SPOLEČNOST PRO VĚDU O LABORATORNÍCH ZVÍŘATECH

CLASA

Proceedings of the 23rd Conference on Laboratory Animals Science

September 29 – October 1, 2021
Olomouc, Czech Republic

The 23rd Conference on Laboratory Animal Science, organized by the Czech Laboratory Animal Science Association CLASA (Společnost pro vědu o laboratorních zvířatech SVLZ), was held on September 29 - October 1, 2021, in Hotel Flora, Olomouc, Czech Republic. More than 100 scientists, veterinary experts, representatives of biomedical research organizations, universities and animal welfare authorities participated in the meeting. The presented lectures were focused on laboratory animal welfare and protection, new experimental methods and procedures and the current status of alternative *in vitro* methods as replacement of animal experimentation according to the 3Rs principle. Representatives of animal welfare authorities shared information on the implementation and amendments of regulations concerning the protection of animals used for scientific purposes.

THE ANIMAL CENTER FNUSA-ICRC: MICE AND RATS BEHAVIORAL RESEARCH LABORATORY IN BRNO

Bartelik, A.[1], Odehnalová, E.[1]
[1]*International Clinical Research Center, St. Anne's University Hospital Brno, Brno, Czech Republic*

The Animal Center (AC) is a part of the International Clinical Research Center, St. Anne's University Hospital Brno (FNUSA-ICRC) and, as a core facility, performs *in vivo* procedures and provides specialist equipment for scientific teams. AC was established in 2010 as a joint project of collaborating institutions, primarily focused on experiments on large animals. Through cooperation with the Institute of Molecular and Translational Medicine in Olomouc, in 2019, a behavioral laboratory for mice and rats was created. The team of AC provides expertise in the design of *in vivo* experimental plans, selection of suitable animal species for the experiment, prepares and conducts the experimental procedures, collects samples. Offers professional services and consults animal research experiments in neurology, surgery, neurosurgery, dermatology and cardiology, also for the pharmaceutical industry to test newly developed pharmaceuticals and medical devices. As well as AC supports the process of licensing and authorization of projects to the Ethical Committee and supervises the procedures. The AC behavioral laboratory enables carrying out procedures on rodents with the use of sophisticated instruments: IntelliCage, PhenoMaster, MotoRater and MultiConditioning. These systems give the possibility to perform tests of animals' activity, their emotionality, learning and memory skills, social behaviors, locomotor activity, metabolic parameters as feeding and drinking analysis, body weight monitoring, behavioral changes in pharmacological treatment, both short-term and long-term observations, up to several weeks in one experimental process, and phenotyping of new transgenic animal models. The equipment enables to provide procedures with animals and to observe their behavior with minimum human intervention. All these means allow for more reproducible data, minimizing animal stress, and improving animal welfare according to 3R rules. Our AC is involved in cooperation in international projects, as INTERREG, COST_TEATIME, which give the opportunity to develop common solutions and test guidelines, which have an impact on the repeatability of experiments on animals.Information is available on our website: https://www.fnusa-icrc.org/animal-center/.

Supported by the INTERREG program V-A Austria – Czech Republic, project no. ATCZ40 and by the project no. LQ1605 from the National Program of Sustainability II (MEYS CR).

DYSFUNCTION OF VAGAL AFFERENT NERVES IN RESPIRATORY AND GIT DISORDERS

Brozmanova, M.[1], Konarska, M.[1], Plevková, J.[1]
[1]*Department of Pathophysiology, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic*

Excessive and irritable coughing associated with airway inflammation belongs to pathological cough. A variety of mediators associated with airway inflammation overstimulate vagal airway fibres including C-fibres leading to airway hypersensitivity and hyperreactivity. Airway afferent nerves are in connection with the gastrointestinal tract including esophageal sensory afferent nerves. Like airway C-fibres, stimulation of esophageal nociceptive C-fibres are involved in visceral pain. Effective antitussive and antinociceptive therapy is an important but largely unmet medical need. Our long-term goal was to contribute to the understanding of the mechanisms of activation and hypersensitivity of vagal afferent nerves in respiratory and esophageal diseases. We focused on receptors detecting inflammation and other noxious stimuli in these tissues. We used a cough challenge model in awake guinea pigs with experimental model of inflammation using standard tussigen stimuli such as citric acid or capsaicin before and after pretreatment with tested receptor inhibitors. On the other side, extracellular electrophysiology of vagal nerves was used with stimulation of receptors using selective agonists and antagonists. We found increased sensitivity of cough reflex in guinea pigs with experimental allergic rhinitis that was inhibited by pretreatment with leucotrienes antagonist. The TRPA1 activation-initiated cough was relatively modest compared to cough initiated by the TRPV1 activation. In accordance with cough studies, *ex vivo* single fibre recordings showed that TRPA1 agonists were approximately 3-times less effective than capsaicin in evoking sustained activation of the cough-triggering tracheal jugular C-fibres. Preinhalation and continuing inhalation of NaV1.8 inhibitor A-803467 blocked capsaicin-induced cough ($p<0.01$). A similar response was observed in electrophysiological studies where the bradykinin-induced action potential discharge in jugular C-fibres was by 50 % inhibited by NaV1.8 blockers. Long-term or intensive action of pro-inflammatory and pro-nociceptive mediators on nerve endings in the respiratory and gastrointestinal systems results in dysfunction of the vagal nerves manifested by increased activation and hypersensitivity, leading to excessive regulation of cough reflex and visceral nociception. Recent scientific findings support a concept that targeting voltage-gated sodium channels NaV1.7 and NaV1.8 is a rational strategy forward for the effective treatment of pathological cough and visceral pain.

This work was supported by VEGA 1/0020/19.

DEVELOPMENT OF THE NUMBER AND SPECIES OF EXPERIMENTAL ANIMALS IN THE USER FACILITY OF UNIVERSITY OF VETERINARY SCIENCES BRNO

Hostovský, M.[1], Hostovská, L.[1]
[1]*Department of Animal Breeding, Animal Nutrition and Biochemistry, Faculty of Veterinary Hygiene and Ecology, University of Veterinary Sciences Brno, Czech Republic*

The use of experimental animals in science and research remains an essential means of understanding the basic mechanisms underlying research into prevention, diagnosis and treatment of human and animal diseases. The basis of quality research to the highest standards of animal care and all experiments should include 3R: replacement, reduction and refinement. However, these three principles have also proved to be an area of common ground for researchers who use animals and for those who oppose their use. It has also been recognized that the acceptance of 3Rs can improve the quality of science and well-designed experiments can minimize the number of used animals and excessive stress, pain or suffering, also often provide better and more reliable data. The principle of reduction seems to be the least controversial, but its application has highlighted the difficulties in providing appropriate professional statistical methods, especially in academic research facilities. In some cases, concern about implementing reduction strategies can lead to the use of a few animals, leading to inconclusive results and failed experiments. The main objective of this study was to evaluate the number of experimental animals used in the user facility of the University of Veterinary Sciences Brno in terms of experimental animal species and purposes of experiments in the period 2009-2019, determine trends in the use of experimental animals, evaluate statistically significant differences and compare data with available statistics of used experimental animals issued by the Ministry of Agriculture of the Czech Republic. Another goal was to check, based on the obtained results, whether in the academic sphere there is a reduction in the number of animals included in the experiments and thus the fulfillment of the 3R concept. In the monitored period 2009-2019, a total of 89,963 experimental animals were used in the user facility of the University of Veterinary Sciences Brno, when fish were the most used with the total number of 63,016 (74 %). In terms of the purpose of the experimental project, it was found that most animals were used for basic research (71 %). The total number of experimental animals used in experiments in the monitored user facility for the period 2009-2019 significantly correlates with the total number of experimental animals used in the Czech Republic ($p<0.01$). Regression analysis revealed the relationship between the total number and the observed period. The regression model is considered statistically significant ($p<0.05$). Decreasing trend of using experimental animals was confirmed by this study and thus the 3R concept of reducing the number of animals in experiments in the user facility of the University of Veterinary Sciences Brno is fulfilled. However, there is still a need for a greater focus on the application of the 3R strategy animal research and testing in the academic environment, not only by improving the standards of care for experimental animals and their welfare, but also by introducing new methods for evaluating the results of experiments or using alternative methods to better animal experiments design.

WHAT HAS CHANGED IN THE IMPLEMENTATION OF NEW APPROACH METHODOLOGIES IN THE 21ST CENTURY: OBSTACLES, CHALLENGES AND OPPORTUNITIES

Kandarova, H.[1,2]
[1]Institute of Experimental Pharmacology and Toxicology, Centre of Experimental Medicine, Slovak Academy of Science, Bratislava, SlovakRepublic, [2]Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak Technical University, Bratislava, Slovak Republic

The development of alternative methods to animal experimentation has progressed rapidly over the last 40 years. Knowledge of alternative methods and their use in the risk and safety assessment of chemicals, pharmaceuticals and biologicals has become essential for modern toxicologists.
Alternative methods (alternative toxicology tests) are methods able to:

- **reduce** the number of animals necessary in a trial,
- **refine** toxicology procedures to make them less painful or stressful to laboratory animals, or,
- **replace** animals with non-animal (*in vitro*, *ex-vivo* or *in silico*) systems.

These three principles, also known as the "3Rs", were defined already in 1959 by W.M.S. Russell and R.L. Burch in their well-crafted and scientifically valid writing on this subject, "The Principles of Humane Experimental Techniques" (Russell and Burch 1959) [1,2]. In the 21st century, a combination of *in vitro*, *ex-vivo* and *in silico* methods has an irreplaceable role in the assessment of the toxicology profile of compounds and is conducted before any animal testing is considered. Advanced alternative approaches and their combinations are also used for the screening of safety assessment of mixtures and final products before clinical trials. Several alternative methods, which were scientifically validated and accepted by competent regulatory bodies, can be used for regulatory toxicology purposes, thus reducing or entirely replacing live animals needed for the tests. The acceptance of the alternative methods as valuable tools of modern toxicology has been recognized by regulators, including OECD, ECHA, FDA and EPA. The presentation provides an overview of the topic "Alternative methods in toxicology". It focuses on the implementation of the New Approach Methodologies (formerly called alternatives) into the EU law, provides an insight into the validation process [3,4], informs on the main validation centres and European centres promoting concepts of the 3Rs. The presentation also will critically discuss the role of NAMs in modern toxicology and summarise the obstacles to the effective implementation of these novel technologies.

Supported by the projects APVV-19-0591, DS-FR-19-0048 and VEGA 2/0153/20.

References:
1. Russell W.M.S., Burch RL. (1959). The Principles of Humane Experimental Technique. Methuen and Co., London, UK, 238 p.
2. Kandarova and Letasiova (2011) Alternative methods in toxicology: pre-validated and validated methods. Interdisc Toxicol. 2011; Vol. 4: 107-113.
3. OECD (1990). Scientific Criteria for Validation of In Vitro Toxicity Tests. OECD Environment Monograph No. 36.
4. OECD (2005). Guidance Document No.34 on the Validation and. International Acceptance of New or Updated Test. Methods for Hazard Assessment. Adopted in June 2005.

HEMOCOMPATIBILITY TESTING OF TUBES FOR CHANDLER LOOP SYSTEM BASED ON IN VITRO EVALUATION

Nalezinkova, M.[1], Roupcova, M.[2], Plodikova, P.[2], Myslivcova Fucikova, A.[1]
[1]Department of Biology, University of Hradec Kralove, Hradec Kralove, Czech Republic; [2]Research Institute of Organic Syntheses Inc., Pardubice, Czech Republic

Hemocompatibility testing is dealt with in standard ISO 10993-4, which allows testing both *in vivo* and *in vitro*. This research is focused on *in vitro* testing, using human blood with the aim of minimizing the use of animals, but also trying to mimic real conditions in the human body as far as possible. The aim was to find a suitable tubing for testing a medical device with human blood in the Chandler Loop system. The Chandler Loop is a device which simulates the circulation of human blood in a closed vascular system. It is composed of a rotor to which the blood-filled tubing is attached. These tubes are closed in a circle. Temperature control is provided by a thermostat with water heated to 37 °C, in which the tubes are immersed. The tested material (such as stents) is placed in the tube, or alternatively the tube is made from this material. Blood interacts with the tested material during circulation. Blood collected from healthy human donors was fasted into anticoagulant tubes in the morning, then coiled in the Chandler Loop in various tubing types, and blood counts were measured. The first tested material was a noDOP tubing of approx. 4.75 mm diameter. It was made of phthalate-free soft-PVC with the plasticizer TEHTM (noDOP) intended for the medical industry. Another sample was a hose from MPH of approx. 4.8 mm diameter. It was a PVC hose designed for use in healthcare. The final one was BPT tubing, with a biocompatible fluid surface and of approx. 4.8 mm diameter. It was silicone tubing designed for a peristaltic pump. All tubes were cut to a length of 30 cm and twisted into a circle. Platelet and leukocyte activation was determined based on the decrease in their number. Hematology analyzer Celltac Alpha MEK-6500 was used to measure platelet (PLT) and leukocyte (WBC) counts. In addition, platelet activation was verified by platelet factor 4 (PF-4) assay. ELISA kits (CUSABIO) were used for the measurement of PF-4 concentration. Tubing made of medical material (polyvinyl chloride) from MPH showed the best results, mainly due to low platelet activation (PF-4 6.26-7.31 ng/ml, compared to positive control PF-4 results 66.99-89.45 ng/ml). MPH tubing type was chosen from the test tubes for the Chandler Loop system. However, the use of noDOP or BPT tubing is also possible. All hoses have some disadvantages and affect certain blood parameters. In addition, any manipulation of the blood and natural changes after blood collection affect the results. For these reasons, it is important to compare the results of the tested medical devices with a negative control that consists of a tube filled with blood without any additional material.

Acknowledgements: The research was supported by the specific research project of the University of Hradec Králové no. 2113.

SEX AS AN IMPORTANT VARIABLE IN BIOMEDICAL RESEARCH

Plevkova, J.[1], Budaj, T.[1], Matloobi, A.[1], Brozmanova, M.[1], Honetschlager, J.[2]
[1]Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin, Department of Pathophysiology, Slovak Republic; [2]Institute of Molecular Genetics of the ASCR, Czech Centre for Phenogenomics BIOCEV, Vestec, Czech Republic

The main role of research in medicine is to provide relevant knowledge which, after successful translation to clinical practice, improves the quality of healthcare. The sex bias which is still present in the majority of research disciplines prefers male subjects despite legislation changes in the US grant agencies and European research programme Horizon 2020. Male subjects (cells, animals) still dominate in preclinical research and it has detrimental consequences for women's health and the quality of science. Opposite bias exists for data obtained mainly in animal models utilizing female subjects (e.g. research in multiple sclerosis, osteoporosis) with skewed outcomes for men affected by these diseases. Either way, scientists are producing results which compromise half of the population. Assumptions that females as cohorts are more variable and another assumption that the oestrous cycle should be tracked in case the females are enrolled in preclinical studies were proven wrong. Variability of male versus female cohorts is comparable and does not only stem from hormonal levels. The widespread prevalence of sex differences in human diseases ultimately requires detailed experiments performed on both sexes, unless the studies are specifically addressing reproduction or sex-related behaviors.

ALTERNATIVE METHODS FOR VERIFICATION OF THE SAFETY AND EFFICACY OF THE TEST SUBSTANCE

Popper, M.[1], Petřík, M.[1], Nový, Z.[1], Bendová, K.[1], Weiss, J.[1], Frantíková, K.[1], Schneiderová, T.[1], Hajdúch, M.[1]
[1]Institute of Molecular and Translational Medicine Faculty of Medicine and Dentistry of Palacký University, Olomouc, Czech Republic

The use of animal models in human and veterinary research has a long tradition. The first records of the possible use of animals for scientific purposes date from the 4th (Aristotle) to the 3rd (Erasistratus) century BC. Determining the safety and efficacy of test items in the preclinical phase of research is still standard practice before a clinical trial is performed and a new drug is placed on the market. According to § 26 par. 5 let. j) point 2 of Act No. 378/2007 Coll.: "The results of pre-clinical tests (toxicological and pharmacological) must be submitted with the application for registration." Efforts to replace animals, laboratory, farmed or wild, in experimental procedures and studies that could cause discomfort, pain and suffering, are correct and justified, but much greater progress has been made in reducing the number of animals and in establishing and monitoring gentle treatment of animals. These 3 principles: replacement, reduction and refinement, abbreviated 3R, were described as key elements of a strategy to achieve humane experimental methods as early as 1959 in "The Principle of Humane Experimental Technique" by W. M. S. Russell and R. L. Burch. 10 years later, the Fund for the Replacement of Animals in Medical Experiments (FRAME) was established, which actively promotes the development and promotion of procedures leading to the strengthening of 3R principles in scientific research. The peer-reviewed international scientific journal "Alternatives to Laboratory Animals" has been published since 1983. The 3R principle was first incorporated into EU law in Directive 2010/63/EU on the protection of animals used for scientific purposes. The original wording and interpretation have been improved since the 1960s, and the Directive also covers and applies to the breeding, care and welfare of animals when they are housed, whether or not they are subject to scientific progress. In the Czech Republic, the issue of protection, breeding and experiments on animals (animals reproduced only for scientific purposes) is legislatively based mainly on Act 246/1992 Coll., on the protection of animals against cruelty, and Decree 419/2012 Coll., on protection of experimental animals, as amended. Already established methods that help in particular to avoid and reduce the number of animals include: molecular methods, cell and tissue cultures, mathematical modeling, advanced imaging methods, human volunteers, human tissues and organs. Promising alternative methods developed with the intention of potentially replacing the use of animals in preclinical research include: organoids, organ-on-a-chip systems, computer simulation - modeling of pharmacokinetics based on physiological mechanisms and parameters (Physiologically Based Pharmacokinetic). These alternative methods which are currently being developed should provide valuable information in the future based on new data, but do not provide such comprehensive and complex data compared to the animal model. Although the efficacy of a test substance can be determined in the first step on an organoid derived from a specific organ, it is not entirely possible to determine the safety of application to other organs. This uncertainty in the data obtained increases even more in studies requiring monitoring of the efficacy and safety of test substances in pathologically altered organs and/or whole macro-organisms due to spontaneous and/or induced disease. Although complete replacement of animals in biomedical research is not appropriate at present and in the near future, it is possible to further reduce the number of animals used by better planning procedures and responding flexibly throughout the experimental design based on the data obtained. Gentle handling and strictly defined and controlled procedures with animals will further ensure accurate results with a low degree of variation in the same experimental group.

Supported by the European Regional Development Fund – Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868). The authors received financial support from the Czech Science Foundation (Project number: 19-10907S).

THE USAGE OF MICROFLUOROSCOPY IN IN VIVO AND IN VITRO EXPERIMENTS

Scheer, P.[1,2], Hložková, J.[1,2], Suchý, P.[2], Mikulík, R.[1,3], Sepši, M.[3]
[1]FNUSA-ICRC, Brno, Czech Republic; [2]FaF MUNI, Brno, Czech Republic; [3]LF MUNI, Brno, Czech Republic

In vivo experimental practice seeks to use identical or analogous imaging methods as in clinical practice in the "translation of knowledge". Therefore, we have high-resolution ultrasounds for small rodents, micro-CT, MRI for small rodents as analogues to ultrasound tomographs, CT and MRI for humans. Sometimes the nature of the experiment requires a sciascopy inspection of the procedure or process being performed. In clinical practice, a C-arm is useful for this purpose. The fluoroscope is its analogue available today. According to our information, the only commercially available device is a system from Glenbrook Technologies. The system is modular and can be customized. The advantage of the system is the low radiation rate (less than 350 μSv/h) associated with zero administration in relation to SUJB. Furthermore, the system has a high resolution stated in pairs of lines (LP) of 20 LP/mm compared to standard C-arms, where the best one has a resolution of max 6 LP/mm, most 2-3 LP/mm. Unlike clinical C-arms, the system does not allow the X-ray and detector to rotate, but this problem can be solved by rotating the animal under the X-ray in the tube. The use of the device is identical to that of classical sciascopy – angiography (selective and non-selective), catheterization, monitoring of swallowing and its disorders, orthopaedic applications, and our established monitoring of thrombolysis in radiolabelled fibrin clots both *in vitro* and *in vivo*.

The contribution was created thanks to the support of the project of the Ministry of Education, Youth and Sports CZ.02.1.01/0,0/0,0/16_026/0008451.

SENSITIZATION POTENTIAL OF MEDICAL DEVICES DETECTED BY IN VITRO AND IN VIVO METHODS

Svobodova, L.[1,2], Rucki, M.[1], Vlkova, A.[1], Kejlova, K.[1], Jirova, D.[1], Dvorakova, M.[1], Kolarova, H.[2]
[1]Centre of Toxicology and Health Safety, National Institute of Public Health, Prague, Czech Republic;[2]Department of Medical Biophysics, Institute of Molecular and Translational Medicine, Faculty of Medicine and Dentistry, Palacky University in Olomouc, Czech Republic

Medical devices have to be tested before marketing in accordance with ISO EN 10993-10 in order to avoid skin sensitization. This standard predominantly refers to the *in vivo* test, however, it doesn't exclude the use of *in vitro* methods, which have been sufficiently technically and scientifically validated for the purpose of medical devices testing. It is foreseen that due to the complexity of the sensitization endpoint, combination of several methods will be needed to address all key events occurring in the sensitization process. The objective of this follow-up study was to evaluate the sensitization potential of real samples of medical devices using a combination of *in vivo* (LLNA DA, OECD TG 442A), *in chemico* (DPRA, OECD TG 442C) and *in vitro* (LuSens, OECD TG 442D) methods and to enhance testing strategy for the safety assessment of medical devices extracts. This limited study aims to optimize the use and preparation of extracts, with reference to our previous study (https://doi.org/10.14573/altex.2008142). A good agreement between *in vitro* and *in vivo* results was achieved regarding the absence of skin sensitization potential, however, discrepancies in positive classifications have been recorded. A testing strategy is suggested in which negative results are accepted and any positive results in the *in chemico* or *in vitro* tests are followed up with a third *in vitro* test and evaluated in accordance with the "2 out of 3 approach". This strategy may reduce and/or replace animal testing of skin sensitization in the field of medical devices in accordance with the ethical and scientific issues connected with the use of experimental animals.