Anne's Hospital, Brno, Czech Republic

The coronary artery diseases are the leading cause of the death worldwide. The study of heart failure requires viable animal models that mimic human cardiac diseases, such as myocardial infarction. In our study we are presenting a freeze-thaw injury induced myocardial infarction model – a cryonecrosis model, which was validated by a magnetic resonance examination. Five healthy rats were anesthetized (xylazin 5 mg/ kg BW + ketamin 35 mg/kg BW + diazepam 5 mg/kg BW), intubated using 16 G cannula and were artificially ventilated (TOPO, Kent Scientific). A left-sided thoracotomy (4th ICS) was performed and then after fixation of the pericardium the heart was exposed. Cryonecrosis (CN) was produced by attaching a liquid-nitrogen cooled steel tool with the diameter of 6 or 8 mm on the left ventricle (LV) free wall on the area of heart apex for 60 s. Thoracotomy was closed in a standard manner. During postoperative care amoxicilin clavulanate was administrated IM daily and every other day a dose of tolfetamic acid was administrated. Five months after CN, rats were sacrificed and hearts stored in a 10 % solution of formaldehyde. All imaging was performed on a 9.4T NMR system (Bruker-Biospec 94/30 USR). For *in vivo* measurement IntraGate FLASH was used. Three sets of anatomical images in long-axis, short-axis and four-chamber view were acquired (FOV 4×4 cm, slice thickness 1 mm, matrix 256×256, TR 40 ms, FA 25°, 10 cardiac phases). Each explanted CN heart and one healthy rat heart was put in a container filled with Fomblin, a perfluoropolyether (Sigma-Aldrich, Missouri, USA). T1 weighted images in long-axis, short-axis and four-chamber view were acquired using RARE sequence with parameters as follows: FOV 1.5×2.3 cm, slice thickness 0.7 mm, matrix 256×256, TR 666 ms, number of averages 4. *In vivo* measurement showed a decrease in LV mass in the area of cryonecrosis. The same area revealed motion abnormalities in the cine images. Comparison of healthy and CN heart demonstrated significant structural changes in myocardium tissue afflicted by CN. In the future we are planning to implement T1 mapping and perfusion imaging, which would allow us to quantify the fibrotic tissue.

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HOW DOES THE HOST SHIFT BIAS THE INFECTIVITY OF TRICHINELLA PARASITE?

Z. Hurníková[1], C.M.O. Kapel[2]
[1]Institute of Parasitology, Slovak Academy of Sciences, Košice, Slovak Republic, [2]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark

The biological characteristics of *Trichinella* isolates, particularly those relating to infectivity and pathogenicity, can show considerable variation because they are subject to strong host influences, notably those associated with immune and inflammatory response. Infectivity and persistence of muscle larvae in host tissue, and its tolerance to hostile environment after the death of the host are the three most important biological features, determining *Trichinella* transmission in nature. Knowledge on biological characteristics of individual *Trichinella* genotypes in different host is therefore crucial in specific risk factor evaluation. The aim of our study was to determine the effect of host shift on infectivity of six *Trichinella* species – three encapsulating (*T. spiralis*, *T. native, T. britovi*) and three non-encapsulating species (*T. pseudospiralis*, *T. papuae, T. zimbabwensis*) in mice and foxes. Experiment was scheduled in four execution phases: passage in mice, passage in foxes, passage in mice following the single carnivore passage, and passage in mice after fox + mice passage. Mice were infected in six groups according the infective dose (100, 200, 400, 500, 600, and 700 larvae) for each *Trichinella* species in each experimental phase. Foxes were infected in two groups, using infective doses 10000 and 15000 larvae for each species. On day 35 post infection, animals were euthanized and necropsied. Muscle samples were examined individually using artificial digestion method. The parameters of intensity of infection and reproductive capacity were calculated as LPG (number of muscle larvae per gram of tissue), and reproductive capacity index – RCI (mean number of larvae recovered/number of larvae inoculated). The experiment demonstrated profound differences in infection levels for six *Trichinella* species; the infectivity varied significantly among species and among particular passages. A significant difference in RCIs between passages suggesting the influence of the change of hosts on the infectivity of *Trichinella* parasites was observed. Whereas after several passages in mice the patterns of infectivity were more or less well balanced in spite of the change of infective dose, after the change of the host from mouse to the fox considerable changes in infectivity in most of *Trichinella* species were detected. After passaging in mice without carnivore passage, the dynamics of infection were constant. Highly significant differences were found in the infectivity of *T. spiralis* comparing to all other species ($p<0.001$), whereas the differences were insignificant between all other

species. In the passage after fox, strong fluctuations in curves of infection were observed. The patterns of the dynamic of infection showed low infective doses (100, 200 larvae respectively) to be more effective than higher infective doses. That was true for all *Trichinella* species except *T. zimbabwensis* and *T. pseudospiralis* that showed increasing infection pattern according to the dose up to the infective dose 500 larvae in passage after fox. The success to establish high infection by lower infective doses after the change of host species is very likely similar also in natural conditions, where the probability to ingest lower doses of infective larvae is higher than to become infected with high infective doses. After sequential passage in mice, the influence of the change of the host species revealed to be less significant in all species but *Trichinella spiralis*. While in all species the dynamic of infection was similar to the pattern before the fox passage, the infectivity of *T. spiralis* rapidly declined from maximum by infective dose 100 larvae up to the top infective dose. For all species, the passage after fox + mice was most critical according the effectivity of infection and the RCIs were the lowest within all phases of infection. A strong influence of the change of host species on the level of infectivity of all *Trichinella* species involved in our study was observed suggesting that the degree of variation in *Trichinella* parasites reflects the variety of hosts to which adaptation is necessary.

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IN VIVO TESTING OF NEW SUBSTANCES WITH POTENTIAL ACTIVITY AGAINST *MYCOBACTERIUM TUBERCULOSIS*

P. Kubíčková[1], O. Pavliš[1], K. Bílková[1], J. Roh[2], V. Klimešová[2]
[1]Military Health Institute, Biological Defence Department, Techonin, Czech Republic, [2]Charles University in Prague, Faculty of Pharmacy, Hradec Kralove, Czech Republic

An experimental murine model of tuberculosis was prepared for evaluation of therapeutic efficacy of the antimycobacterial active substances determined by *in vitro* tests. All *in vivo* experiments were performed according the Czech law No. 246/1992 Sb., on animals protection against brutalization. Laboratory mice were infected with *Mycobacterium tuberculosis intranasally*. Afterward therapy with new prepared substances was established. The compounds were administered *orally* in the form of liquid solution. Stabling conditions of experimental animals (temperature, humidity, etc.) were observed continuously. Visual manifestation of proceeded infection and relevant changes of tested animals were observed during the experiment. Tested compounds were administered for 2 and 3 months. Tissue samples from spleen and lungs for determination of CFU (colony forming units) were taken after euthanasia. CFU are used as a measure of the number of microorganisms present in a sample. The antitubercular effect of studied substances was evaluated in comparison with untreated control and control treated with standard antituberculosis drugs (isoniazid, rifampicin and pyrazinamide).

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VASCULAR MINIPORT FOR PERMANENT VENOUS ACCESS IN RAT

A. Litvinec[1], J. Svoboda[2], J. Otáhal[2]
[1]Department of Husbandry and Zoohygiene of Laboratory Animals, Institute of Physiology of the Czech Academy of Sciences, Prague, [2]Department of Developmental Epileptology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Animal vascular ports are usually used for blood sampling, parenteral drug delivery or administration of agents for imaging. Commercially available animal ports are designed for single-use. The price is rather high. In order to reduce study costs for vascular miniport we have adapted and tested low-price, metal-free mice vascular access miniature port described by Fiebig *et al.* (2013) for use in rat. Our miniature vascular access port (MVAP) is composed of generally used medical equipment (silicon, cannula and tubing). Microvascular ports were implanted into the interscapular region and the cannula was placed into the jugular vein of the rat. MVAP can be repeatedly accessed by transcutaneal puncture with thin needle. The port is filled with saline and small amount heparine to prevent blood clotting on the tip of the catheter. Leaking tests were conducted to ensure it practical use in preclinical imaging. Repeated injections of different contrast or radiotracing agents (ULTRAVIST 300, FDG) followed by CT-PET imagining was used to visualize the port tightness. The MVAP is reliable, easy to assemble alternative to commercially available animal injection ports and is thus suitable for animal research and preclinical imagining techniques.

Fiebig T, Figueiredo G, Boll H, et al.: A low cost metal-free vascular access mini-port for artifact free imaging and repeated injections in mice. *PLoS One,* 8, e65939, 2013. doi: 10.1371/journal.pone.0065939

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EXPERIMENTAL MODELS OF ACUTE LUNG INJURY AND THEIR IMPORTANCE FOR CLINICS

D. Mokrá, A. Čalkovská
Biomedical Center Martin and Department of Physiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovakia
mokra@jfmed.uniba.sk

Acute lung injury (ALI) can originate from different direct (pulmonary) or indirect (extrapulmonary) reasons. In severe cases it can prograde to acute respiratory distress syndrome (ARDS). In an acute or exudative phase there dominate diffuse bilateral alveolar injury, increased permeability of the alveolar-capillary membrane, neutrophil-mediated inflammation and hypoxemia. The exudative phase is within several days followed by a fibroprolipherative phase with various degree of fibrosis, neovascularization and healing. Despite intensive research in this field, mortality of ALI/ARDS is still very high and at the moment there is no relevant treatment except of ventilatory support. Recently, several pharmacological approaches (e.g., pulmonary vasodilators, corticosteroids, antioxidants, methylxanthines etc.) and non-pharmacological approaches (gene and cell therapy) appeared to be at least partially effective for ALI/ARDS. However, evaluation of novel types of the therapy in the laboratory conditions continues. Regarding wide spectrum of triggering factors and complexity of pathogenetic changes, different types of experimental models of ALI/ARDS are used: 1. models with primary injury to capillary endothelium, such as model of pulmonary embolism after i.v. delivery of oleic acid, model of pulmonary endothelium injury caused by i.v. delivery of lipopolysaccharide (LPS) etc.; 2. models with primary injury to alveolar epithelium, e.g. model of aspiration of the gastric juice elicited by i.t. instillation of hydrochloric acid (HCl), model of surfactant insuffiency caused by repetitive lung lavage with saline, model of lung injury caused by i.t. instillation of LPS, model of lung fibrosis induced by i.t. instillation of bleomycine, model of lung injury caused by excessive ventilation or hyperoxia etc. Nevertheless, utilization of the results for the clinics can be partially limited by several factors: 1. ALI/ARDS in humans is usually caused by a complex action of several factors including comorbidities and genetic predispositions, whereas the models of ALI/ARDS are usually induced by a single triggering factor; 2. inter-species differences in response to the type of injury or innate immune responses etc., which can cause different response to the treatment in animals and in humans. Despite the mentioned limitations the use of experimental models enables better understanding the pathophysiology of ALI/ARDS and helps to find out more efficient therapeutic approaches.

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GENETICALLY MODIFIED MAIZE ADDED TO WISTAR RAT'S FEED

J. Ondrejková, R. Aláčová, K. Ambrušová, A. Kebis, J. Kovrižnych, M. Kuricová, A. Líšková, E. Rollerová, E. Szabová, J. Tulinská, D. Zeljenková
Slovak Medical University in Bratislava, Bratislava, Slovak Republic

Expansion of biotechnology and genetic engineering brought a new term GMO – genetically modified organisms to the awareness of scientific community and general public. In recent years, a technology development of genetically engineered plants has occurred. Due to using the new technologies and the cultivation of GM plants and crops that are used for food preparation, it is necessary to properly assess their impact on the health of the human population using the animal models in experiments. From the perspective of public health, it is important to assess the risks due to exposure of the entire human population. The international project GRACE also contributes into the risk assessment, and likewise to the unification of the methodologies used to test the GM crops. The GRACE project (GMO Risk Assessment and Communication of Evidence) is funded by European Commission within the 7th framework program. One of objectives of this project is to conduct 90-day animal feeding trials, animal studies with an extended time frame as well as analytical *in vitro* studies on genetically modified maize, in order to comparatively evaluate their use in GM plant risk assessment. We present results of 90-day feeding study with a GM maize MON810 Pioneer variety and its near-isogenic non-GM variety. The rat feeding study was performed by taking into account the EFSA Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee, 2011) and the OECD TG 408. Three dietary treatments represent the groups of near-isogenic control, 11 % GMO and 33 % GMO. Regarding the results, no statistically significant differences in feed consumption and in body weights of animals were observed in male as well as female rats in all three experimental groups. In 11 % GMO diet and 33 % GMO diet groups the concentration levels of Ca and urea levels increased with statistical significance when compared to the control group. The Na concentration levels significantly decreased in all GMO groups against to the control group. The alanine transferase (ALT) and aspartate aminotransferase (AST) activities were significantly increased in the serum of female rats being fed the 33 % GMO diet if compared to the animals receiving the control diet. However, the ALT and AST activities measured in the serum of 33 % GMO-fed female rats are in the same range as the historical ALT and AST data collected by the breeder company for control animals of the same strain, age and gender. Furthermore, the ALP activity was similar to that of the control diet-fed animals and no sign of liver injury was observed in the gross necropsy as well as in the histopathological analyses. Hematological changes were observed in all test groups. No signs of morbidity and mortality were observed throughout the 90-day feeding period. Histological changes were sporadically observed in the control and 33 % GMO groups. Results show that the Pioneer MON810 maize at two various levels of diet did not cause significant adverse effects on health of male and female Wistar rats after ninety day exposure.

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A NEW MULTIMODAL CENTER FOR ADVANCED PRECLINICAL IMAGING (CAPI) AT THE FIRST FACULTY OF MEDICINE IN PRAGUE

L. Sefc[1], V. Sykora[2]
[1]Center for Advanced Preclinical Imaging (CAPI) and [2]Centre of Experimental Biomodels, First Faculty of Medicine, Charles University in Prague, Prague, Czech Republic

First Faculty of Medicine of the Charles University in Prague has recently opened a multimodal imaging center equipped with four preclinical imaging instruments. The Center for Advanced Preclinical Imaging (CAPI) was created with support from the European Operational Programme Research and Development for Innovation (OP RDI). The modalities used in CAPI include magnetic particle imaging (MPI), magnetic resonance (MRI), computed tomography (CT), positron emission tomography (PET), single photon computed tomography (SPECT), optical imaging (OI), planar X-ray and Cherenkov detector. CAPI is a part of national research infrastructure for biological and medical imaging (Czech-BioImaging), a member of the ESFRI large pan-European research infrastructure Euro-BioImaging. CAPI will provide an open access to a wide range of imaging technologies and expertise to all scientists in the Czech Republic and also to those from European Union. The already established contacts include academic and industry partners focused on development and testing of contrast agents (radioisotopes, optical, and magnetic tracers) as well as custom synthesis of various ligands. http://capi.lf1.cuni.cz/en

CRUCIAL QUESTION OF GNOTOBIOLOGY

I. Šplíchal, A. Šplíchalová
Laboratory of Gnotobiology, Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Nový Hrádek, Czech Republic

A French pediatrician Tissier postulated in 1900 that fetuses develop in sterile conditions of the uterus. Newborns are microbiologically colonized during or immediately after birth. Different ways of colonization are in vaginally born newborns and in Caesarean section born newborns. The majority of microbiota is located in the gastrointestinal tract (GIT). The microbial settlement of the newborn GIT is influenced also by other factors as nutrition (breast milk × formula), antibiotics, weight and maturity of the newborn (term × preterm), and possible using of probiotics. The microbiota of the GIT is predicted but non-defined. A term ´gnotobiology´ originates from Greece words gnotos (known, defined) and bios (life). An adjective ´gnotobiotic´ is commonly used for microbiologically defined hosts including of sterile (germ-free) ones. The gnotobiology has developed since the end of the ninetieth of 19th century and has helped to understand the impact of the microbes on the health of their host. Additional impulses in gnotobiotic animal models have been dated since the beginning of this century. New methods of study of microbiota on nucleic acid level – e.g. PCR, high-throughput RNA analysis and next generation sequencing have offered new possibilities to study influence of microbiota on the health of their host. Our possibilities of detection were enriched for highly sensitive detections of nucleic acids – general compounds of cells including of microbial ones. More than one century accepted Tissier's statement dealing with sterile conditions of prenatal development has been doubted. Before more than one century accepted Tissier's statement should be refused an additional research in the field of possible prenatal microbiome is required. At first it is necessary to demark clear borderline between possible prenatal microbiome and inapparent intrauterine infections. Does the presence of nucleic acids really confirm the existence of prenatal microbiome? Why we have not had any reports dealing with microbiome of germ-free laboratory rodents that are bred for generations in sterile isolators? At least these answers would contribute to solve the crucial question of gnotobiology: Does prenatal development occur in the sterile conditions?

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CONFIDENCE REGION DETERMINATION AND BIOPHYSICAL INTERPRETATION OF WATER-MOLECULE DIFFUSION PARAMETERS DETERMINED BY MAGNETIC RESONANCE DIFFUSION TENSOR AND DIFFUSION KURTOSIS IMAGING IN THE MOUSE BRAIN

Z. Starcuk Jr.[1], E. Drazanova[1,2], L. Dvorakova[1,3]
[1]Institute of Scientific Instruments, Czech Academy of Sciences, Brno, Czech Republic, [2]Department of Pharmacology, Faculty of Medicine, Masaryk University, Brno, Czech Republic, [3]Department of Biomedical Engineering, Brno University of Technology, Brno, Czech Republic

Random motion of water molecules in soft tissues, detected by diffusion-weighted magnetic resonance imaging techniques and quantified most frequently by the apparent diffusion coefficient, diffusion tensor (DTI) or diffusion kurtosis (DKI) models, turns out to be a useful marker of pathological changes, such as those associated

with Parkinson's disease or stroke, and a useful basis for white-matter neural fiber tractography. The standard measurements are characterized by the number of diffusion encoding directions and the '*b*-values' [s/mm^2], characterizing the amount of diffusion weighting. The purpose of this work is to analyze the role of a seldom emphasized experimental parameter, the diffusion time Δ, on the diffusion model validity and the impact on confidence intervals of diffusion parameters. Monte-Carlo simulation was used to test the effect of diffusion time on whether the local diffusion appears free (characterized by a Gaussian distribution, broadening in time) or restricted (non-Gaussian distribution, converging to uniform distribution). The simulated results were compared with the DTI and DKI models used. For the diffusion weighting gradient orientations used in real experiments, another Monte-Carlo simulation was used to estimate diffusion parameter confidence regions. The simulated results were compared with selected mouse-brain data acquired in a 9.4T animal MR scanner. With the average free-diffusion travel distance of $(2D\Delta)^{1/2}$ it is obvious that by increasing of Δ the travel distance increases the volume probed: whereas with small Δ, small subcellular-sized volumes are probed (e.g. 4 μm for Δ of 8 ms), long diffusion times Δ increase the probed volume in excess of the cell size (e.g. 10 μm for Δ of 50 ms). These distances are comparable with cell sizes and hence adjusting Δ may be seen as selecting the tissue microstructure probe size. The transition between free and restricted diffusion was examined with a simple 1D-3D Monte-Carlo water molecule mobility model, which confirmed the distribution function evolution and demonstrated the transformation of the exponential signal model $\exp(-b.D)$ used in DTI through $\exp(-bD+b^2D^2K/6)$ used for DKI to a leveling-off model definitely necessary for $b>3/D/K$ when the kurtosis model fails to properly model further signal loss with growing *b*. Larger number of parameters or model inappropriateness are connected with larger confidence regions. In conclusions, the simulations show that statements about the diagnostic value of markers derived from the DTI and DKI models must always be assigned to a particular combination of the diffusion time Δ and *b*-value because the model parameters, such as fractional anisotropy, may significantly depend on the choice of Δ, which calibrates the measurement from probing local intracellular environments to the whole cell shapes.

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GENOTOXICITY OF WASTEWATER FROM HEALTH CARE FACILITIES

A. Vlková[1,2], Z. Wittlingerová[1], M. Zimová[1,2], G. Čárová[1,2], K. Kejlová[2], S. Janoušek[2]
[1]*Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic,* [2]*National Institute of Public Health, Prague, Czech Republic*

Health care facilities use for therapeutic purposes, diagnostics, research, and disinfection a high number of chemical compounds such as pharmaceuticals (e.g. antibiotics, cytostatics, antidepressants), disinfectants, surfactants, metals, radioactive elements, bleach preparations, etc. Hospitals consume significant amounts of water (in the range of 400 to 1200 liters/day/bed) corresponding to the amount of wastewater discharge. Some of these chemicals are not eliminated in wastewater treatment plants and are the source of pollution for surface and groundwater supplies. Hospital wastewater represents chemical and biological risks for public and environmental health as many of these compounds might be genotoxic and are suspected to contribute to the increased number of cancers observed during the last decades. The changes of the genetic information can have a lethal effect, but more often cause tumor processes or mutations in embryonic development causing serious defects. The quantification of risk associated with these chemical pollutants is extremely difficult as they usually occur in concentrations too low to allow analytical determination and the synergic effects of mixtures cannot be evaluated by means of chemical analytical methods. Therefore, genotoxicity is preferentially evaluated using biological tests which do not require the exact knowledge of toxicant identity and physical-chemical properties of the wastewater sample. The most widely used tests of genotoxicity are based on bacteria *Salmonella typhimurium* and *Escherichia coli* (i.e. Ames test, umuC test and SOS chromotest). In monitoring studies higher plants (namely Allium cepa) have been recognized as excellent genetic models to detect environmental mutagens. Due to relative simplicity, sensitivity to genetic damage, low cost of experimentation and small amount sample required these short-term bioassays have proved to be an important tool in genotoxic studies. The presented poster summarizes the current situation in wastewater pollution from health care facilities with regard to genotoxicity and suitable biological tests.

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PROTECTIVE EFFECT OF AMLODIPINE ON RAT BONE TISSUE AFTER ORCHIDECTOMY

H. Zivna[1], I. Gradosova[2], V. Palicka[2], S. Hubena[2], K. Svejkovska[2], P. Zivny[2]
[1]*Radioisotope Laboratories and Vivarium, Charles University in Prague, Faculty of Medicine in Hradec Kralove, Hradec Kralove , Czech Republic,* [2]*Institute of Clinical Biochemistry and Diagnostics, Charles University in Prague, Faculty of Medicine and University Hospital in Hradec Kralove, Hradec Kralove, Czech Republic*

Cardiovascular diseases together with osteoporosis most frequently occur in older people. Antihypertensive agents are very effective in reducing high blood pressure. Recently, however, interest has been focused on whether these agents intervene in bone metabolism and in what manner. Amlodipine, a dihydropyridine calcium channel blocker of the third generation, is indicated in the treatment of hypertension, vasospastic and chronic stable angina pectoris. Our study aimed to investigate the effect of amlodipine on bone metabolism in orchidectomized rats. Eight-week-old albino Wistar rats (Biotest Ltd., Konarovice, Czech Republic) were divided into three groups. The operations and sacrification were performed under ether anesthesia. The sham-operated control group (only scrotal incision, SHAM) and the control group after bilateral orchidectomy (ORX) received the standard laboratory diet (SLD, VELAS, plc., Lysa nad Labem, Czech Republic). The experimental group after bilateral orchidectomy (ORX+AML) received SLD enriched with amlodipine for 12 weeks. Bone marker concentrations in serum of PINP, OPG and IGF-1, and the levels of CTX-I, BAP and BMP-2 in a bone homogenate were measured using ELISA. The homogenate was prepared from the tibia. Bone mineral density (BMD) was measured by dual energy X-ray absorptiometry. The femurs were used for biomechanical testing, three-point bending and femoral neck fracture using a special electromechanical custom-made testing machine (Martin Kosek & Pavel Trnecka, Hradec Kralove, Czech Republic). Statistical analysis was performed using the program NCSS 2007 (Number Cruncher Statistical System, Kaysville, Utah, USA). Bone markers (CTX-I, BAP, BMP-2) in ORX were higher versus SHAM. In ORX+AML there was a decrease in PINP, CTX-I, BAP, BMP-2 and OPG versus ORX. IGF-1 was decreased in ORX versus SHAM. In ORX+AML it was increased versus ORX. In ORX, a decrease was demonstrated versus SHAM in BMD of the whole body, in the lumbar vertebrae and in both femurs. In ORX+AML there was an increase in BMD of the whole body versus ORX. Three-point bending test revealed a decrease in maximal load values in ORX versus SHAM. After amlodipine administration there was an increase in the left femur versus ORX. In conclusions, amlodipine is capable of mitigating the negative effects of orchidectomy and could be a good prevention of osteoporosis. Amlodipine has a positive effect on bone metabolism by decreasing bone turnover. It can be conjectured that amlodipine administration to patients with diagnosed osteoporosis probably confers a protective effect on the bone tissue, at least in hypertensive patients.

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