ISSN 1580-4003

THE SCIENTIFIC JOURNAL OF THE VETERINARY FACULTY, UNIVERSITY OF LJUBLJANA

SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Supplement 18

3rd Congress of the SLAS
and
1st joint SLAS - CroLASA meeting

Proceedings

Ljubljana, 15-16 June 2017

Volume 54 Suppl 18

Slov Vet Res • Ljubljana • 2017 • Vol 54 • Supplement 18 • 1-72

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3. kongres SDLŽ in 1. skupno srečanje SDLŽ in CroLASA
Ljubljana, Slovenia
15-16 June, 2017

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Sponsors / sponzorji:	Mikro + Polo d.o.o., Slovenia; FELASA, United Kingdom; Merck d.o.o. Slovenia, Slovenia; FIBO d.o.o., Slovenia; Angelini Pharma, Slovenia, Sanolabor d.d., Slovenia; INNOVO Ltd. Hungary, Hungary; IN VIVO, Slovenia; World Courier, AmerisourceBergen, Slovenia; VODA 902 d.o.o., Slovenia

The contributions are not proofread. / Prispevki niso lektorirani.

ISSN 1580-4003

Printed by / tisk:	Tiskarna Ozimek, Trzin, Slovenia
Issued by / izdal:	Society for Laboratory Animals of Slovenia
Edition / izdaja:	1st edition, 120 copies, free copy
Indexed in / indeksirano v:	Agris, Biomedicina Slovenica, CAB Abstracts, IVSI Urlich's International Periodicals Directory, Science Citation Index Expanded, Journal Citation Reports / Science Edition
Web page / spletna stran:	http://www.slovetres.si/

Society for Laboratory Animals
of Slovenia (SLAS)

Croatian Laboratory Animal
Science Association (CroLASA)

3rd Congress of the SLAS and 1st joint SLAS - CroLASA meeting

Proceedings

Ljubljana, 15-16 June 2017

Scientific and Organizing Committee

Chair: Martina Perše

Treasurer: Simona Kranjc

Members: Maja Čemažar, Simon Horvat, Gregor Majdič, Tatjana Pirman, Miša Korva, Andreja Erman, Viktorija Smerdu, Maja Lang Balija, Julija Erhardt

Welcome

Dear Colleagues,

On behalf of the Scientific and Organizing Committee it is a great pleasure to welcome you to the 3th Congress of Society for Laboratory Animals of Slovenia (SLAS) and 1st joint meeting of SLAS and CroLASA entitled Ethical use of laboratory rodents in biomedical research.

Nowadays, ethical considerations and animal welfare has become important issue in all parts of biomedical research. The use of animals in biomedical research is allowed only when there is no other alternative and when the number and suffering of animals used in experiment is reduced to minimum to achieve a valid scientific objective. It has been widely recognized that the most reliable results can be attained when suffering of the animals is avoided. Assessment of animal welfare/suffering has become fundamental part of designing and conducting experiments as well as regular statistical reporting. Care for animal welfare should be implicated in every step of animal experimentation, in procedures and husbandry. Although in the past years advances have been made in the treatment of animals used for scientific purposes, there is much more that remains to be done to advance all of the 3R principles.

In the last years SLAS has recognized the need for state of the art knowledge on welfare assessment, function of animal welfare bodies, severity classification of procedures, use of analgesia and anesthesia in rodents etc..

All these burning topics are now addressed at the Congress by renowned and experienced experts from leading research institutions. We believe that this event will contribute to increased awareness of animal welfare in research and better biomedical science.

We would like to thank to all the speakers, all our sponsors and FELASA that enabled this unique event.

At the same time we would like to express deepest respect and gratitude to all the animals involved in research.

We look forward to welcoming you in Ljubljana,

Znan.sod.dr. Martina Perše

President of the Scientific and Organizing Committee

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From in *vivo* to *in vitro*: a long and winding road

Coenraad F.M.Hendriksen

Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands & Institute for Translational Vaccinology (Intravacc), Bilthoven, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands & Institute for Translational Vaccinology (Intravacc), Bilthoven, The Netherlands

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All existing regulations on animal experimentation, including Council Directive 2010/63/EU, emphasize the importance to develop and implement 3Rs alternatives; methods or strategies to replace, reduce and/or refine the use of animals in biomedical research and education. This reflects the general feeling in our society (including the scientific community) to move away from using laboratory animals; the sooner, the better. For animal welfare reasons, but also because animal research is expensive and faces limitations in mechanistic understanding. Next, an increasing number of studies report about a questionable translatability to the human situation.

Researchers tend to respond to the 3Rs expectations in society by making unrealistic claims on what they can achieve. Unfortunately, however, in practice the development of 3Rs alternatives is less simple as people often believe they are and the way to successful implementation of such an alternative is almost never a straight line.

This presentation will discuss current opportunities and limitations of 3Rs alternatives, this in the context of relevance and reliability of animal models. It also will provide an analysis of the factors that may slow down or speed up progression in the process from development to actual use.

My presentation will use vaccine quality control as a case study to illustrate these factors; a discipline in research and testing which is characterized by its extensive animal usage (estimated to be approximately 15% of total animal usage in Europe) and high severity scoring.

We have been active in the area of 3Rs alternatives and vaccine quality control for the last few decades; developing new methods and strategies for vaccine testing ranging from Refinement, such as the implementation of humane endpoints in animal experimentation, to Replacement , such as cell-based assays for demonstration of vaccine safety. Next, we have coordinated and participated in large scale validation studies and collaborated with industry and with regulatory authorities. Sometimes we have been successful, but as often we have failed in bringing 3Rs to regulatory acceptance and routine use. At the end, however, also long and winding roads bring you to your destination and it is obvious that 3Rs alternatives are increasingly being used, for ethical and for scientific reasons.

Reproducibility issues of animal research

Martina Perše

Medical Experimental Centre, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

Despite intensive effort and financial stimulation for the development and implementation of the methods to replace animal experimentation use of animals in biomedical research is still indispensable. The experiments on animals remain to play an important and yet unreplaceable role in the investigation of pathogenesis of many human diseases as well as testing potential treatment strategies. Consequently animal experimentation in EU is a subject of strict regulation and complex process of evaluation and supervision. Use of animals is allowed only when all legal, scientific and ethical standards are met. Replacement, reduction and refinement are basic principles of the EU legislation with the purpose to provide reliable and reproducible results.

However, recent analyses of publications discover concerning prevalence in the number of studies that cannot be reproduced. Since scientific progress is achieved by robust experiments that generate reliable and reproducible results, reproducibility of animal research is becoming big issue in scientific community. It was already recognized that reasons for such situation are multifarious and no single party is solely responsible. In an attempt to improve reproducibility and validity of publications, some actions and strategies have already been taken. Presentation will summarize recent literature on this subject and highlight factors affecting reproducibility of publications with the aim to encourage scientific audience to pay attention not only to 3R principles but also to reproducibility of their publications.

Know your FELASA

Hanna-Marja Voipio

FELASA and Laboratory Animal Centre, University of Oulu, Finland

SLAS and CroLASA are two of the 21 member associations in FELASA – Federation of European Laboratory Animal Science Associations. During almost forty years of existence, FELASA has grown to be a significant operator in laboratory animal science, representing 27 countries today. FELASA advocates responsible scientific conduct with the animals in the life sciences with especial emphasis on ensuring animal welfare according to the 3Rs principles.

The best known FELASA activity may be the triennial conference, the largest LAS occasion in Europe. After the first symposium in Germany 1981, twelve symposia have been organized in co-operation by FELASA and member associations. Another key element are the working groups, resulting in widely recognized recommendations, like the Health monitoring guidelines.

Harmonizing the use of animals for scientific purposes, including the education and training of people working with laboratory animals is an aim embedded in The Council Directive 2010/63/EU. FELASA started similar action already in the 1990s, by developing the recommendations of education and training for different professional groups. The quality of this education can be ensured by the FELASA's accreditation board for education and training, accrediting courses – nowadays also outside Europe. Continuous professional development is in FELASA's agenda, for example in the form of Severity classification and reporting workshops, soon available in all European countries. This may be a model to harmonize and disseminate knowledge also in future.

Today's FELASA is networking widely in Europe and globally, maintaining relations with national and international bodies and associations. FELASA is an appreciated stakeholder within the European commission and the Council of Europe, representing the views and opinions of the European LAS community.

The work in FELASA is based on the volunteer contribution. Each constituent association has a member in the FELASA board and experts to new working groups are requested through associations; fresh ideas are always needed. Take a look at the FELASA's website: www.felasa.eu. Are you interested? Contact your national Laboratory Animal Science Association and offer your active participation.

Biomarkers for the recognition and assessment of pain and stress in laboratory rats and mice

Klas Abelson

Department of Experimental Medicine, University of Copenhagen, Copenhagen, Denmark

Pain and stress in laboratory animals is a major concern when performing experiments on animals. Pain and stress may both have negative impact on the animal's well-being, which is ethically problematic, and affect the experimental data in an unexpected manner, which could compromise the scientific quality. In order to control and alleviate pain and stress, we must learn to recognize and assess these phenomena at an early stage. There are several methods for pain and stress assessment, including physiological parameters, biomarkers and behavioural and clinical signs. This presentation will give an overview of the various methods, and discuss their applicability and validity as well as advantages and disadvantages in various experimental situations, with particular focus on our most recent research on the use of biomarkers.

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Animal welfare bodies - their role and mission

Birgit Ledermann

Novartis Pharma AG, Basel, Switzerland

The EU directive 2010/63/EU states that Animal Welfare considerations should be given the highest priority in the context of animal keeping, breeding and use. Breeders, suppliers and users should therefore have an animal-welfare body in place with the primary task of focusing on giving advice on animal-welfare issues. In countries other than the EU, *e.g.* the US, the IACUC (institutional animal care and use committee) has a similar role which is also established in other countries with a less strict animal welfare legislation in institutions and that have an AAALAC (association for assessment and accreditation of laboratory animal care) accreditation. In Switzerland, there is no internal body or committee required and the role of the Animal Welfare Officer is currently not obligatory. However, the Swiss Animal Welfare Legislation is one of the strictest worldwide and the institutions working with animals including academia and Pharma industry usually have implemented this role.

The members of animal welfare bodies/IACUCc/AWOs shall include Person´s responsible for the welfare and care of animals, *e.g.* Animal Welfare Officer, designated veterinarian and representative from scientific research.

The duties of animal welfare bodies/IACUCs/Swiss AWO shall include:

- Advice of staff dealing with animals on matters related to the welfare of animals
- Advice on the procedures of the 3Rs, replacement, reduction and refinement
- Information of recent scientific developments in the field of 3Rs
- Implement procedures for the internal review of operational processes regarding monitoring, reporting and follow-up in relation to the welfare of animals housed or used in the establishment
- Post approval monitoring, *e.g.* looking at the effect of the procedures to the animals used in the respective experiments and advise for potential improvements with regard to the 3Rs.
- Advise on rehoming of animals and provide rehoming schemes (important for large animal spees)
- It is very much important that the animal welfare bodies/IACUCs/AWOs are perceived as partners by the scientists and not as a “a “police” function. These functions support a culture of care and thereby ensure best Animal Welfare which will enable better science.

Basic and continuing education – FELASA perspective

Ana Isabel Santos

FELASA and NOVA Medical School, Universidade NOVA de Lisboa, Lisboa, Portugal

Accreditation of the training programs for persons working with laboratory animals under the Directive 2010/63/EU envisages that Laboratory Animal Science (LAS) training will be accepted around Europe. This same Directive as also included in the legal framework the requirement for Continuing Professional Development (CPD). The Federation of Laboratory Animal Sciences Associations (FELASA) accreditation system has become increasingly recognized as a robust way of improving training and ensuring a European golden standard in LAS basic education and training. The FELASA accreditation recognizes, supports and enhances the quality of training; establishes a more uniform platform of competence of those trained, so enabling greater mobility of researchers and animal care staff; enables the identification and sharing of good practice; and provides independent reassurance for National Authorities and the public about the competence of those working with laboratory animals.

In 2013 the accreditation system has been reviewed and adapted for program accreditation of the functions A, B, C and D or Designated Veterinaries and has kept accreditation for programs for Specialists in LAS.

The FELASA Accreditation scheme provides the possibility of accrediting courses specific for one function or for more. Allows the Course Organizer to issue modular certificates. Courses aiming to become accredited or FELASA accredited programs must be comprised of at least the “Core Modules” for that Function and at least one species or group of species.

FELASA has also developed guidance on how a CPD scheme could be introduced and has based this on some principles:

- People working with animals should have and maintain the state of the art knowledge and skills;

- Continuing Professional Development should be available and organised in a flexible way;

- CPD should be considered as a process that starts with the first training steps before working with animals and continue through the working career;

- The system should be based on the award of credits over a certain period of time as requested in the Directive;

- There should be a process for review and endorsement of CPD activities which are for inclusion in a CPD program.

Live animal imaging: preclinical imaging solutions by Bruker

Francesco Moneta

Preclinical Imaging Division - Bruker BioSpin, Milano, Italy

Drawing on over 50 years of expertise in the life and animal sciences, Bruker designs preclinical imaging systems that deliver outstanding research results. Furthermore, in designing its product line, Bruker has always placed an emphasis on providing optimal, humane treatment of study animals while they are being imaged.

Performing *in vivo* imaging with Bruker instruments has minimal to no adverse effects on study animals, allowing the same animal to be imaged multiple times over the course of an experiment. This has the benefit of minimizing data variability, of documenting true progression in an animal model, and of reducing the overall number of animals required for any given study. Stop Sacrificing – choose the right modality for your application.

Mouse genetic modification: state of the art and interferences

Boris Jerchow

Research Animal Facilities, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Over the last three decades a toolbox of techniques has been developed to engineer the genomes of virtually any species. During the same time, in biomedical research, the mouse has become the most important laboratory animal. Mice need little space, have a short reproductive cycle, and in most physiological aspects remain comparable to man. However, one of the most important reasons for its predominance is that, over many years, the mouse has been the only mammal that allowed fairly straight forward complex genome engineering. To date, a multitude of mouse models with genetic alterations have been generated, readily available to researchers around the globe. Examples are lines that mimic human disease, make it possible to visualize gene expression *in vivo*, and lines that allow the controlled activation or inactivation of specific genes. Recently, genome engineering has reached the next level with the advent of CRISPR/Cas9 technology. Still, the basic paradigms of genetic alteration remain the same. Likewise, pitfalls remain the same and must not be overlooked when generating new models at increased speed and with increasing numbers. In my presentation, I will discuss state of the art technology and highlight some common aspects that, when unnoticed, may lead to unexpected experimental outcomes.

How to assess welfare in genetically altered rodents?

Anne Zintzsch, Elena Noe, Monika Reißmann, Kristina Ullmann, Stephanie Krämer, Boris Jerchow, Reinhart Kluge, Claudia Gösele, Hannah Nickles, Astrid Puppe, Thomas Rülicke

Working Group of Berlin Animal Welfare Officers and Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany

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Genetically altered (GA) animals are frequently used research models with continuously increasing numbers. Apart from its scientific value, a genetic alteration can compromise an animal's wellbeing. However, the large variety of phenotypes is challenging when it comes to welfare assessment and severity classification, which plays an essential role in pro- and retrospective severity assessment.

In Germany, national guidance has been developed on a basic welfare assessment and documentation of strain characteristics (1,2). Recently, the Working Group of Berlin Animal Welfare Officers devised an example driven guideline on how to classify different phenotypes into severity categories. The Guidelines on severity assessment and classification of genetically altered rodents (3) contain examples of symptoms and syndromes caused by genetic alterations. Examples are assigned to a particular severity category (none, mild, moderate, severe) including recommendations for monitoring and refinement strategies. This guideline will contribute to the harmonisation of severity assessments of genetically altered mice and rat lines within Europe.

The presentation gives an overview about the idea of welfare assessment of GA rodents and demonstrates the approach of severity classification on the basis of examples.

R*eferences*

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2. Bundesinstitut für Risikobewertung. Severity Assessment of genetically altered mice and rats - Version 2: Recommendation no. 002/2016 by the National Committee (TierSchG) dated 9 September 2016.

3. Zintzsch A, Noe E, Reißmann M, et al. Guidelines on Severity Assessment and Classification of Genetically Altered Mouse and Rat Lines. Working group report of Berlin Animal Welfare Officers. Lab. Anim., paper accepted.

Cryopreservation of laboratory rodents: possibilities, advantages and limitations

Martina Maria Dorsch

Institute for Laboratory Animal Science, Hannover Medical School, Germany

Cryopreservation of embryos and gametes of laboratory rodents is an important tool to protect the experimenter against the loss of valuable animal models. Possible reasons are genetic drifting, inbreeding depression, or infections. Also conceivable are failures of facility equipment and environmental disasters. In addition cryopreservation of strains that are not under actual scientific use frees up space and reduces shelf costs for maintaining a vital colony. It is strongly recommended to establish a genetic back up for every valuable and unique strain at least. Different options/methods have gained acceptance: embryo-freezing, sperm-freezing, or freezing of ovaries. The decision for one option depends on the availability of donor animals, availability of time, and genetic requirements. Additional requirements are methods for assisted reproduction such as embryo-transfer or *in vitro* fertilization. Today, the Institute for Laboratory Animal Science of Hannover Medical School is looking back to a more than 30 years of experience in cryobanking. In my talk I will show the feasibility of the different methods to store and save valuable mouse, rat, and guinea pig models. As every method has its pros and cons, I will also point out unsolved problems and limitations of cryobanking.

The human tumor spheroids as a model for gene electrotransfer

Simona Kranjc, Maja Čemažar, Gregor Serša

Department of Experimental Oncology, Institute of Oncology Ljubljana, Ljubljana, Slovenia

Gene electrotransfer, is a non-viral approach to target variable genes, which by using of short high voltage electric pulses induce transient permeability in the cell membrane and thus enabling the passage of variable molecules (plasmid DNA, antisense oligonucleotides, siRNA, shRNA) into the cell. Spheroids, as three dimensional cell cultures, provide possibilities to evaluate gene therapy *in vitro* and to obtain reliable results for better planning and understanding of gene electrotransfer *in vivo*. In order to obtain the best approach in treatment of spheroids with therapeutic gene the optimization of gene electrotransfer has to be performed. Thus, the aim of the study was to evaluate gene electrotransfer of plasmid encoding GFP (enhanced green fluorescent protein, pEGFP) into different human spheroids.

Human tumor spheroids were formed from the colon cancer carcinoma cells (HT-29 and LoVo), prostate cancer carcinoma cells (Du145) and A549 lung adenocarcinoma cells. To obtain spheroids of appropriate size (400 μm in diameter) and shape at day two after seeding cells, a forced floated and hanging drop methods were tested. Gene electrotransfer of spheroids with pEGFP (10 μg) was performed by application of electric pulses at different voltage over distance ratio (500-700 V/cm, 5 ms, 8 pulses). The growth of spheroids, the expression of EGFP and the effect on invasion and migration were monitored at different time points after gene electrotransfer.

The highest transfection efficiency up to 70% using electric pulses at 500 V/cm was obtained 48 hours after gene electrotransfer of HT-29 spheroids. The growth of spheroids at these conditions was decreased for 26% compared to control. A slightly lower transfection efficiency of 56% in Du145 and LoVo spheroids was achieved by optimal pulses at 600 V/cm, which decreased growth by 34% and 9% respectively. The lowest transfection efficiency around 10% was achieved in A549 spheroids at chosen optimal pulses of 500 V/cm, which minimally decreased spheroid growth by 14%. However, their transfection could not be improved even by increasing voltage or by changing the duration time of pulses, resulting only in decreased spheroid growth. Gene electrotransfer with pEGFP had no effect on migration and invasion of different spheroids.

In conclusion, the tumor spheroids were demonstrated as an appropriate *in vitro* tool for further studies of gene electrotransfer, especially colon cancer spheroids. However, in order to obtain even more predictable spheroid model for the research *in vivo*, co-cultures of tumor cells with fibroblast and endothelial cells in the future will be performed.

Color matters: they would choose if they could (see)!

Krämer Stephanie, Tina Nitezki, Nadja Schulz

German Institute for Human Nutrition

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Introduction: Employing the principles of standardization concerning laboratory animal husbandry only exiguous changes of the habitat can potentially influence animal's physiology or result in unexpected responses performing standard behavioural tests. Routinely, mice chow is dyed with inert food coloring in order to avoid irritations when different types of diets are dispensed. Given the fact that the dye per se has no effects on food odor or flavor, we wanted to test the hypothesis, that the color of chow has an impact on food uptake in laboratory mice.

Material and Methods: In order to determine exact food and water intake groups of 7-8 weeks old male mice of three different strains (B6, BALB/c, DBA/2J; n= 12 per strain) were single-housed in pheno master cages (TSE Systems). After acclimatization standard mice chow (SD) in different colors (green, red, blue, yellow) was administered. Cages contained two feeding tubes, which by turns were filled with food of the same or different color, thereby exchanging the position of the tubes daily. Food and water intake was monitored as a side-by-side comparison of the different color combinations for each combination for seven days. Before applying a new combination animals were allowed to equilibrate for seven days on SD.

Results: All animals had an average daily food intake of 5 g (no differences in water uptake). In B6 mice no preferences were observed, when food of the same color was offered. Interestingly, when food of different colors was administered a significant aversion concerning blue food and a significant attraction concerning yellow food could be observed. In BALB/c and DBA/2J mice again no differences concerning food consumption of the same color could be determined, but furthermore no distinct pattern of denial or favoritism related to the different colors occurred.

Conclusion: Mice strains were selected due to their capabilities of developing a physiological visual sense. B6 mice are classified as normal sighted, BALB/c is representative for common albino strains and DBA/2J mice carry mutations resulting in retinal ganglion cell alterations. Results suggesting, that normal sighted mice would be selective concerning food color. Nevertheless, this does not influence quantity of food intake, whereas visually impaired mice showed no preferences, as expected.

Stereotypies in FVB/NJ mice and their impact on metabolism

Tina Nitezki, Fabian Schumacher, Julia Bornhorst, Anne Petrick, Burkhard Kleuser, Stephanie Krämer

University of Potsdam and German Institute for Human Nutrition, Nuthetal, Germany

Standardized housing conditions of laboratory mice deviate from the natural environment in various aspects and might require adaptation. Behavioural adaptation allows animals to adjust to environmental changes and leads to species' characteristic behaviour. If an animal is unable to adapt to environmental conditions, abnormal behaviours like stereotypies might occur. Since it remains unclear to what extend stereotypic behaviour influences the individual's metabolic phenotype, this study investigated behaviour of FVB/NJ mice (stereotypy-prone) and compiled the impact of behavioural deviations on physical activity and animals' metabolism. For this, after characterization of maternal care, 35 animals of the F1 generation were kept individually from weaning age. For 12 weeks they were observed, faecal samples were obtained and body weight was determined. Additionally, behavioural tests, metabolic parameters and physical activity were investigated. Cerebral serotonin and dopamine content, faecal cortisol and corticosterone levels and hepatic glycogen, muscular triglyceride and glycogen levels were assessed. Almost independently from maternal care, more than half of the pups developed stereotypic behaviour, showing increased activity and motility, associated with a gender-dependent lower body weight and comparably lower fat and higher muscle proportions. Significant differences in organ weights were found, whereas the animals did not differ in cerebral serotonin and dopamine contents.

A goal of refinement is to improve experimental animals' husbandry conditions in order to prevent discomfort and associated behavioural and/or physical impairments. Before releasing appropriate recommendations fundamental groundwork is essential to understand the nature of behavioural deviations. This work contributed strongly to this aspect and clearly showed that stereotypies in FVB/N mice vary concerning their locomotion pattern, are obviously not directly genetically or behaviourally transmitted from the parental generation to offspring and have notable impact on metabolic parameters. The effects observed seem to be independent from standardized husbandry conditions, pointing to individual variables, such as animal personality, which have to be taken much stronger into concern.

Gene modificaton and therapeutic genome editing *via* extracellular vesicles delivery of CRISPR/Cas system

Duško Lainšček, Lucija Kadunc, Mateja Manček Keber, Roman Jerala

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The CRISPR/Cas system emerged as the highly potent tool for genome engineering and regulation of gene transcription. This novel gene editing tool consists of Cas9 enzyme, representing the scissors and RNA complex as the precise targeting component. TracrRNA and crRNA, an RNA complex, guides endonuclease Cas9 to target the desired genome site, which bears a short PAM sequence, that is recognized by the Cas9 protein. Target DNA is then cleaved and double stranded breaks of targeted DNA are repaired with cell repair mechanisms. Using CRISPR/Cas9 system we can make knockout models by introducing indel mutations or knock in models by codelivery of a donor DNA that reacts like a DNA template for the repair. Another important benefit of CRISPR/Cas system is highly efficient gene expression alteration. By using catalytically inactive endonuclease dCas9 that possesses no activity and acts only as a binding tool to DNA to recruit heterologous activation or repression domains we can regulate gene expression. CRISPR/Cas technology is highly versatile and in principle very simple as we can modify the targeting by simply including a short RNA segment. One of the additional important features of CRISPR is that it can function in basically any type of cells or organism, so far it has been tested in more than 50 different species. However the efficiency of its delivery into cells, particularly for safe therapeutic *in vivo* applications, remains a major bottleneck. Extracellular vesicles, released by cells, can mediate the transfer of different functional molecules. We have shown the efficient packaging and delivery of the CRISPR/Cas system via extracellular vesicles to target cells, combining the advantages of both technologies. Extracellular vesicles can transfer the functional Cas9 or designed transcriptional regulator dCas9-VPR in a combination with appropriate targeting gRNAs, enabling genome editing or regulating gene transcription. Delivery and robust genome editing and gene upregulation function was shown for cell lines, primary cells and in the animals. *In vivo* delivery of dCas9-VPR/sgRNA by using extracellular vesicles demonstrated therapeutic efficiency in a mouse model of liver damage, which opens the path towards therapeutic applications.

Mouse genotypes drive the liver and adrenal gland clocks

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Background: Circadian rhythms regulate a plethora of physiological processes. Perturbations of the rhythm can result in pathologies which are frequently studied in inbred mouse strains. Genotype of different mouse strains effects circadian physiology: free-running activity rhythms, feeding cycles, phase-shifting effects etc.

Methods: We compared expression patterns of two mouse strains: C57BL/6JolaHsd and 129S2/SvPasxC57BL/J6 in dark – dark (DD) and light – dark (LD) experimental conditions. With exome sequencing and data mining we searched possible single nucleotide variants (SNVs) that could explain the difference in expression patterns. With the use of Imputed Mouse SNP Resource (http://csbio.unc.edu/imputation/) and data from ChIP seq experiments in circadian settings we evaluated if SNVs in clock protein binding regions are more frequent than in open chromatin regions that are considered as transcription active places.

Results: Expression of the majority of core clock and output metabolic genes is phase delayed in the C56BL/6J line compared to 129S2 in the adrenal glands and the liver. Circadian amplitudes are generally higher in the 129S2 line. Exome sequencing data proposed that mouse lines differ in SNVs in the binding regions of clock related transcription factors in open chromatin regions. One of the possible mechanisms of differential circadian expression could be the entrainment and transmission of the light signal to the peripheral organs. This is supported by the genotype effect in adrenal glands that is largest under LD, and by the high number of single nucleotide variants in the Receptor, Kinase and G-protein coupled receptor Panther molecular function categories. Different phenotype of these two mouse strains and changed amino acid sequence of the Period 2 protein possibly contributes further to the measured differences in circadian gene expression. We can conclude that genotype of mouse lines defines the circadian gene expression patterns.

Effect of mesenchymal stem cell transplantation after immune preconditioning of the recipient suffering from acute kidney injury

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L16

Introduction: Mesenchimal stem cells (MSCs) were shown to be one of the promising tools in regenerative medicine. Several studies demonstrated that the administration of MSCs may result in amelioration of acute kidney injury (AKI) in different animal models. Although studies on animal models have shown that MSCs can repopulate damaged tissue, reduce apoptosis, promote mitosis and secrete a number of immuno-regulatory molecules, one of the major problems influencing the efficacy of stem cell therapy is the poor MSCs survival following transplantation. One of the strategies to improve MSCs survival following their transplantation is to reduce inflammatory overload of damaged tissue with immunosuppressive agents, such as antithymocyte globuline (ATG).

Methods: In order to evaluate underlying mechanisms of MSCs on ATG immuno-suppressed animal model of AKI, MSCs distribution and gene expression of selected inflammatory and oxidative proteins in various internal organs were analyzed. After 3 months survived animals were sacrified and their tissues collected for patohystologic analysis.

Results: Our results show that immuno-suppression may improve MSCs transplantation through various molecular mechanisms. However, three months after MSCs transplantation mouse that recovered from cisplatin toxicity and showed no signs of illness was euthanized and examined. Histology revealed lymphohystiocytic infiltrates in the lungs and kidney suggestive of late onset rejection of engrafted MSCs. In addition, histology revealed expansive subpleural tumor.

Conclusion: immunosuppression with ATG followed by MSCs transplantation improved early functional and morphological parameters of AKI in mice. However, late onset tumorigenesis raises serious concern, at least in case of MSCs transplantation in severely immuno-suppressed recipients.

Revisited: bone marrow chimerism after bone marrow transplantation in non-conditioned mice

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It was long believed that bone marrow (BM) transplantation could successfully lead to chimerism only with prior conditioning. This belief is still alive, even though there are some mouse model studies in which chimerism was achieved after BM transplantation in non-conditioned mice. It has become clear that engraftment in non-conditioned mice is possible, however, the procedure requires immunocompatible grafts with extremely high numbers of nucleated BM cells that can be a challenge to prepare and to transplant successfully.

The aim of our study was to perfect a protocol for the isolation of high numbers of BM cells from one mouse and their subsequent transplantation into non-conditioned female BALB/c mice in order to achieve substantial donor chimerism. In accordance with the principles of 3R (Replacement, Reduction, Refinement) our aim was also to use the lowest number of donor animals as possible, for which we tested different isolation protocols.

The BM cells were best isolated by crushing the main bones (femur, tibia, humerus, iliac crest, and the spine), collecting the cell suspension and performing red blood cell lysis. 39 to 82 million nucleated BM cells were transplanted in four separate doses. Chimerism in BM, spleen, lungs, blood, and FACS sorted myeloid and lymphoid subpopulations was measured with the qPCR at 2, 6, and 12 weeks after transplantation.

At 12-17 weeks after transplantation, the recipients contained 9.2% ± 1.3% donor cells in BM, 5.0% ± 1.8% in spleen, 1.3% ± 0.3% in lungs, and 7.8% ± 1.4% in blood samples. Purified neutrophils, B, and T cells exhibited 6.4% ± 1.2%, 6.8% ± 1.3%, and 2.9% ± 0.9% chimerism, respectively. Our study confirmed some of the previous reports on chimerism obtained after BM transplantation in non-conditioned recipients. qPCR showed to be an accurate and a reliable method for chimerism detection in this setting. In addition, we isolated high numbers of BM cells per mouse, meeting the objectives of 3R.

Non-conditioned BM transplantation can offer important clinical advantages for the treatment of certain clinical conditions. Transplanted cells can also be an efficient gene delivery system. A non-conditioned syngeneic sex-mismatched mouse model could lead us into a new era of treatment and rejuvenation.

The FELASA severity assessment/classification workshop

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Directive 2010/63/EU introduced the requirement for the classification of procedures (Article 15) during the application for project authorisation to use animals in scientific procedures. It also introduced the requirement to report the actual severity experienced by each animal used in a procedure. Both these processes provide opportunities to refine the adverse effects of procedures.

Consistency of assignment of severity categories across Member States is a key requirement.

The session will use a number of animal models to illustrate the severity process from inception of the project, through monitoring during the course of the procedure, to the final assessment of actual severity at the end of the procedure. The impact of refinement on the potential adverse effects and consequences to the assigned severity will be highlighted. The session will also cover a number of issues being encountered in ensuring a consistent application of actual severity reporting.

New developments in anaesthesia and analgesia for laboratory animals

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Tremendous advances have been made in refining analgesia and anaesthesia. However progress has often been frustratingly slow and a number of practical problems remain. We still lack data on the clinical efficacy and duration of action of many analgesic agents, and there is considerable uncertainty as to the required duration of analgesic therapy. Management of large numbers of animals remains a particular problem, and the potential role of novel products, such as slow-release formulations and novel techniques such as epidural and intrathecal routes of administration have not been properly evaluated.

In the field of anaesthesia, research workers normally aim to use an anaesthetic regimen that is safe, effective, and that is appropriate for the particular purpose of their study. Given the wide range of species used in research, and the varying scope of different research projects, it is clear that there can be no single “best” anaesthetic regimen. However in all situations the aim should be to select an anaesthetic that best meets these goals. Most publications report use of appropriate methods, but some could be significantly improved. Use of less optimal methods may be due to a failure to appreciate the considerable species variations that occur, and in particular the differences in response between man and other mammals. A second problem is the apparent failure to appreciate the potential side effects and interactions between anaesthetic agents and the animal model being studied.

Despite these problems, the future in relation to refinement of laboratory animal anaesthesia and analgesia looks bright. The considerable increased interest in this field from research scientists, veterinarians and animal technologists will lead to wider use of analgesics and adoption of improved anaesthetic protocols. More importantly, it is essential that investigators are provided with sources of up-to-date, accurate information, and given sufficient training to be able to critically review their own current practice, to ensure it is still fit for purpose.

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Animal breeding conditions reflect the quality of cellular models

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Primary cell cultures are in many cases regarded as the gold standard in biomedical research of cell metabolism, diseases, toxicology and drug testing. As human primary cells are scarce, basic and clinical research relies heavily on animal models that must be thoroughly validated. We have noticed that animal breeding conditions and treatment prior to cell isolation can influence the physiology of isolated cells in culture that can have profound effect on cellular responses to treatments and can change the applicability of the model. Animal nutrition can quickly alter the composition of gut microbiota, which impacts the physiology of host organism and can even lead to pathological changes. Furthermore, acute and chronic stress has been shown to influence the physiology of certain organs, especially heart and liver. Our data from the experiments, only separated by a few years, imply that changes in animal nutrition and stress levels initiated up to minutes before cell isolation could alter the cell stress response of primary rat hepatocytes in culture for days after isolation and lead to variations in sensitivity to apoptosis triggering. Therefore, we have proposed the hypothesis that acute and chronic conditions of animal breeding, especially diet and stress levels, are reflected in the physiology of the isolated primary cells. Careful consideration and recording of animal diet, manipulation and treatment up to the cell isolation may result in cellular models that better match the conditions *in vivo*, therefore improve their applicability, reproducibility and standardization. This would considerably reduce the need for testing on laboratory animals.

The hepatocyte specific Cyp51 knock-out mouse model HCyp51 doxy+/doxy+: a better choice to investigate NA(F)LD induced HCC

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Hepatocellular carcinoma (HCC) represents the second leading cause of cancer-related mortality worldwide. The major risk factors for it´s development are different infections as well as non-alcoholic (fatty) liver disease (NA(F)LD), the hepatic manifestation of metabolic syndrome. Based on the literature, HCC has accounted for approximately 90% of liver cancer cases. At the same time, 20% of HCC cases could develop from NA(F) LD, because of chronic inflammation- and oxidative stress-induced fibrosis. Generally, the female-to-male ratio averages between 1:7 in NA(F)LD related HCCs. After fifty, when concentration of sexual hormons falls and the hepatocellular demages and liver inflammation increase in women, HCC becomes 1.5-fold more frequent in females in comparison to male.

We applied the hepatocyte knockout mouse model of lanosterol 14α-demethylase (Cyp51) (HCyp51-/-) to evaluate the age-related changes in HCC development due to cholesterol imbalance in the liver. The second hepatocyte time-dependent knockout mouse model of Cyp51 (HCyp51 doxy+/doxy+) is used for easier and sufficient hepatocytes isolation and studies for cell specific molecular mechanisms in hepatocarcinogenesis. Histopathology was performed on liver sections. Hepatic gene expression was assessed by expression profiling and qPCR.

We concluded that development of HCC is age-dependent and exhibits in our HCyp51-/- mouse model sexual dimorphism with a male to female ratio of 1:2. First HCC cases were observed at year 1 with a rising number at later age.

For investigation how blocked cholesterol synthesis influences NAFLD progression towards HCC, which molecular mechanisms are hallmark for hepatocarcinogenesis and if they are happening in the hepatocytes or in other liver cells, establishment of the appropriate in-vivo model is essential. Hepatocytes as parenchymal cells represent 52% of total cells in mouse liver while the rest are non-parenchymal cells.

Our data show that HCyp51 doxy+/doxy+ model is appropriate for hepatocytes isolation and in-depth studies of molecular mechanisms in NALFD induced tumorogenesis.

Neuronal cultures from fresh and frozen embryonic brain tissue

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Cultivation of embryonic brain cells is a common technique. Aside from the costs of experimental animals, equipment, and reagents, it also requires careful planning. The time between planning and performing an experiment is at least 25 days, since animals have to reach the desired developmental age, plus additional 7 to 10 days for the cell culture to mature. Furthermore, the number of embryos and required culture dishes is not predictable. This led us to consider freezing the unused embryonic tissue as well as freezing the remaining cell suspension. We tested two freezing media HBSS and Hibernate E, as they can both maintain cultured cell outside the cell incubator. Both media were supplemented with 10% dimethyl sulfoxide and 10% foetal bovine serum. Best yield (65 to 80%) was achieved when cortices were cut into small pieces and slowly frozen in Hibernate E with additives, while HBSS and cell suspension failed to yield viable cells. The viability of frozen and fresh cells was similar. Cell cultures established from frozen tissue were equivalent to cell cultures from fresh tissue regarding the neuronal and astrocyte morphology as well as glutamate toxicity. This results show that residual brain tissue no longer needs to be discarded, but can be frozen and plated at an appropriate time. Freezing also facilitates research as the time between planning and performing an experiment is reduced to 7 or 10 days.

Effects of single dose of cyclophosphamide in young and old mice

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Cyclophosphamide (CP) is an alkylating agent, which is widely used in the treatment of many neoplastic diseases. Beside nephrotoxicity, one of well known side effects of treatment with CP is hemmoragic cystitis, which develops early during CP treatment and limits therapy with this drug. The urinary bladder damage and subsequent recovery are well described due to extensive experiments on animal models, where pathogenesis and regeneration were studied after single dose of CP. In addition to its immediate toxicity, it exerts also a delayed toxicity on mice, which is reflected in damage of haemopoietic and lymphoid organs and even in animal death.

In our study, we investigated the effect of CP on the urinary bladder epithelium and the regeneration process after CP injection in young adult (2-3 months) and old (20 months) mice after single *i.p.* injection of CP (300 mg/kg) with light and electron microscopy.

No significant changes in clinical picture or behaviour were observed in young adult mice during the whole duration of experiment (14 days). The only finding observed was slight body weight loss in the first few days after treatment. In contrast, old mice showed clinical signs of progressive illness (lack of grooming, body weight loss, hunched back, piloerection, ataxia) that resulted in death. Consequently, according to the human endpoint protocol one third of old mice were euthanised between the day 2 and day 6 after CP injection.

Autopsy revealed severe heterogeneous pathological changes in the gastrointestinal tract (erosions, inflammation), liver, kidneys, spleen and lymphatic glands, indicating systemic toxicity and multiorgan injury in old mice. No significant changes were observed in young mice. However, the effect of CP on urinary bladder epithelium and its recovery were in accordance with the reports in the literature.

We concluded that old mice are more susceptible to CP systemic toxicity in comparison to young mice. Thus, lower dose of CP is recommended when older mice are used in experiment.

In vitro correlate of *in vivo* antivenom neutralization potency assay for efficiency assessment of antivenom purification steps

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Downstream processing of antivenom from hyperimmune plasma consists of series of purification steps. The whole process requires constant evaluation of antivenom yield in each purification step, specifically during developmental phase.

Evaluation of antivenom efficiency is still based on the *in vivo* neutralization assay in mice (medium effective dose determination; antivenom ED_{50}), which cannot be determined without previous venom toxicity test (determination of the median lethal dose; LD_{50} of venom). Both assays require the use of large number of experimental animals that feel pain and suffering. Therefore, the tendency of each laboratory is to develop a method that will fully or at least partly replace tests on mice, in accordance with the principles of 3R.

To monitor the efficiency of individual purification steps in antivenom production, we have developed ELISA for monitoring venom-specific antibodies, in which antibodies isolated from hyperimmune plasma by protein A affinity chromatography are used as a standard. Knowing that Protein A does not bind all horse IgG classes with an equal affinity, affinity purified antibodies might have different venom neutralization potency in comparison to original population of polyclonal antibodies in the starting hyperimmune plasma. From that reason we compared the neutralisation potency of hyperimmune plasma and affinity purified horse IgGs and proved them comparable. Thus, ELISA using on affinity purified immunoglobulin as a standard was proved suitable for monitoring the efficiency of antivenom purification process.

Keywords: antivenom production, in process control, snake antivenom, ED_{50}, snake venom, LD_{50}, the development of alternative tests

P5

An arguable model of hyperphagia – gold thioglucose administration

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Introduction: Obese mice models are essential in the research field of adipositas, metabolic syndrome and diabetes mellitus. An experimental model of obesity can be induced by administration of gold thioglucose (GTG) to mice. After transcending the blood-brain-barrier the GTG molecule interacts with regions of the ventromedial hypothalamus, thereby primarily targeting glucose sensitive neurons. When these neurons are impaired, mice become insensitive to satiety effects of glucose, develop hyperphagia and become obese due to enhanced food uptake.

Material and Methods: In a pilot study for optimizing dosage and body weight development 5 weeks old male C57BL/6 mice were treated with low dose GTG (0.5 mg/kg BW, *ip*, n=10) or saline (n = 5), respectively. Animals were provided with a physiological amount of standard diet (5 g per animal) for the first 24 h after induction, in order to prevent overeating and associated gastric dilatation. Mice were housed under standard housing conditions. Cages were provided with wooden shavings and mice had free access to fresh tap water. Application of substances was performed at 8 am. Animals were monitored until 6 pm and did not show any signs of discomfort.

Results: 24 hours after GTG injection all animals had passed away due to gastric overload and subsequent circulatory shock. Animals developed severe attacks of hyperphagia during nighttime, and as the amount of provided chow was restricted, mice exhibited unforeseen allotriophagia and ingested embedding material.

Conclusion: These observations strongly suggest, that an initially restricted feeding regimen is contraindicated concerning GTG application and that the perception of hunger reaches an allotriophagic phenotype. Presumably, the impulse of excessive food intake was a strong driving force, therefore the actual degree of suffering in the GTG induced model of hyperphagia should be estimated in further investigations and must be adapted in terms of the 3R-rule, especially under the aspect of refinement.

Refinement of the procedure of 5/6-nephrectomy in rats, a representative model of chronic-progressive kidney disease

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Implementing the “3Rs” replacement, reduction and refinement in animal experimentation is worldwide an acknowledged ambition. Thereby, refinement of applied techniques, targeting on scaling down extend of pain, suffering or distress in animals as low as possible, seems to be hampered by sufficient availableness of scientific information. As the implementation of analgetic regimen seems to be a matter of course, it is of great importance to achieve comparable progress concerning refinement of experimental techniques combining animal welfare and enhancement of model validity. Scientists all over the world potentially can contribute to this important issue by sharing their scientific experiences, beyond that supporting further development of concise and alienable guidelines for estimating pain and suffering of laboratory animals.

Chronic kidney disease (CKD) endangers human health worldwide. Even if in the last decade some progress concerning renal therapy focusing on the blockade of the renin-angiotensin-aldosterone system (RAAS) and on blood pressure control has been made, there still is no convincing therapeutic strategy of halting disease progression. Therefore, not only from a scientifically point of view, but primarily aiming on protecting patients from the need of dialysis or organ replacement, suitable pharmacological strategies have to be found. Investigations focusing on a better understanding of renal pathology and thereby hopefully detecting new molecular targets for intervention are essential for escaping the “renal dilemma”.

In terms of progressive renal models 5/6-nephrectomy in rats is approved and highly reproducible. Even if in the current paperwork information concerning surgical procedures is provided, the content of this scientific work deals with details, intending to minimize unforeseen loss of animals (100% survival rate), improve fast recovery indicated by postsurgical steady body weight development, abate suffering and provide a highly comparable analogon to the human course of chronic kidney disease (delineated through histiological outcome and renal functional parameters), thereby representing a substantial contribution to the 3R's aspect of refinement.

LABexpert - Laboratory animals data management solution

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Considering how complex is keeping all records for laboratory animals today and to follow all legal requirements of Animal protection act (1), a custom managed software LABexpert was designed. Precisely, the software offers an integrated management and tracking of the animal supply, quarantine, and integration of animals in experiments, final destiny of animals (way of sacrificing) and disposal of carcasses. Upon arrival of laboratory animals in the organization they received, unique traceability number according to their purpose (type of license), which accompanies them to their final destiny. The program offers a platform to describe all procedures that were performed on experimental animals, system for management of collected samples (tissue, blood), as well as database for obtained results in the experiment. The fate of individual animal is monitored and recorded chronologically. Furthermore, the program allows accurate recording the status of laboratory animals in all stages of the trial, multiple users to search in the database and simultaneous generation of an electronically protocols, records, reports, required by our legislation. Due to central database of all performed experiments at institution, a better control over the use of animals in experiments and the improvement in the implementation of 3Rs were achieved.

In conclusion, the LABexpert program in Microsoff Access improved the supervision of the use of animals in the experiments and further goal is to upgrade it in the program on the data server, Microsoft SQL Server Express.

(1) ZZZiv-UPB3, Official Journal RS, no. 38/2013 and Rules on animal testing (Official Journal RS, no.37/2013).

P8

EBI2 provides a molecular link between cellular oncogene expression, cell proliferation and B cell malignancies

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The G protein-coupled receptor (GPCR) Epstein-Barr virus-induced gene 2 (EBI2 or GPR183) was originally discovered as the most upregulated gene in Epstein-Barr virus (EBV) infected Burkitt lymphoma cells. EBI2 is a chemotactic receptor, expressed in immune cells and mediates B cell and follicular dendritic cell migration in response to its endogenous ligands, oxysterols. Recent studies have shown that downregulation of EBI2 is important for B cell participation in germinal center (GC) reactions. Knockout of the EBI2 gene facilitates B cell migration to GCs, whereas retrovirus-induced expression retains the B cells in the extrafollicular area within secondary lymphoid organs. Furthermore, previous studies have revealed that EBI2 induces cell proliferation and a strong G protein-coupled signaling similar to the oncogenic herpesvirus-encoded GPCRs CMV-US28 and HHV8-ORF74, which may contribute to the maintenance of immune cells that express his receptor. To elucidate the role of EBI2 in B cell and lymphoma development, we generated transgenic C57BL/6 mice expressing the human EBI2 (hEBI2) under the control of the immunoglobulin heavy chain promoter and intronic enhancer, to induce expression in B cells (designated IgH-hEBI2).

We show that B cell-targeted expression of hEBI2 in B6 mice leads to lymphoma development and premature death. B cell-targeted expression of hEBI2 in mice not only leads to an expanded CD5+ B1a B cell subset from a young age, but also to the development of a late-onset chronic lymfocytic leukemia (CLL)-like disease with lymphomatous transformation and premature death. In addition, the B2 cell compartment, and, as a consequence, the GC-dependent humoral immune response, is compromised. B cells expressing hEBI2 are characterized by elevated proliferation and upregulation of the cellular oncogenes c-Myc and BCL-2.

Obtained results are highly similar to the changes detected in CLL patients and identify EBI2 as a promotor of B cell malignancies. Therefore our findings suggest C57BL/6 mice expressing hEBI2 as a model to study the origins and development of CLL, which is the most common hematological cancer in the world.

Reference: Niss Arfelt K et al. Blood 2017; 129: 866-78.

P9

Ethics of animal experimentation - personal viewpoints from the course participants and veterinary medicine students

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In the past six years questionnaires regarding the ethics of animal experimentation were completed by the participants of the Course in laboratory animal science, who would be able to work in the future with laboratory animals on different levels according to the valid regulation on animal experimentation in Slovenia and Europe, as well as with students of veterinary medicine in 1st year at the subject Anatomy of domestic animals and in the 3rd year at elective subject Anatomy of laboratory and exotic animals.

The aim was to observe and compare participants, who will be involved in handling or experimenting with laboratory animals. We were interested in their views about man's duties to animals and if there are any differences between compared groups.

Participants of the course and students have used Animal Ethics Dilemma website, which helps users to obtain a better understanding of their own ethical views and those of others. It is a computer maintained educational instrument, developed primarily for veterinary students. The programme is constructed as a computerized role-play game involving a number of case studies that users can either 'play' or explore. Website tool provokes user as a 'provocation engine' to reflect critically on the standpoint (contractiarism, utilitarism, the animal rights view, relational view, respect for nature, hybrid view) regarding different ethically problematic situations and defines user's personal viewpoint based on attraction to alternative perspectives. The user experiences a role-play situation with a number of alternative choices. Choices made by the user lead to new dilemmas in which further decisions need to be made – challenging the user's initial reactions. The dilemmas help to bring out the way in which ethical arguments relate to the situations described. Students and participants were asked to complete survey before listening to lectures or course and to choose between different answers offered as soon as possible on the basis on the affiliation of their first thought.

We have observed that students and participants of the Course in laboratory animal science have hybrid viewpoints regarding experiments on animals. They are similar in the strongest component of the view, however not in all other viewpoints.

P10

Safe and simple procedure to restrain rats for blood sampling from tail vein

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Everyday laboratory practice and teaching procedures include handling laboratory rats in order to collect blood samples from the tail vein. For that purpose, a restraining device is needed. It is generally accepted to restrain the rat either by wrapping it into the towel or by using one plastic cylinder that can be adapted to animal size. Exposing the rat to such a procedure is potentially stressful and detrimental to obtaining a proper sample. Also, while trying to place the rat in the restraining device, the animal might resist the procedure. Here we propose a simple, effective and harmless way to restrain the rat. Two plastic cylinders are needed. Their diameter is such that the smaller cylinder can be placed within the bigger one. As smaller cylinder we used plastic bottle. The cover and the bottom of the bottle were removed, leaving a cylinder that is narrow on one end. The cylinder with bigger diameter has a cover on each end. On one end of a bigger cylinder, the cover has only one hole, which is suitable to place the rat's tail. On the other end of the same cylinder, the cover has several smaller holes to enable a free flow of air for breathing. Both covers are firmly attached to the outer cylinder. Once the rat is placed within the cylinders, it cannot move and stays immobile within a cylinder of smaller diameter. This way, the tail is freely accessible, making blood sampling from the tail vein fast and easy. Using two such cylinders can make blood sampling from the tail vein safe and simple. The need for touching the rat is minimal, since both cylinders can be placed into the cage so that the rat can freely enter the smaller cylinder while moving around the cage. Advantage of such a device is low cost, ease of use and minimal physical contact with the rat when the animal is already inside the smaller cylinder. Using such a device reduces potential stress in the laboratory rat caused by blood sampling and eliminates need for anaesthesia.

Isolation, cultivation, and characterization of mesenchymal stromal cells from mouse bone marrow

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Mesenchymal stromal cells (MSCs) are cells isolated from various tissues (*e.g.* bone marrow, adipose tissue, umbilical cord) exhibiting medically interesting features. The set of medical applications using MSC is growing steeply. Mouse MSCs are commonly used as model systems for the development of medical treatments.

Despite numerous attempts to create a standardized method for isolation and expansion of mouse MSCs, obtaining a homogenous population of large numbers of MSCs is still problematic. The purpose of our study was to develop an isolation protocol yielding high numbers of MSCs per individual mouse, their expansion *in vitro*, and determination of their phenotype using flow cytometry.

Previously, researchers have used only hollow bones to aspirate the bone marrow, often using a pool of 4—5 mice in order to generate enough MSCs for further applications. Using the bone crush method, we isolated the bone marrow cells of a single mouse from femur, tibia, humerus, iliac crest (legs) and the spine. MSCs were isolated based on their attachment to the bottom of the culture flasks. MSCs from legs and spine were cultured separately and analyzed in different culture conditions (O_2 levels, various growth mediums and growth densities).

Slow growth and contamination with various blood cells are known main problems in murine MSC cultures. We have managed to grow MSCs successfully only at 5% O_2 in the complete medium MesenCult, supplemented with MesenPure. Using this culture conditions, we were able to obtain MSC cultures with more than 90% CD45neg in passage 2. By measuring the β-galactosidase activity mouse MSCs senescence status was determined, and the wound-healing assay was used to test their migration capacity. Taking into account all analyzed parameters, MSCs isolated from the spine showed different characteristics than the MSCs isolated from the legs. Spine MSCs were less senescent with better proliferative and migration capacities. Their cell-surface antigen profile was similar for CD45, CD44, CD29 and heterogeneous for CD73 and CD105.

Our research indicates that the spine shows promise as a good source of MSC, however further functional tests are required to confirm their developmental potential and the ways in which they differ to MSCs isolated from leg bones.

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Computational support for management, tracking and analysis of animal samples

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We present the design and implementation of the information system as a support system for management and tracking of animal collected samples, as well as analysis of acquired data within the laboratory research environments. The proposed information system supports various task, which can be divided among (1) collection, storage and identification of samples, (2) data management and (3) computational analysis of acquired data. The system was implemented solely with open-source tools and platforms, which makes it accessible to wide scientific community. Moreover, the system is easy to maintain and can be customized according to the users' demands. The system is designated for the use in the field of laboratory animals and incorporates several experimental workflows, *i.e.* from the initial collection of samples to the biochemical analysis of obtained data. It supports data import and export in a CSV format, which makes it compatible with the majority of commercial software products for data management and analysis. We strongly believe that the proposed system presents an excellent alternative to the popular general-purpose computational tools for data management and analysis that are currently prevalent within research laboratories.

3R opportunities in QC laboratory performing Pharmacopoeial *in vivo* assays

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Quality control (QC) laboratories in pharmaceutical companies face several strict regulations dictated by different health authorities in order to assure safe, efficient and quality medicines to the patients. At the same time these laboratories are engaged to follow high intenal ethics and norms.

Biological control is a QC laboratory which needs to follow the Pharmacopoeial guidelines and current Good manufacturing practices (cGMP). This means that each single method, system or eqipment must go through validation and qualification processes in order to assure that every result obtained is accurate and reliable. Practically, this can result in more work upfront, also requiring possible increased use of laboratory animals as these represent the testing system for *in vivo* analytical methods. However, once the method is in a validated status, no or very little test are to be repeated.

The Pharmacopoeial chapters are clearly and exactly written and there is not much space for different interpretation or simple implementation of 3R rules. One needs to be creative in finding what is possible to do within the frame. This includes all 3Rs: reduction (*e.g.* using of animals more than once, whenever allowed by the method), refinement (*e.g.* continuous improvment of the method, trending, reviewing and analyzing historical results, keeping the operaters skilled) and replacement (*e.g.* seeking other methods which might once substitute *in vivo* methods with the *in vitro* ones).

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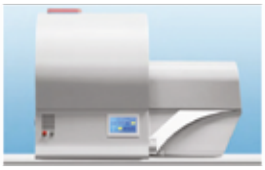

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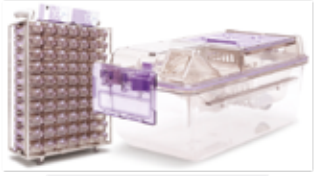

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