ALTERNATIVES IN THE TESTING OF SKIN SENSITISATION POTENTIAL OF CHEMICALS

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Chemical-induced adverse effects such as contact allergy as well as skin irritancy are of major importance in human clinical dermatology and during the development of new industrial chemicals. Since clinical and histological features of allergic and irritant contact dermatitis are similar, the differentiation between both types of dermatitis in the preclinical and clinical evaluation of chemicals remains difficult. However, underlying immunological mechanisms are thought to be fundamentally different. For allergic skin immune responses, it is well established that antigen presentation induces T-cell activation and the formation of antigen-specific memory T cells. In contrast, irritant skin reactions are believed to activate the immune cascade independent of the antigen presentation pathway by inducing proinflammatory mediators and cytokines that directly recruit and activate T cells. Thus, irritant skin reactions are defined to be "non-specific" reactions that do not result in the induction of antigen-specific memory T cells. The majority of tests for predicting allergenicity of chemicals use guinea pigs or mice with biphasic, long-term protocols comprising a sensitisation phase (induction) and an elicitation phase (challenge). Most common are the Buehler's occluded patch test and Magnusson and Kligman guinea pig maximization test (comprised in OECD Test Guideline 406), where contact reactivity is assessed by a subjective local erythema score and determined from the frequency of animals exhibiting a positive response. Some alternative tests were proposed for the identification of skin sensitisation potential of chemicals in order to reduce the stress and painful procedures in laboratory animals. One of them MEST (Mouse Ear Swelling Test) comprises both the induction phase and the elicitation phase of the immune response, but the painful procedures during the testing has been hardly avoided in this test. Most promising in this sense was LLNA (Local Lymph Node Assay). In contrast to guinea pig models and the MEST, the LLNA is based upon the detection of a primary immune response as a function of auricular lymph node activation following topical application of chemicals on the dorsal surface of ears. So such a stressing procedures as intradermal injection and removing the fur on the site of topical application were eliminated. The activation of lymph node is measured by the incorporation of ^{3}H-methyl thymidine into the DNA of proliferating lymphocytes in draining lymph nodes. The LLNA does not include a challenge phase. The endpoint of interest is the stimulation index giving the ratio of thymidine incorporation in lymph nodes from dosed animals compared to the incorporation in lymph nodes from vehicle-treated control animals. After proper validation studies was the LLNA incorporated to a set of testing methods of OECD and EU (OECD Test Guideline 429 and EU testing method B.42). Further in the paper there it is described the modification of LLNA, which does not require the usage of radioactive-labelled materials and include also the step with measuring the ear thickness. This later procedure allows distinguishing some potential „false positives" e.g. chemicals which cause the proliferation of lymph node cells by above mentioned "non-specific" reactions.

THE USE OF ANIMALS IN RESEARCH: ARGUMENTS FOR AND AGAINST

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Since the 20th century, medical discoveries were principally supported by animal experimentation which has led to much public and scholarly discussion about its correct use as well as its moral and ethical foundations. From a medical point of view, experiments in animals seem to be unavoidable because they enable and considerably speed-up development of new therapeutic strategies. On the other hand they are publicly criticized because in many instances they could be replaced or at least restricted by the use of alternative techniques such as molecular genetics, tissue culture and computer modeling.

THE USE OF ANIMALS IN PROJECTS: COMMON PROBLEMS AND PROPOSED SOLUTIONS.

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In this study, we focused on five experimental projects from our laboratory and investigated the numbers of animals used as was declared in the project documents. In some studies, the number of animals declared in the project proposals was lower than in the actual performed experiment. In one experiment, the animals were used for training purposes for new transplant methodology. In this project only were the numbers of animals used similar from project proposal to the experimental stages. For the other projects, the use of animals for training was unnecessary because the methodology in Langerhans Islets laboratory was already well established and therefore not needed. In all the projects, a typical mistake was missing data about number, sex, age and weight of the animals. The aim of this study was to identify mistakes which occurred during the process about missing information and discrepancies and to suggest solutions to correct them. This could prevent the need to submit additional study amendments as it happened in the evaluated projects.

DETERMINATION OF VETERINARY CONDITIONS, CONTROL AND SUPERVISION OF EXPERIMENTS ON ANIMALS.

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In compliance with the international standards experiments on animals can be performed in the Czech Republic solely according to the legal requirements. Pursuant to Art. 4 (3) of Act No. 166/1999 Coll., on veterinary care and amending certain related laws (Veterinary Act) the keeper intending to use animals for experiments shall be obliged to apply to the regional veterinary administration or the Municipal Veterinary Administration in Prague (hereinafter referred to as the "RVAs") for specification of the veterinary conditions. Veterinarians supervise experiments on animals from the commencement of an experiment by participating as members in the expert commissions of user establishments, which discuss and approve experimental projects. RVAs supervise the fulfilment of duties imposed upon breeding, supplying and user establishments by Act No. 246/1992 Coll. on the protection of animals against cruelty and other implementing legal regulations. For the purpose of standard conduct of control and supervisory activities of the veterinary administration authorities the following methodological guidelines were published in 1995 and last amended in 2007: Methodological Guidelines of the State Veterinary Administration of the Czech Republic No 2001/04/EPIZ dated February 1, 2000 and amended April 25, 2007 "Determination of veterinary conditions, control and supervision of experiments on animals". Veterinary conditions of the experimental project represent the set of animal health requirements and restrictions relating to diseases, which are launched before the commencement of an experiment, in the course of and after the completion of an experiment; precautionary measures to guarantee health safe animal products produced in the course of and after the completion of an experiment; measures for veterinary protection of environment; and other measures arising from veterinary legislation for breeding and use of experimental animals. Since 1993 approximately 10-150 of such supervisory activities have been conducted each year in the Czech Republic. Non-compliances were found just in a few isolated cases.

GENETICS OF SUSCEPTIBILITY TO INFECTION *LEISHMANIA MAJOR* IN MICE

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L. major causes cutaneous pathology in humans, but induces systemic pathology in mice, which, in many respects, resembles the systemic disease caused in humans by *L. donovani*. Initial studies indicated that resistance or susceptibility to *L. major* might be determined by the activation of different classes of T helper lymphocytes, but later studies have revealed a complexity of responses. We have mapped genes controlling clinico-pathological and immunological manifestations of *L. major*-induced disease using recombinant congenic (RC) strains derived from the susceptible background strain BALB/c and the donor resistant strain STS/A. Each of 20 RC strains contains a different random subset of approximately 12.5% and 87.5% genes of a donor and a common background strain, respectively. We have mapped *Lmr* (*Leishmania major* response) genes in the most resistant strain CcS-5; an intermediate strain CcS-20; and a susceptible strain CcS-16. We found 17 novel *Lmr* (*Leishmania major* response) loci: *Lmr3-19* and described their effects on organ pathology and systemic immune reactions. These Lmr loci control thirteen different combinations of pathological and immunological symptoms. Seven loci control both organ pathology and immunological parameters, 10 influence immunological parameters only (Havelková et al. *Genes Immun* 2006; 7: 220). This finding extends the paradigm for the genetics of host response to infection to include numerous genes, each controlling a different set of organ-specific and systemic effects. Final molecular identification of *Lmr* genes can lead to understanding of the prevalent pathogenetics pathways associated with different *Lmr* genotypes with ensuing possibilities of individual therapy.

ENVIRONMENTAL STRUCTURE, WELFARE AND BEHAVIOR OF RHESUS- *(MACACA MULATTA)* AND BARBARY MACAQUES *(MACACA SYLVANUS)* IN CONDITIONS OF LABORATORY BREEDING, ZOO AND FREE RANGING

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Rhesus macaque *(Macaca mulatta)* and barbary macaque *(Macaca sylvanus)* are very often bred species of primates both in zoological gardens and in laboratory primates breeding centres. From the findings based on the observations of rhesus macaques in the laboratory breeding, and observations of barbary macaques in the conditions of zoo egress and free outdoor enclosure with the different type of enrichment, we can say that the enrichment of the cages results in expressively increased activity, although if it would be really effective, we must combine more types of enriching item, because macaques will quickly loose interest in toys. But every type of enrichment is welcomed. The comparison of all three types of housing brings interesting results, mainly in angle of composition of behaviour. Locomotive behaviour is in the condition of zoo runs composed mainly by active movement. Social environment, which is very important for all primates, is mostly applied in zoo, too. But it is necessary to become aware of fact, that laboratory breeds cannot provide full natural species-specific behaviour of animals, because there are limitations based on the types of many different experiments. But these experiments are inevitable parts of research work, especially in medicine, and that is why it is necessary to continually improve breeding environment of the laboratory animals.

EVALUATION OF ENVIRONMENTAL STRUCTURE ON THE BEHAVIOUR OF RHESUS MONKEYS *(MACACA MULATTA)* IN LABORATORY CONDITIONS

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In former years, the main goal for laboratory breed was to create simple and functional quarter for animals. The quarters had to contain only the animal and as few as possible of other elements, which could affect health and physiological processes of the animal and at the same time enable obtaining preferably undistorted output data. This goal was soon accomplished, but it was quickly found that the animals, although safe and with sufficient food, were not thrive well over physical and especially psychical side. It was especially noticeable on laboratory primates, who live in nature in groups with advanced social behaviour, and are also very locomotionally active. Some problem was in first zoological gardens. Therefore people started to more widely deal with the questions of animal welfare, their psychical and physical well-being, and the effort for enabling animals to behave on their natural species-specific behaviour. The research in this area still continues, and the question of animal welfare is getting into the consciousness of the public. In zoological gardens, the solution of this problem is simpler than in the environment of laboratory breeds, where exists much greater restrictions by virtue of the types of experiments. Though, even in laboratories, the living conditions of singly caged laboratory animals are improving. As our findings about complex ethology of primates, which can be very easily observed in laboratory conditions, are improving, the number of proposals for enrichment of breeding environment increases. These proposals can be used not only in laboratory breeds, but in every place where the primates are bred in captivity. In order to improve the quality of life for rhesus macaques at a research facility of rhesus macaques *(Macaca mulatta)* an improved and enriched housing system was introduced to ensure that the rhesus macaques are housed in compatible pairs in a complex and stimulating environment with access to individual outdoor enclosures. All animals are captive-bred at the facility and were raised and housed until selection in large harmonious families. Since frequent experimental procedures, like e.g. frequent dosing, blood sampling, food intake measurement, and weighing, under minimal distressed and controlled housing conditions without disturbing the entire former family group were needed, formation of adult male-female pairs was introduced to fulfil the animal's strong basic need for social companionship. The newly developed enriched indoor cages are made with flexible walls to enable either pair or group housing and a crush-back wall that combined with positive reinforcement training ensure safe, secure, and minimal stressful restraining of the animals for experimental procedures. Each double cage is furthermore connected to an enriched outdoor enclosure which allows the animals exercise options and a dynamic three-dimensional space to fulfil their natural arboreal behaviour related to foraging, exploration and vertical flight reaction. The newly developed housing system could covers the rhesus macaques´ basic natural needs to a much higher degree than traditional experimental housing system and thereby contributes to improved animal welfare.

EUROPEAN LEGISLATION NEWS: APPENDIX A OF EUROPEAN CONVENTION FOR THE PROTECTION OF VEREBRATE ANIMALS USED FOR EXPERIMENTAL AND OTHER SCIENTIFIC PURPOSES (ETS NO. 123)

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Since the adoption of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123) important progress has been made in science and new techniques. Therefore, a revision of the legislation was

urgently needed. Since 2002 the work to revise the Appendix A of the Convention began and the Revised Appendix A was issued in June 2007. The Appendix outlines minimum standards for the care, housing and welfare of animals used in laboratory based research. The revised Appendix A includes additional animal groups to those in the original and these are now also afforded protection under the Convention. The tables in the Appendix give details of standards of care and dimensions of animal enclosures. The full version of the Appendix was published (also in the Czech language) on the EU-website. FELASA (Federation of European Laboratory Animal Science Associations), in which take part also the Czech Laboratory Animal Science Association (SVLZ), issued the FELASA "EUROGUIDE". This booklet provides an abbreviated and "user-friendly" v of the revised Appendix A.

NEW INFECTIOUS AGENTS INVOLVED IN REGULAR HEALTH MONITORING OF LABORATORY RODENTS

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All breeding and experimental facilities should be regularly checked for presence of infectious agents. The worldwide exchange of transgenic animals between animal facilities increases the risk of contamination and positive findings of serious infection result usually to rederivation or long term and expensive therapy of animal colony. Since the year 2000, the list of agents regularly monitored should increase about at least three discovered important infections. Theirs presence have been also confirmed in the Czech Republic. **1. *Helicobacter sp. (H. hepaticus, H. bilis)***, method PCR, diagnostics from the year 2000. Positive findings with prevalence *H. hepaticus*, mainly at mice, including pathological lesions (hepatocytomegaly, cholangiohepatitis chronica, typhlocolitis, hepatocellular tumour). **2. *Rat Respiratory Virus* (RRV)**, unclassified virus, method histopathology of lungs at age 10-12 weeks, staining by H&E. Diagnostics from the year 2005. Positive findings sporadically. Pathological lesions: interstitial pneumonia with infiltration of mononuclear cells (mainly lymphocytes and macrophages) and with typical perivascular cuffs from mononuclears. Differential diagnostics of Paramyxoviruses (mainly Sendai virus) by serology is necessary. **3. *Mouse Norovirus* (MNV)**, non-enveloped RNA virus belongs to *Caliciviridae*. Antibodies against MNV detected by ELISA. Diagnostics from the second half of the year 2006. No pathological lesions are found at immunocompetent mice, but seroconversion is detectable very often. Some transgenic strains of mice (lacking interferons αβ and interferon γ receptors) are known extremely sensitive developing meningoencephalitis, cerebral vasculitis, pneumonia or hepatitis with final exitus.

SINGLE-PASS RAT SMALL INTESTINAL IN SITU PERFUSION METHOD AND THE POSSIBILITIES OF ITS UTILIZATION IN EXPERIMENTS

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The rat small intestinal in situ perfusion method is commonly used in pre-clinical experiments. This model enables to study the rate and the extent of absorption and biotransformation of xenobiotics in the condition of whole organ constitution. In this model, the native architecture of the small intestine is maintained with respect to the circulation such that the extents of metabolism, absorption, and secretion can be studied simultaneously. Perfusion can be carried out as a bilateral (both mesenterial system and luminal part are perfused) or as a unilateral (mesenterial or luminal part is perfused). The intestine can be ligated in various intestinal segments (for creation of closed loops) and following intraluminal single-dose drug administration can be studied the segmental absorption and thus potentially specified the absorption window. In this preparation, the mesenterial system (arteria mesenterica cranialis – inflow; vena portae - outflow) and/or the intestinal lumen are cannulated. The perfusion proceeds under the constant condition (perfusate flow and pH, thermo-stable box). In our laboratory, we studied the competition influence of L-carnitine on the rate of absorption of acetylcholinesterase inhibitors, the usage of methotrexate in the differentiation of the mechanisms of intestinal absorption of various substances. Further, we especially examined the bioequivalence of the atypical drug formulations (suspension, core). The bioequivalence of these drug formulations in the perfusion in situ conditions were compared with the results in vivo. These findings give a good perspective for the utilization of this experimental model as a tool for the pre-clinical predictive bioequivalence testing.

EXPERIMENTAL PIG: THE RELATIONSHIP BETWEEN INTESTINAL DESINTEGRATION OF TABLETS DRUG FORMULATIONS SCANNED WITH MICROCAMERA AND BIOAVAILABILITY OF ACTIVE COMPONENT IN SYSTEM CIRCULATION

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The process of dissolution of two different tablet batches (technology: „A" = pressed granules, „B" = pressed micropellets) after their endoscopic intraduodenal administration in anaesthetized pigs was scanned using diagnostic microcamera (capsule endoscopy „EndoCapule Olympus", scanning frequency of 2 pictures/sec.). The nine-hourly limit of microcamera function enabled to observe the disintegration process until the caecum reaching. Simultaneously, the plasma time profiles of active component („N") desintegrated from the tablets were detected. The desintegration of tablet „A" was perceptible in duodenum (till 60th min) and culminated in proximal jejunum (at 3rd hour). Just single grains from desintegration granulate were noticeable in distal jejunum (at 4th hour). The plasmatic level of „N" was detectable from 30th min, the maximal concentration in the form of peak reached at 3rd hour. There was observed the rapid bioelimination decrease between 4th and 5th hour. Tablet „B" was partly desintegrated in duodenum (till 2nd hour), the release into the particular fragments reached in jejunum (between 3rd and 6th hour). The pellets were fractionalized into the suspension in terminal ileum (at 8th hour). The maximal plasmatic concentration of „N" expressed a character of „steady state" (between 3rd and 8th hour) but reached of maximum at 30% lower in comparison with tablet „A".

The demonstration of relationship of time dimensions between desintegration view of solid dosage form in the intestine and the released drug bioavailability give a methodical promise for research of absorption mechanisms of xenobiotics in different small intestinal regions.

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OXYURIDA IN THE COLONIES OF LABORATORY RODENTS AND LAGOMORPHS

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Laboratory rodents and lagomorphs are one of the most common laboratory animals, used in experiments worldwide to evaluate different biological parameters. Therefore, the quality and reliability of laboratory animals have a major influence on research and its benefits on human health. Animals that are standardized as much as possible are important prerequisites for reproducible animal experiments. Only healthy, well-cared for animals yield valid scientific data and thus enjoin us to the highest standards of care. Pinworms (*Nematoda: Oxyurida*) are common contaminants in most laboratory rodents and lagomorphs colonies. Every conventional colony is probably infected with oxyurids. Important and common oxyurids affecting laboratory

rodents and lagomorphs are *Syphacia spp*. (mouse, rat and hamster), *Aspiculuris tetraptera* (mouse, rat), *Paraspidodera uncinata* (guinea pig) and *Passalurus ambiguus* (rabbit). Although pinworm parasites of laboratory rodents are generally considered to be relatively non-pathogenic, and infections are generally regarded as symptomless, no specific clinical signs appear even in heavy infections. It has been suggested that infections affect weight gain, growth rate, and general health. Various disorders of intestine, as impaction, intussuspection and rectal prolapse were associated with heavy infection. Pinworm infestation has been however found to interfere with the occurrence of induced adjuvant arthritis and with intestinal electrolyte transport. The aims of this study are a brief overview of common Oxyurids species occurring in laboratory rodents and lagomorphs, their life cycles, mode of transmission and ways of eradication.

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COMPARISON OF HEALTHY AND HHTG (HEREDITARY HYPERTRIGLYCERIDEMIC) RAT MODELS IN THE STUDIES OF LIPID METABOLISM

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HHTg (hereditary hypertriglyceridemic) rats were developed as a genetic model of human hypertriglyceridemia from the colony of Wistar rats. They exhibit elevated fasting and postprandial triglycerides, impaired glucose tolerance, hyperinsulinemia, insulin resistance and oxidative stress so they can be used as a model of metabolic syndrome. Our goal was to compare expression of several genes involved in lipid metabolism in healthy rats with expression in HHTg rats on either standard laboratory diet (STD) or high-cholesterol (1% w/w) diet (HCD). Rats were fed ad libitum for 3 weeks with STD or HCD (n=5-7). RNA was isolated from liver samples, reverse transcribed with SuperScript II and submitted to real-time PCR using SYBR Green PCR Master Mix in ABI PRISM 7700 Sequence Detection System. Data was normalized to hypoxanthine-guanine phosphoribosyltransferase (HPRT). Genes of interest were: peroxisome proliferator-activated receptor (PPARα), acyl-CoA oxidase (ACO), cytochrome P450 4A1 a 4A2 (CYP4A1, 2), liver X receptor (LXRα), sterol regulatory element-binding protein (SREBP-1c), CYP7A1, ATP-binding cassette (ABCA1, ABCG5, 8) and fatty acid synthase (FAS). Genes with significantly higher expression in HHTg rats: STD-CYP7A1 (3.4-fold) HCD - CYP7A1 (4.1-fold), ABCA1 (1.8-fold), SREBP-1c (3.4-fold) Genes with significantly lower expression in HHTg rats: STD - ABCG5 (1.8-fold), ABCG8 (2.4-fold), FAS (1.5-fold) HCD - ABCG8 (2.2-fold) Genes with no significant change in expression in HHTg rats: PPARα, LXRα, ACO, CYP4A1, CYP4A2. Our data shows differences in basal expression of genes important for lipid metabolism in HHTg rats and different reaction of each model in regulation of expression of these genes after increased intake of cholesterol.

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PRE-CLINICAL EVALUATION OF VETERINARY MEDICINAL PRODUCTS – RESIDUE STUDIES UNDER THE CONDITIONS OF GOOD LABORATORY PRACTICE

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Residues of veterinary medicinal products and their metabolites remaining in human food made from animals treated with these products are strictly controlled by European law. Residue studies in farm animals should fulfil not only the legislative condition but also routine conditions of the veterinary practice. This article evaluates two different dosage regimens of a Veterinary Medicinal Product (VMP): A) Dosage of VMP per 1 kg of body weight – VMP should be administered in such volumes of water or feed that are voluntarily consumed by the animals without leftovers. In the case when some leftovers occur, use of a gastric tube becomes necessary. This way ensures precise dosing of the VMP, reliable and unified term of dosing cessation and, hence, homogeneous data for a statistical evaluation. B) Dose of the VMP per 1 litre of drinking water or 1 kilogram of food – In this case, the VMP is administered via water or food *ad libitum* usually with slight overdose in attempt to create so-called worst-case situation and to ensure that all experimental animals receive at least the recommended dose. Comparing Dosage Regimens A and B is can be concluded that Dosage Regimen A ensures more accurate dosing for all experimental animals and is in compliance with the rules of the Good Laboratory Practice. On the other hand, Dosage Regimen B does not ensure accurate dosing for all animals, but considering that under-dosing is eliminated, it can be acceptable for drug registration. Moreover, results obtained using Dosage Regimen B correspond more accurately with the routine veterinary practice.

THE CLINICAL EVALUATION OF LOCAL TOLERANCE AT THE INJECTION SITE AFTER ADMINISTRATION OF VETERINARY MEDICINAL PRODUCTS

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The article presents two similar methods of clinical evaluation at the injection site: 1) Percentage evaluation of local reactions – Ratio of animals with to animals without tissue lesions is expressed as percentage. Swelling extent is measured in cm^2 (score of 1 to 4, with 1 = normal and 4 >10 cm^2). Pain at the injection site is also evaluated using score (1 = no pain and 4 = severe pain). 2) Score evaluation of local reactions – Incidence of tissue lesions is estimated by means of following parameters: temperature (score: 0 = normal, 1 = warm, 2 = hot), hardness, swelling (score: 0 = normal, 1 = very slight, 2 = slight, 3 = moderate, 4 = severe), pain (score: 0 = none, 1 = slight, 2 = moderate, 3 = severe). Swelling extent is measured in cm^2. Clinical examination consists of visual check of swelling characteristics, pain intensity, increased tissue density upon palpation, temperature (heat at he injection site), and measurement of the injection site with a calliper. During a study, clinical examination is assessed at scheduled post-treatment intervals (4, 24, 48, 72 and 96 hours, in case of lesion persistence pending 14 days). Reversibility or irreversibility of the changes is considered. This examination is accompanied with serum biochemistry and gross pathology and histopathology examination at the injection site. The both described methods of clinical evaluation of the local tolerance are part of the packet of pre-clinical tests and both are accepted by European Authorities.

ORGAN DISTRIBUTION OF L-CARNITINE UPON ITS VARIABLE DOSING IN RAT

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We have recently reported that L-carnitine (L-CAR) enhances the inhibitory effect of 7-methoxyacrine on acetylcholinesterase in the CNS. We have shown the existence of a therapeutic window with a maximum at the dose of 300 mg/kg. The enhancement of the inhibitory effect was not significant at both higher and lower doses of L-CAR. In order to shed more light on this observation, L-CAR pharmacokinetics was monitored (according to plasma levels) within 24 hrs following the administration of 300, 600 a 1200 mg/kg doses p.o. Subsequently, a kinetic biodistribution study (in the liver, heart and brain) was performed after p.o. administration of the same doses. The concentrations of free and esterified L-CAR in the plasma and the tissues were determined by HPLC with the following results: a) a higher

L-CAR dose led to a proportional increase of its plasma levels (linear pharmacokinetics); b) in a dose-dependent fashion, the level of free L-CAR in the liver increases, while that of esterified L-CAR remains the same (probably due to the biotransformation capability of the liver); c) the highest L-CAR concentration is in the heart, where the administration of a higher dose does not give rise to a higher L-CAR concentration level (the high endogenous L-CAR levels may prevent further influx); d) increasing the dose caused decrease of the level of free L-CAR in the brain tissue, with the highest level of free and simultaneously the lowest level of esterified L-CAR being reached upon the administration of the dose of 300 mg/kg.

THE USE OF CONFOCAL LASER SCANNING ENDOMICROSCOPY IN EXPERIMENTAL PIGS - THE INITIAL EXPERIENCE IN THE CZECH REPUBLIC

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Confocal laser scanning endomicroscopy (CLSE) has to be used for microscopic imaging at a quality approaching that of histopathologically prepared samples. CLSE becomes possible due to the integration of a miniaturized confocal microscope in the distal tip of a conventional endoscope (Pentax and Optiscan Imaging). During CLSE a laser light source delivers blue excitation light at a wavelength of 488 nm. Fluorescence substances in the tissue absorb this light and emit green-yellowish light at a longer wavelength 510 – 580 nm by themselves. Only fluorescence light coming from a specific focal plane is detected afterwards by the endomicroscopy system. Light from outside the focal plane is rejected. This results in high-resolution images providing an excellent image quality for visualization of tiniest details. Digital images of cells magnified 1000-fold appear in real time on a computer screen, which enables immediate detection of changes in cellular structure without the need for a biopsy. Due to this magnification the endoscopic images enable recognition of structures to the size of cell nuclei. The strength of CLSE is not only the high magnification of tissue structures but also the possibility to acquire images. CLSE images are showing the horizontal view of approximately the upper two thirds of the mucosa (250 µm). The optical slice thickness is 7 µm, with a lateral resolution of 0.7 µm. Confocal image data are collected at a scan rate of 0.8 frames per second (1024 x 1024 pixels) or 1.6 frames per second (1024 x 512 pixels). The field of view is 500 x 500 µm, and the range of the z-axis is 0 – 250 µm below the surface layer. We have worked up a method of ex-vivo CLSE of the oesophagus, stomach, small and large intestine in experimental pigs after previous i.v. administration of 10% acriflavine (7.5 mg per kg). The introduction of this method is a starting point for further experimental studies.

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LOCAL AND SYSTEMIC CYTOKINE RESPONSE IN THE COURSE OF INFECTION

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Cytokine response in the course of infection may be valuable prognostic tool. Inflammatory cytokines are elevated in most cases locally whilst plasma cytokines are very sophistically downregulated with the exception of "*cytokine storm*" in life threating septic state. Gnotobiotic pig model used in our laboratory for studies of cytokine pattern in infectious states consists of two unique models: **foetuses** and **germ-free pigs**. Inoculation of gramnegative bacteria into the amnion of pig foetuses demonstrated extensive ability of the amniotic epithelium to produce various inflammatory cytokines. Concentrations of interleukin-18 in amniotic fluid have shown tight correlation with the virulence of gramnegative bacteria used for the inoculation and with the damage of amniotic epithelium as confirmed by electron microscopy. So, IL-18 level in amniotic fluid could serve as a prognostic marker of intrauterine infection. Germ-free pigs can be used also as an exquisite model for infectious diseases in immunodeficient patients, especially due to their immature adaptive immunity in the gut and absence of antibodies in this site. Such a model has actually great relevance due to an enormous increase of non-typhoidal *Salmonella* which causes 1.3 billion cases of intestinal disease including 3 million deaths annually with bacteremia in 50% of infected individuals in HIV endemic regions. Gnotobiotic pigs were used as a model of human *Salmonella* enteritis and systemic disease and human enteritis caused by enteropathogenic *E. coli.* The convenience of germ-free pigs for cytokine studies is supported by the uniformity and reproducibility of results obtained from these animals.

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THE ROLE OF ANIMAL MODELS IN PSYCHIATRIC RESEARCH

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Animal models are very useful tools in the research of human diseases. Although modelling the full symptoms of mental diseases is indeed unrealisable, a significant amount of evidence has documented some parallels in brain morphology, neurochemistry and behaviour between human beings and other mammals. This shows that many features of human behaviour are shared with other mammal species, due to common evolutionary process. Behaviour as a reflection of brain activity then becomes a suitable instrument for searching for similarities to pathological processes underlying human diseases, including psychosis. Models of schizophrenia, which is thought to be a major psychosis, are prominently based on a pharmacological, neurodevelopmental or genetic approach. The vast majority of laboratory animals used in psychiatric research are rodents, with whom we can evaluate many motor patterns and postures. Hyperlocomotion in rodents is thought to be equivalent to positive symptoms in humans. Moreover, an exploratory activity in open field tests can be used as a marker of vulnerability to stress or anxiety. Social withdrawal can be investigated in tests using some dyadic social interactions. Prepulse inhibition of the startle response is a valid method for testing sensorimotor gating, which is impaired in patients suffering from schizophrenia. Finally, mechanisms of cognitive impairment can be experimentally examined in tests utilising methods based on working memory and learning. We conclude that animal models play an important role in psychiatric research. However, the quality of experimental results is closely connected with the level of understanding of the behavioural biology and ethology of the species used.

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THE ROLE OF INFECTION IN A NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA

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The etiology of schizophrenia (SCH), a complex neuropsychiatric disorder, is still unclear, but environmental insults during pregnancy are associated with its late manifestation. Primarily maternal infectious processes, both bacterial and viral in origin, are involved in negative insults of on-going brain development. The induced alterations are connected with activation of microglia and the immune system. The activated microglia produce a broad spectrum of neuroactive and neurotoxic compounds, including proinflammatory cytokines (including quinolinic acid, QUIN), with long-term effects on the neuronal state. The aim of this study was to determine the late consequences of neonatal exposure to the bacterial endotoxin lipopolysaccharide (LPS) and pro-viral factor gp120 (an antigenic marker of retroviral infection)

in comparison with the effect of QUIN in an animal model of SCH. Male rats pups (N = 100) received a daily i.p. injection of either the examined drugs (LPS - 2mg/kg; gp120 - 10 μg/kg; QUIN - 10mg/kg) or saline on postnatal days (PD) 4 - 8. The young adult rats (PD50) were assessed for schizophrenia-like behaviour (prepulse inhibition, PPI). Both LPS and gp120 induced impairment in prepulse inhibition. In contrast, no alteration of PPI was registered in QUIN- treated rats. We summarize that the neonatal (systemic) administration of bacterial endotoxin or viral protein alters some indicators of psychotic-like behaviour in postpubertal rats that are relevant to SCH.

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GENES CONTROLLING CYTOKINE PRODUCTION AND THEIR RELATIONSHIP TO SUSCEPTIBILITY TO INFECTIOUS DISEASES

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Cytokines interleukin 4 (IL-4) and IL-10 are implicated in an array of biologic effects on several cell lineages. The imbalance of cytokine expression has been observed in infectious, atopic and autoimmune diseases, and in some cancers, which aroused interest to regulation of this process. Genotype belongs to important factors influencing cytokine level. Mouse strains BALB/c and STS differ in production of IL-4 and IL-10 after stimulation of splenocytes by Concanavalin A; BALB/c and STS being a high and a low producer, respectively. To map genes responsible for these strain differences, we used recombinant congenic (RC) strains of the BALB/c-c-STS/Dem (CcS/Dem) series. Each of the twenty CcS/Dem strains carries a different, random set of approximately 12.5% genes of the donor strain STS on the background of BALB/c genes. RC strain CcS-20 with intermediate expression of both IL-4 and IL-10 between BALB/c and STS was selected for further studies. Analysis of (CcS-20xBALB/c)F2 hybrids led to mapping of a locus *Cypr1* (cytokine production 1) that controls IL-4 production, and loci *Cypr2* and *Cypr3*, which influence production of IL-10. In addition, the relationship between the levels of these two cytokines depends on a locus *cora1* (correlation 1) (Kosařová et al. *Immunogenetics* 1999; 49:134). *Cypr1-3* and *cora1* co-localize with loci that determine resistance to bacteria and parasites (Havelková et al. *Genes Immun*. 2006, 7:220; Lipoldová and Demant, *Nat. Rev. Genet*. 2006; 7:294). The co-localization of loci controlling cytokine level and resistance to infections suggests that these resistance genes operate through regulation of cytokine expression.

METABOLIC CHANGES IN LIVER AND THYMUS INTERSTITIUM OF RATS WITH CHRONIC LIVER FAILURE - USE OF MICRODIALYSIS METHOD IN EXPERIMENT ON LABORATORY RATS

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Microdialysis is method enable to follow metabolic changes directly in interstitial space of tissues. We compared substrate utilisation in rats with liver fibrosis or cirrhosis using microdialysis. We followed interstitial concentration changes of urea, glucose, lactate and glycerol in peritoneal cavity, liver and thymus. Methods: Male Wistar rats (n=18) were divided into 3 groups. Group INT: intact rats. Group BDL: rats 14 days after bile duct ligation. Group CIRH: liver cirrhosis was induced by chronic tetrachloromethane administration. Under pentobarbitale anaesthesia microdialysis probes CMA 20 was inserted into organs, perfused by Ringer's solution (75μl/h), samples were collected during 6 hours. Statistical analyses were performed using SigmaStat (Jandel Scientific, CA, USA). Results: Periodical degradation of pentobarbitale by intact liver and fluctuating depth of anaesthesia increases interstitial blood perfusion. Glucose (mmol/l) decreased especially intraperitoneally in BDL (0.80…0.61). Glucose in liver was higher in INT (1.11…0.90) versus BDL (0.56…0.38) or CIRH (0.95…0.36). Thymic lactate (mmol/l) did not decrease in INT (0.30…0.25 - this signifies thymocyte proliferation) versus CIRH (0.22…0.10). Thymic glucose was stable in INT (0.81…0.71) but not in BDL (0.80…0.45) and CIRH (0.63…0.47). Lower availability of glucose and lactate production was in BDL and CIRH (thymocytes not proliferated). **Conclusions:** Liver damage influenced metabolism of basal metabolites in distant organs. Glucose, lactate and glycerol are essential for cell proliferation. Decrease of interstitial concentrations of these metabolites could be an explanation of damages in distant organs. The impairment of glucose metabolism should be the cause of pathophysiological changes in thymus.

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BONE MINERAL DENSITY IN RATS WITH CHANGES OF IRON METABOLISM

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Aim of study was to assess the influence of dietary iron overload and repeated blood withdrawals in male Wistar rats on bone mineral density (BMD) in different regions of interest: R1 – distal spine, R2 – proximal tail, R3 – femur. Methods. Male Wistar rats (n=32) were fed by standard laboratory diet (SD, 27 mg Fe/kg) or by iron enriched diet (FE, 400 mg Fe/kg). Chronic iron losses were induced by repeated blood withdrawals (-w signed groups) from retroorbital plexus. SD and FE group underwent one blood withdrawal in 9th week of experiment, groups SD-w and FE-w underwent the blood withdrawal once a week, 9 times total. All rats were sacrificed by exsanguination from abdominal aorta and BMD (g/cm^2) was measured by Hologic Delphi A (Hologic, Waltham, MA, USA). Statistics: *t-test* using SigmaStat software (Jandel Scientific, CA, USA). Results are presented as mean±SEM. Results. BMD of SD group: R1 0.217±0.004; R2 0.210±0.008; R3 0.181±0.009; FE group: R1 0.222±0.003; R2 0.217±0.007; R3 0.185±0.015; SD-w group: R1 0.227±0.006; R2 0.222±0.005; R3 0.181±0.012; FE-w group: R1 0.230±0.005 ($p<0,05$ vs. SD); R2 0.234±0.004 ($p<0,05$ vs. SD); R3 0.176±0.007 ($p<0,05$ vs. FE). Conclusions. Male Wistar rats fed by iron enriched diet with blood withdrawals had in comparison with rats without blood withdrawals and rats on standard diet: the increase of BMD in distal spine and tail region and not significant decrease of BMD in femoral region. We stated that higher iron turnover with stimulation of haematopoesis support bone formation in spine vertebrae.

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INFLUENCE OF DIFFERENT DIETS ON LIVER DAMAGE IN WISTAR RATS

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Aims: 1) to prepare standard laboratory diet (SLD), diet enriched with 4 % of cholesterol (CHOL) or with 1 % of orotic acid (ORO), and methionin-choline deficient diet (MCDD) 2) to observe biochemical markers of liver damage in serum. **Methods:** After institutional approval 24 male Wistar rats were divided into four groups and fed by specially prepared defined diets during 29 days *ad libitum*. Diets were prepared in our laboratory and differ in the only one parameter (higher cholesterol, orotic acid, methionin-choline deficiency). Components used for diet preparation were: milk casein, tree fiber, maize starch, maize oil, sugar, mix of vitamins and minerals (with or without DL-methionin and choline-chloride). We prepared diets by used kneading-trough, meat-mincer and fruit-drier. The hardness of diets was sufficient

for comfortable teeth abrasion. Rats were sacrificed by exsanguination from abdominal aorta and obtained serum was used for cholesterol, HDL-cholesterol, LDL-cholesterol, triglyceride concentration (mmol/l) and ALT activity (μkat/l). **Results:** Total cholesterol was in SLD 2.28±0.33, CHOL 3.05±0.22, ORO 1.95±0.24, MCDD 1.78±0.08. HDL-cholesterol was in SLD 1.37±0.13, CHOL 1.07±0.06, ORO 1.51±0.17, MCDD 0.93±0.06. LDL-cholesterol was in SLD 0.21±0.08, CHOL 0.54±0.05, ORO 0.11±0.02, MCDD 0.13±0.02. Triglyceride was in SLD 1.41±0.21, CHOL 3.27±0.43, ORO 0.65±0.17, MCDD 2.32±0.19. ALT in SLD 0.75±0.09, CHOL 0.71±0.07, ORO 1.74±0.40, MCDD 0.55±0.16. **Conclusions:** Cholesterol rich diet leads to significant increase of total cholesterol and glucose serum concentrations. Orotic acid impaired liver LDL particle metabolism and leads to steatotic liver damage with ALT elevation.

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THE INFLUENCE OF DIETS INDUCING LIVER STEATOSIS ON METABOLIC CHANGES IN WISTAR RATS

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Aims: We prepared standard laboratory diet (SD), diet enriched by 4 % of cholesterol (CHOL) or by 1 % orotic acid (OA), and methionin-choline deficient diet (MCDD). We observed serum markers of lipid metabolism and liver damage. **Methods:** We used 24 male Wistar rats fed by below mentioned defined diets during 29 days ad libitum. Diets were prepared in our laboratory and differentiated only in one parameter (cholesterol, orotic acid, methionin-cholin). For diet preparation we used: milk casein, tree fiber (cellulose), maize starch, maize oil, sugar, mix of vitamins and minerals (with or without DL-methionin and cholin-chloride). Rats were sacrificed by exsanguination from abdominal aorta and serum was used for determination of selected biochemical markers. Statistics: unpaired t-test, OneWayANOVA SigmaStat (Jandel Scientific Corp.). **Results:** Total cholesterol concentration in groups (mmol/l): SD 2.28±0.33; CHOL 3.05±0.22 ($p<0.01$ vs. OA, MCDD); OA 1.95±0.24; MCDD 1.78±0.08. HDL-cholesterol (mmol/l): SD 1.37±0.13; CHOL 1.07±0.06; OA 1.51±0.17; MCDD 0.93±0.06 ($p<0.05$ vs. OA); LDL-cholesterol (mmol/l): SD 0.21±0.08; CHOL 0.54±0.05 ($p<0.05$ vs. OA); OA 0.11±0.02; MCDD 0.13±0.02. Triglyceride (mmol/l): SD 1.41±0.21; CHOL 3.27±0.43 ($p<0.001$ vs. all groups); OA 0.65±0.17; MCDD 2.32±0.19. Glucose (mmol/l): SD 5.93±0.21; CHOL 8.15±0.28 ($p<0.01$ vs. SD); OA 8.25±0.47 ($p<0.01$ vs. SD); MCDD 7.01±0.70. ALT (ukat/l): SD 0.75±0.09; CHOL 0.71±0.07; OA 1.74±0.40 ($p<0.01$ vs. all groups); MCDD 0.55±0.16. **Conclusions:** Increased cholesterol content in diet leads to significant lipid and glucose changes in serum. Orotic acid inhibited of LDL production in liver, this leads to liver damage.

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CHANGES OF CHOLESTEROL METABOLISM IN RATS WITH CHOLESTEROL ENRICHED DIET

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The aim of study was to assess impact of long-term dietary cholesterol overload on cholesterol metabolism and liver regeneration in rats. Methods. After institutional approval male Wistar rats were divided into 4 groups. Rats were fed with standard laboratory diet (SD) or by cholesterol enriched diet (CHOL) during 29 days. Partial hepatectomy (PH) was performed on 28th day of experiment. The ^{14}C-cholesterol was administered p.o. 1 hour after PH, and ^{3}H-thymidin i.v. 1 hour before rats were sacrificed (18 or 24 hours after PH, i.e. groups: 18-SD or 24-SD, and 18-CHOL or 24-CHOL). Liver DNA synthesis (by ^{3}H-thymidin), cholesterol concentration were estimated in serum (mmol/l) and ^{14}C-cholesterol activity in liver (Bq/g of liver tissue). Statistics: *t-test* (mean±SEM) was performed using SigmaStat software (Jandel Scientific, USA). Results. Synthesis of liver DNA (Bq/mg DNA): 18-SD: 2.29±0,44 ($p<0.05$ vs. 18-CHOL); 24-SD: 11.21±1.10 ($p<0.001$ vs. 24-CHOL); and 18-CHOL: 1.11±0.26; 24-CHOL: 6.09±0.82. Liver ^{14}C-cholesterol: 18-SD: 1467±235; 24-SD: 1895±402; and 18-CHOL: 1082±159; 24-CHOL: 845±162; serum cholesterol: 18-SD: 1.39±0.13 ($p<0.05$ vs. 18-CHOL); 24-SD: 1.01±0.07 ($p<0.001$ vs. 24-CHOL); 18-CHOL: 1.94±0.13; 24-CHOL: 1.32±0.08. Conclusion. Partial hepatectomy led in SD rats to decrease of serum cholesterol concentration associated with increase of exogenous cholesterol absorption from intestine.

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LIVER REGENERATION IN RATS WITH LIVER STEATOSIS INDUCED BY STEATOGENIC DIETS

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Aim of study was to determinate the influence of diets on liver regeneration after partial hepatectomy (PH) in Wistar (W) and Prague hereditary hypercholesterolemic rats (P). Methods: Male W and P rats were divided into groups and fed by followed diets for 29 days: group 1: standard laboratory diet (W-SD, P-SD), group 2: diet with 4 % of cholesterol (W-CHOL, P-CHOL), group 3: methionin-cholin deficient diet (W-MCDD, P-MCDD), group 4: diet with 1 % of orotic acid (W-OA, P-OA) and then PH was performed. The rats were sacrificed 24 hours after PH. We estimated serum insulin concentration (ng/ml, EIA, RD Systems, USA) before and after PH. Liver DNA synthesis was determined by methyl ^{3}H- thymidine (Bq/mg DNA, Beckman Coulter, USA). Statistics: *t-test* (mean±SEM) was performed using SigmaStat software (Jandel Scientific, USA). Results: Rats of both strains fed by CHOL and OA diets had liver steatosis. Insulin before PH: W-SD 1.94±0.24; W-CHOL 1.13±0.20; W-MCDD 1.92±0.22; W OA 2.51±0.31; P-SD 0.68±0.17; P-CHOL 0.80±0.27; P-MCDD 0.94±0.20; P-OA 1.79±0.43; insulin after PH: W-SD 1.23±0.13; W-CHOL 1.13±0.17; W-MCDD 1.48±0.19; W OA 0.46±0.16; P-SD 0.29±0.08; P-CHOL 0.46±0.12; P-MCDD 0.29±0.06; P-OA 0.11±0.03. Synthesis of liver DNA after PH: W-SD 11.21±1.10; W-CHOL 6.09±0.82; W-MCDD 11.27±1.24; W-OA 3.44±0.64; P-SD 2.92±1.27; P-CHOL 4.16±1.00; P-MCDD 5.44±1.17; P-OA 2.44±1.07. Conclusion: The liver regeneration was delayed by dietary induced liver steatosis in both strains. Serum insulin concentrations were lowest after PH in rats P, and P rats had delayed liver regeneration in comparison with W rats.

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