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SLOVENIAN VETERINARY RESEARCH

SLOVENSKI VETERINARSKI ZBORNIK

Supplement 14

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1st Congress of Slovenian Society for Laboratory Animals

Ljubljana, 24. januar 2013

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1. Kongres Slovenskega društva za laboratorijske živali
1st Congress of Slovenian Society for Laboratory Animals

Ljubljana, Slovenia
24 January, 2013

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Veterinarska fakulteta Univerze v Ljubljani
Veterinary Faculty, University of Ljubljana,
Cesta v Mestni log 47, Ljubljana

1. Kongres Slovenskega društva za laboratorijske živali

1st Congress of Slovenian Society for Laboratory Animals

Zbornik povzetkov / Proceedings

Ljubljana, 24. januar 2013

Pozdravni nagovor

Spoštovani kolegi,

V imenu Slovenskega društva za laboratorijske živali vas z velikim veseljem vse lepo pozdravljam na 1. kongresu Slovenskega društva za laboratorijske živali.

Čeprav je bilo Slovensko društvo za laboratorijske živali ustanovljeno že leta 2001, smo z aktivnostmi v okviru društva začeli šele pred dvemi leti. Čas začetka aktivne poti društva sovpada s sprejetjem evropske Direktive 2010/63/EU, ki ureja področje dela s poskusnimi živalmi. V okviru društva smo (v sodelovanju z Etično komisijo Republike Slovenije ter Iniciativnim odborom raziskovalcev) v teh dveh letih aktivno sodelovali pri pripravi predpisov oziroma uvajanju Direktive 2010/63/EU v slovensko zakonodajo.

Kongres organiziramo za vse, ki ste kakorkoli vpeti v delo s poskusnimi živalmi. Prvi kongres Slovenskega društva za laboratorijske živali je namenjen predstavitvi raziskovalnega dela, metod, tehnik, opreme in izkušenj na področju dela s poskusnimi živalmi v posameznih organizacijah, ki ga bodo predstavili prestavniki vseh slovenskih inštitucij s tega področja.

Del programa je namenjen predstavitvi Direktive 2010/63/EU in novostim, ki jih le ta prinaša v slovensko zakonodajo.

Posebna zahvala gre našim sponzorjem, ki so omogočili ta dogodek.

Veselimo se srečanja z vami na 1. kongresu Slovenskega društva za laboratorijske živali.

Znan.sod.dr. Martina Perše
Predsednica Slovenskega društva za laboratorijske živali

Welcome

Dear Colleagues,

On behalf of the Slovenian Society for Laboratory Animals it is a great pleasure to welcome you to the 1st Congress of the Slovenian Society for Laboratory Animals.

The Slovenian Society for Laboratory Animals was created in 2001. However, the first activities of the Society started two years ago. From the beginning of this active path, the Slovenian Society for Laboratory Animals (in collaboration with the Ethical Committee for laboratory animals of the Republic of Slovenia and the Committee of researchers working with laboratory animals) has taken important part in the process of implementation of the EU Directive 2010/63/EU.

The mission of the 1st Congress of Slovenian Society for Laboratory Animals is to bring together all Slovenian organizations and companies that are using experimental animals and to gain insight into their current work, techniques and the equipment they use and possess.
Thus, the 1st Congress of the Slovenian Society for Laboratory Animals will mostly serve as presentation of research, methods, equipment and experience by representatives from all institutions in Slovenia that are using experimental animals either in research or in pharmaceutical development.
Special part will be devoted to the introduction of European Directive 2010/63/EU and the changes it brings to the Slovenian legislation in the field of laboratory animal science.

We would like to thank our sponsors who genereously support this event.

We look forward to welcome you to the 1st Congress of the Slovenian Society for Laboratory Animals.

Znan.sod.dr. Martina Perše
President of the Slovenian Society for Laboratory Animals

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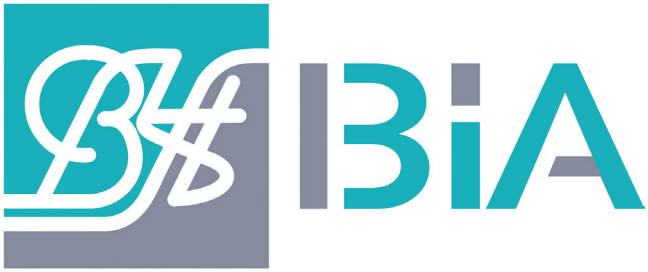
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PROGRAM / PROGRAMME

8.30 – 9.00	**Registration**
9.00 – 10.00	Dragica Ornik: **Presentation of a new EU directive and Slovenian legislation** (The administration of the Republic of Slovenia for food safety)
10.00 -10.20	***Coffee break***

Predsedstvo / Chairpersons: M. Černe, G. Majdič

10.20 - 10.30	Gorazd Drevenšek: **Preclinical pharmacological and toxicological studies and experimental animals** (Faculty of Medicine, University of Ljubljana)
10.30 – 10.40	Andreja Erman: **Basic and applicative studies of urinary bladder on animal models** (Faculty of Medicine, University of Ljubljana)
10.40 – 10.50	Anton Cerar: **Animal models of human pathology - our experience** (Faculty of Medicine, University of Ljubljana)
10.50 – 11.00	Jure Ačimovič: **Transgenic animal models for studies of cholesterol synthesis** (Faculty of Medicine, University of Ljubljana)
11.00 - 11.10	Mara Bresjanac: ***In vivo*** **methods for the study of brain function in laboratory rodents** (Faculty of Medicine, University of Ljubljana)
11.10 – 11.20	Aleksandra Milutinović Živin: **Rat model of unilateral excitotoxic striatal injury for the study of drugs with potential neuroprotective effects** (Faculty of Medicine, University of Ljubljana)
11.20 - 11.40	Fajko Bajrović, Špela Glišović, Uroš Kovačič, Janez Sketelj, Marko Živin: **Neurobiological research on laboratory rodents** (Faculty of Medicine, University of Ljubljana)
11.40 – 12.00	Maja Čemažar: **The use of laboratory animals in oncology** (Institute of Oncology Ljubljana)
12.00 - 13.15	***Lunch***

Predsedstvo / Chairpersons: A. Milutinovič Živin, S. Horvat

13.15 – 13.30	**Komercialna predstavitev**
13.30 - 13.50	Vladka Čurin Šerbec: **Experimental animals were prerequisite for the development of modern diagnostic reagents and many scientific discoveries** (Blood Transfusion Centre of Slovenia)

13.50 - 14.10	Marjan Slak Rupnik: **Glucose-stimulated events in islets of Langerhans from acute mouse pancreas tissue slices** (Faculty of Medicine, University of Maribor)
14.10 - 14.30	Gregor Majdič: **Laboratory mice in neuroendocrinological and behavioural studies** (Veterinary Faculty, University of Ljubljana)
14.30 - 14.50	Boris Turk: **Animal models as a tool for understanding the role of proteases and their regulation** (Institut Jožef Stefan)
14.50 - 15.10	Manica Černe: **Testing the safety, efficacy and quality of medicinal products for human and veterinary use** (Lek pharmaceutical company)
15.10 - 15.30	***Coffee break***

Predsedstvo / Chairpersons: M. Čemažar, M. Slak Rupnik

15.30 - 15.50	Simon Horvat: **Genetic analyses of obesity using polygenic mouse models and development of transgenic lines for cholesterol biosynthesis functional studies** (Biotechnical Faculty, University of Ljubljana and National Institute of Chemistry)
15.50 – 16.05	Tatjana Pirman: **Animal nutrition research in Biotechnical faculty** (Biotechnical Faculty, University of Ljubljana)
16.05 – 16.10	Irena Rogelj: **Use of laboratory animals for the study of safety and mechanisms of action of probiotic bacteria** (Biotechnical Faculty, University of Ljubljana)
16.10 – 16.20	Mojca Narat: **Study of immune processes in poultry and production of mouse and chicken monoclonal antibodies** (Biotechnical Faculty, University of Ljubljana)
16.20 – 16.25	Dušan Terčič: **Educational and research centre for poultry breeding – genetic resources and infrastructure** (Biotechnical Faculty, University of Ljubljana)
16.25 – 16.30	Klemen Potočnik: **Horses in experiments as productive, pet and therapeutic animals** (Biotechnical Faculty, University of Ljubljana)
16.30 – 16.40	Martina Perše: **Keeping the records and reports in animal experimentation** (Faculty of Medicine, University of Ljubljana)
17.00	Meeting of the Slovenian Society for Laboratory Animals

Vabljena predavanja

Invited lectures

Predstavitev nove EU direktive in slovenske zakonodaje

Dragica Ornik

Uprava Republike Slovenije za varno hrano, veterinarstvo, varstvo rastlin, Območni urad Ptuj, Miklošičeva 5, 2250 Ptuj, Slovenija, E-mail: dragica.ornik@gov.si

Zakonodaja ščiti poskusne živali z vrsto določil in predpisuje pogoje, pod katerimi je dovoljeno izvajati poskuse na živalih, kadar so ti potrebni. Nacionalna zakonodaja temelji na evropski konvenciji ETS 123 in novi direktivi 2010/63/EU. Zakonodaja dosledno zahteva uporabo načela 3R oz. zamenjave, zmanjšanja in izboljšanja pri vzreji, reji in uporabi živali. Definicija poskusa vključuje ravnanja, ki pri živali povzročijo enakovredno ali hujšo stopnjo bolečine, trpljenja, stiske ali trajnih poškodb, kot jo predstavlja vbod igle v živalsko telo. Vse organizacije morajo biti odobrene s strani Uprave Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin in morajo med drugim imeti strokovnjaka za dobrobit živali, Komisijo za dobrobit živali in imenovanega veterinarja. Postopki izdaje dovoljenj za izvajanje poskusov na živalih so obsežnejši; vsi poskusi morajo biti razvrščeni v eno izmed štirih kategorij težavnosti: nepovraten, blag, zmeren, težaven. Odobritev projekta je omejena na poskuse, ki so dobili pozitivno oceno etične komisije. Za nekatere poskuse bo zahtevana retrospektivna ocena. Z objavo netehničnih povzetkov projektov bo o uporabi živali seznanjena javnost. Vsaka država članica je dolžna prispevati k razvoju in potrjevanju alternativnih pristopov, s katerimi bi zamenjali, zmanjšali in izboljšali uporabo živali v poskusih. V ta namen je imenovan referenčni laboratorij Unije, v Sloveniji povezan z Uradom Republike Slovenije za kemikalije.

Presentation of a new EU directive and Slovenian legislation

Dragica Ornik

The administration of the Republic of Slovenia for food safety, veterinary and plant protection, Regional office Ptuj, Miklošičeva 5, 2250 Ptuj, Slovenia, E-mail: dragica.ornik@gov.si

Legislation protects experimental animals with a number of provisions and prescribes conditions under which is permitted to carry out experiments on animals, when they are needed. National legislation is based on the European convention ETS 123 and the new Directive 2010/63/EU. The legislation requires strict adherence to the 3R principles of replacement, reduction and refinement in breeding, rearing and use of animals. Definition of an experiment includes practices that cause an equivalent or higher degree of pain, suffering, distress or lasting harm to the animal, by the introduction of a needle into the animal body. Organizations need to be approved by the administration of the Republic of Slovenia for food safety, veterinary and plant protection. Among others, they must have an expert on animal welfare, animal-welfare body and a designated veterinarian. Project authorisation for carrying out experiments on animals is more extensive; all the experiments must be classified in one of the four severity categories: non-recovery, mild, moderate and severe. The project authorisation is limited to experiments, which have been positively evaluated by ethical committee. For some experiments a retrospective assessment will be requested. The public will be informed about the use of animals by publishing non-technical project summaries. Each Member State is required to contribute to the development and confirmation of alternative approaches, which would replace, reduce and refine the use of animals in experiments. For this purpose is named a reference laboratory of the Union, in Slovenia connected with the Chemicals Office of the Republic of Slovenia.

Vodenje evidenc in poročil pri izvajanju poskusov

Martina Perše[1], Dragica Ornik[2], Boštjan Markelc[3], Tatjana Pirman[4], Tomaž Snoj[5]

[1]Medicinski eksperimentalni center, Inštitut za patologijo, Medicinska fakulteta Univerze v Ljubljani, Zaloška 4 , 1000 Ljubljana; [2]Uprava Republike Slovenije za varno hrano, veterinarstvo in varstvo rastlin, Območni urad Ptuj, Miklošičeva 5, 2250 Ptuj, Slovenija; [3]Oddelek za eksperimentalno onkologijo, Onkološki inštitut Ljubljana, Zaloška 2, 1000 Ljubljana; [4]Katedra za prehrano, Oddelek za zootehniko, Biotehniška fakulteta, Univerza v Ljubljani, Groblje 3, 1230 Domžale, Slovenia; [5]Veterinarska fakulteta, Univerza v Ljubljani, Gerbičeva 60, Ljubljana, Slovenia, E-mail: martina.perse@mf.uni-lj.si

Slovenska zakonodaja predpisuje, da je pri delu s poskusnimi živalmi potrebno voditi številno dokumentacijo, ki jo je potrebno hraniti vsaj 5 let po prenehanju veljavnosti. Vodenje dokumentacije je predpisano zelo podrobno in za vsako posamezno kategorijo organizacij posebej (vzrejna, uporabniška, dobaviteljska, izvajalec). Kljub podrobnemu opisu teh določb so se v preteklosti pojavljale težave pri vodenju dokumentacije v različnih organizacijah iz različnih objektivnih razlogov. V Slovenskem društvu za laboratorijske živali je bila posledično sprejeta pobuda, da bi pripravili univerzalne obrazce evidenc in poročil in se tako v bodoče izognili tovrstnim nepotrebnih težavam, hkrati pa poenostavili delo tako uporabnikom kot nadzornikom.

V okviru Slovenskega društva za laboratorijske živali smo ustanovili delovno skupino, v kateri smo na podlagi preteklih izkušenj ter določb Direktive 2010/63/EU pripravili obrazce evidenc in poročil, ki bodo objavljeni v prilogi Pravilnika o pogojih za izvajanje poskusov na živalih in se bodo večinoma lahko vodili v pisni ali elektronski obliki.

Zaradi omejenosti s časom bomo na predavanju predstavili primer vodenja evidenc in letnih poročil na namišljenem primeru izvajanja raziskav na živalih ter namišljenem primeru znanstveno-raziskovalnega dela na tkivih usmrčenih živalih.

Keeping the records and reports in animal experimentation

Martina Perše[1], Dragica Ornik[2], Boštjan Markelc[3], Tatjana Pirman[4], Tomaž Snoj[5]

[1]Medical Experimental Centre, Institute of Pathology, Faculty of Medicine, University of Ljubljana, Zaloška 4, Ljubljana, Slovenia; [2]The administration of the Republic of Slovenia for food safety, veterinary and plant protection, Regional office Ptuj, Miklošičeva 5, 2250 Ptuj, Slovenia; [3]Department for experimental oncology, Institute of Oncology, Zaloška 2, Ljubljana, Slovenia; [4]Chair of Nutrition, Department of Animal Science, Biotechnical Faculty, University of Ljubljana, Groblje 3, 1230 Domžale, Slovenia; [5]Veterinary faculty, University of Ljubljana, Gerbiceva 60, Ljubljana, Slovenia, E-mail: martina.perse@mf.uni-lj.si

According to Slovenian legislation, work with experimental animals requires the recording of numerous data and documents that must be kept for at least 5 years from the expiry date. Under the national provisions the managing of records is extensive, precise and specific for each category of users or organizations that work with experimental animals i.e. breeders, suppliers and users. In spite of detailed descriptions of these requirements, various difficulties in the managing of records have appeared in different establishments in the past due to various objective reasons.

To avoid unnecessary difficulties and disagreements and to make managing of records easier for users and inspectors the Slovenian Society for Laboratory Animals proposed to prepare universal forms of records and reports. Under this initiative a working group was established in the Slovenian Society for Laboratory Animals. Based on our previous experience and the provisions of the Directive 2010/63/EU we prepared simplified forms of the records and reports, which will be published as Annex of Rules on conditions for experiments on animals. According to our provisions, most of the prepared forms of records will allow keeping the required records in either writing or electronic form.

Due to the limited time of the lecture, we will present an imaginary example of keeping all the required records and reports when executing experiments on animals. We will also present an example of keeping the records and reports when killing the animals solely for the use of their organs or tissues.

Predklinične farmakološko-toksikološke raziskave na poskusnih živalih

Gorazd Drevenšek

Inštitut za farmakologijo in eksperimentalno toksikologijo, Medicinska fakulteta, Univerza v Ljubljani, Korytkova 2, 1000 Ljubljana, Slovenija, E-mail: gorazd.drevensek@mf.uni-lj.si

Na Inštitutu za farmakologijo in eksperimentalno toksikologijo Medicinske fakultete izvajamo raziskave večinoma na živalskih tkivih, organih, in na živalih, v manjši meri pa tudi na humanih tkivih in celičnih linijah. Proučujemo učinke endogenih prenašalcev, zdravil, toksinov, strupov in drugih ksenobiotikov na izoliranih organih, tkivih in celicah ter njihov vpliv na delovanje posameznih organov v fizioloških pogojih in patološko spremenjenih poskusnih modelov, kot npr. hipoksično in ishemično izzvane okvare tkiv. Poleg farmakodinamičnih študij se ukvarjamo tudi s farmakokinetičnimi študijami in farmakoepidemiologijo. Raziskovalno delo poteka na različnih eksperimentalnih modelih, od celičnih sestavin, izoliranih celic (mastociti, astrociti, miociti, endotelijske celice idr.) in tkiv do izoliranih organov (izolirane žile, izolirano srce, izoliran atrij, želodec,) in živali (miši, podgane, budre). Poskusi potekajo tako ob akutnem kot kroničnem odmerjanju zdravil in učinkovin.

Zdravila, učinkovine in prenašalne sisteme proučujemo večinoma v tkivih in organih osrednjega živčevja, srčno-žilnem sistemu, prebavilih. Nekoč obsežne raziskave na histaminskih receptorjih in histaminskem sistemu so danes dopolnile raziskave nevrotrofičnih dejavnikov v astrocitih in sistemu osrednjega živčevja, kot so živčni rastni dejavnik in drugi nevrotrofini. V srčno žilnem sistemu pa so osrednji modeli za preučevanje delovanja zdravil ishemično reperfuzijske okvare srca, ateroskleroza, metabolni sindrom, diabetes. Proučujemo tudi vpliv zdravil na metabolizem kosti. Za raziskave novih ali novejših farmakološko aktivnih učinkovin je mnogokrat potrebno uvesti nove modele, kot je model ateroskleroze na budrah, model venske tromboze za proučevanje antikoagulacijske aktivnosti novih zdravil ali modeli vrednotenja kardio- ali nevroprotektivnih lastnosti zdravil.

Poleg temeljnih raziskav izvajamo tudi aplikativno usmerjene raziskave skupaj s kliničnimi raziskovalci, pa tudi za farmacevtska in druga podjetja. Prednost predkliničnega dela v primerjavi s kliničnim je, da lahko po kroničnem poskusu odmerjanja zdravil žrtvovanim živalim tkiva ali organe odvzamemo in jih biokemično analiziramo, česar v kliničnih poskusih ni mogoče narediti, večinoma za analizo izražanja izbranih genov. Živalski vrsti, ki ju največ uporabljamo za kronične študije sta podgana (Wistar, Goto Kakizaki, gensko spremenjene podgane Wistar z izbitim genom za endotelinski B receptor) ter budre (Dunkin Hartley). Prav te živalske vrste in gensko spremenjene linije omogočajo študije, ki jih na ljudeh zaradi nezmožnosti odvzema tkiv ni mogoče izvesti.

Preclinical pharmacological and toxicological studies on experimental animals

Gorazd Drevenšek

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At the Institute of Pharmacology and Experimental Toxicology, Faculty of Medicine, the most of research is conducted in animal tissues, organs, and animals, and to a lesser extent, also in human tissues and cell lines. Most of the studies are focused on the effects of endogenous transmitters, drugs, toxins, poisons and other xenobiotics on isolated organs, tissues and cells and their role on the functioning of various organs under physiologic conditions and modified experimental models, such as by hypoxia or ischemia induced tissue injuries. Beside the pharmacodynamic studies researhers are engaged in pharmacokinetic studies and pharmacoepidemiology. Research work is carried out in different experimental models of cellular components, isolated cells (mast cells, astrocytes, myocytes, endothelial cells, etc.) to isolated tissues and organs (isolated arteries, isolated hearts, isolated atrium, stomach) and animals (mice, rats, guinea pig). Experiments are conducted by using acute and chronic administration of drugs and other substances.

Drugs, substances and neurotransmitter systems studied are mostly the tissues and organs of the central nervous system, cardiovascular system, and gastrointestinal tract. Once upon extensive research on histamine and histamine system is now complemented by research of neurotrophic factors in astrocytes, and in central nervous system, such as nerve growth factor and other neurotrophins. In the cardiovascular system, a central model for the study of drug effectivity is a model of ischemic reperfusion injury, then atherosclerosis, metabolic syndrome, diabetes. We are also studying the effects of drugs on bone metabolism. For research of new or newer pharmacologically active substances, it is often necessary to introduce new study models, like the model of atherosclerosis in guinea pigs, venous thrombosis model to study the anticoagulant activity of new drugs or evaluation models for cardio-or neuroprotective properties of drugs.

In addition to basic research, also applied-oriented studies are performed in cooperation with clinical researchers as well as pharmaceutical and other companies. The main advantage of pre-clinical research studies in comparison to the clinical ones is the ability to sample animal tissues or organs from the sacrificed animals following chronic drug treatment and use them for biochemical analysis, mostly for expression analysis of selected genes in our case, which cannot be done within clinical trials. Species, which we used the most for study the chronic effects of the drugs are rat (Wistar, Goto Kakizaki genetically modified Wistar rats with a knockout of endothelin B receptor) and guinea pig (Dunkin Hartley). These species and genetically modified lines enable us the study design and tissue collection that cannot be performed on humans.

Temeljne in aplikativne raziskave sečnega mehurja na živalskih modelih

Andreja Erman

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Raziskovalna skupina Inštituta za biologijo celice Medicinske fakultete v Ljubljani se ukvarja s proučevanjem epitelija sečnega mehurja, imenovanega urotelij. Ta triskladni epitelij gradijo bazalne celice, vmesne celice in površinske celice. Slednje so v neposrednem stiku s hipertoničnim urinom, zato s številnimi tesnimi stiki in specifično zgrajeno apikalno plazmalemo tvorijo za organizem življenjsko pomembno krvno-urinsko bariero. Urotelij sesalcev je stabilno tkivo z zelo počasno samoobnovo in nizko proliferacijsko aktivnostjo, ki pa na izzvano poškodbo zelo hitro odgovori z intenzivno proliferacijo in diferenciacijo, kar omogoči hitro obnovo tkiva in krvno-urinske bariere.

Na našem inštitutu potekajo raziskave na *in vitro* gojenih urotelijskih celicah ter na odvzetih tkivih mišk in podgan. *In vitro* raziskave potekajo na primarnih kulturah urotelijskih celic in na trajnih celičnih linijah urotelijskih celic različnih stopenj rakaste transformacije. Na teh modelih proučujemo številne procese celične diferenciacije, proliferacije, regeneracije, migracije, endocitoze in znotrajceličnega transporta. V zadnjem času razvijamo tudi metodo transfekcije urotelijskih celic z mikroinjiciranjem plazmidov za fuzijske proteine z zelenim fluorescenčnim proteinom.

Na odvzetem tkivu laboratorijskih glodavcev potekajo študije ultrastrukture, proliferacije, diferenciacije in celične smrti urotelijskih celic odraslih živali, embrijev, mladičev in starih živali. Za analizo fiksiranih vzorcev tkiva uporabljamo klasično svetlobno mikroskopijo, fluorescenčno mikroskopijo ter presevno in vrstično elektronsko mikroskopijo, razvijamo pa tudi korelativno mikroskopijo.

Poleg temeljnih raziskav izvajamo tudi aplikativne raziskave, kjer na laboratorijskih glodavcih proučujemo regeneracijo urotelija po induciranem luščenju urotelijskih celic, za raziskave eksperimentalnega cistitisa sproženega z direktno instilacijo uropatogene bakterije *E. coli* v sečni mehur laboratorijskih živali, za študij karcinogeneze, sprožene s kemičnim karcinogenom BBN ter za vzpostavitev ortotopičnega modela tumorja sečnega mehurja z instilacijo rakave celične linije direktno v sečni mehur. Namen vseh teh *in vivo* raziskav je analiza celično-bioloških mehanizmov zlasti regeneracije, odzivov na bakterijsko vnetje in karcinogeneze, ki naj bi prispevala k boljšemu razumevanju omenjenih procesov ter iskanju novih načinov zdravljenja.

Basic and applicative studies of urinary bladder on animal models

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The experimental object of research group at Institute of Cell biology of Medical faculty in Ljubljana is the epithelium of the urinary bladder. This three-layered epithelium called urothelium is composed of basal, intermediate and superficial cells. Aforementioned cells are in contact with hypertonic urine and are responsible for blood-urine permeabiliy barrier by numerous tight junctions and specifically structured apical plasma membrane. Mammalian urothelium is a very stable tissue with slow turn-over and low proliferative activity, but after acute injury it responses immediately with intense proliferation and differentiation, which ensure fast renewal of tissue and blood-urine barrier.

At our institute, studies of urothelial cells are performed in *in vitro* conditions and on isolated tissue of mice and rats. For *in vitro* studies, primary cultures and cell lines of urothelial cells at different stages of carcinogenesis are used to analyse processes of cell differentiation, proliferation, regeneration, migration, endocytosis and intracellular transport. Recently, transfection with microneedle delivery of plasmid DNA for fusion protein with GFP is also introduced.

Isolated tissues of laboratory rodents are used for studies of ultrastructure, proliferation, differentiation and cell death of urothelial cells of adult, embryonic, newborn and aging animals. For the analysis of fixed tissue samples we use bright-field microscopy, fluorescence microscopy and transmission and scanning electron microscopy, while correlative microscopy is just developing.

Beside basic research, applicative studies are also performed on laboratory rodents for different studies such as: urothelial regeneration after induced desquamation of urothelial cells, experimental cystitis induced by transurethral catheterisation with *E.coli*, carcinogenesis induced by mitogen BBN and also for the establishment of murine orthotopic bladder tumor model by implanting tumor cells into the bladder. The aim of these *in vivo* studies is the analysis of cellular-biological processes of the regeneration, urothelial response on bacterial infection and carcinogenesis, which could contribute to better understanding of aforementioned processes and to find new therapeutic approaches.

Živalski modeli humane patologije – naše izkušnje

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Živalski modeli so velikokrat predmet kritik zaradi dejstva, da ne odražajo vseh vidikov človeških bolezni. Vendar pa popolna podobnost s človeško boleznijo kot tudi način ustvarjanja živalskega modela ne moreta biti predmet razprav ali merilo pri presojanju dobrega živalskega modela. Pomembno se je zavedati, da ima vsak živalski model prednosti in omejitve, ki jih je treba poznati in upoštevati. Izbira ustreznega živalskega modela je tako odvisna od področja in ciljev raziskave ter značilnosti in omejitev posameznega modela.
Iz izkušenj lahko povemo, da je delo z živalskimi modeli posebna veja znanosti o laboratorijskih živalih, za katero je potrebno imeti specifično znanje in posluh. S prispevkom želimo osvetliti naše znanje in izkušnje, ki smo jih pridobili pri delu s kemično induciranimi živalskimi modeli in izpostaviti številne dejavnike, ki jih je pri delu z živalskimi modeli treba upoštevati. Kot primer na kratko povzemamo značilnosti nekaterih kemično induciranih živalskih modelov (živalski modeli želodčne, kolorektalne in mamarne karcinogeneze, kolitisa ter akutne nefrotoksičnosti), ki smo jih prevzeli in uporabili v Medicinskem eksperimentalnem centru. Navajamo tudi njihove podobnosti s posamezno boleznijo pri ljudeh ter ključne dejavnike, ki lahko vplivajo na zanesljivost rezultatov pri uporabi posameznega modela. Na koncu prispevka smo na podlagi dolgoletnih izkušenj nanizali tudi nekaj priporočil za delo z živalskimi modeli.

Animal models of human pathology - our experience

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Animal models are often criticized due to the fact that they do not reflect human pathology in all aspects of the disease. However, the perfection in the resemblance of human pathology or the mode of production of animal model is not a matter of debate or judgment of a good animal model. It is important to recognize that every animal model has its own advantages and limitations that need to be taken into account. The choice of an animal model should base on the scope and aims of particular study and characteristics and limitations of particular model.

According to our experience, work with animal models is special branch of laboratory animal science that requires specific knowledge and attention. The aim of the present paper is thus to highlight our knowledge and experience obtained due to work with chemically induced animal models and to point out the numerous factors that need to be taken into account when working with animal models. As an example, we briefly introduce characteristics of some chemically induced animal models that have been adopted and used at Medical Experimental Centre (animal models of gastric, colorectal, and mammary carcinogenesis, colitis, and acute nephrotoxicity) and their similarities to corresponding human disease. Main factors that may seriously affect validity of the results when using particular animal model are also exposed. At the end of our paper we mention some experience based recommendations when using animal models.

Transgeni živalski modeli za preučevanje biosinteze holesterola

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Holesterol je zelo pomemben sestavni del membran živalskih celic. Pri tem ima na membrane utrditveni učinek, hkrati pa ohranja njihovo fluidnost. Zato je biosinteza holesterola uravnavana na najrazličnejših nivojih in razumevanje tega procesa je ključnega pomena v humani medicini, saj je holesterol pogosto omenjen v povezavi z boleznimi srca in ožilja. Biosinteza holesterola poteka v dveh fazah. Začetna je izoprenoidna, ki je zelo dobro raziskana, ter kasnejša poskvalenska, za katero pa natančno zaporedje reakcij še ni dokončno razjasnjeno. V naši raziskovalni skupini se ukvarjamo predvsem z raziskavami poskvalenskega dela biosinteze holesterola. Pri tovrstnih študijah kompleksnosti metabolične poti je uporaba transgenih živali pomembna za razumevanje celotnega mozaika biosinteze holesterola. V ta namen smo v sodelovanju s prof. dr. Gregorjem Majdičem pridobili miši z izničenim genom *Crem* (modulator cAMP odzivnega elementa) ter v sodelovanju s prof. dr. Simonom Horvatom miši z izničenim genom *Cyp51* (lanosterol-14α-demetilaza). CREM je transkripcijski dejavnik, ki preko vezave na CRE-promotorske regije DNA vpliva na izražanje genov s CRE-promotorskimi mesti, npr. *Cyp51*. CYP51 pretvori lanosterol, prvi sterolni intermediat v biosintezi holesterola, ki ima ciklično strukturo steroidnega obroča, v mejozo-aktivirajoči sterol, izoliran iz folikularne tekočine ženskih jajčnikov. V prostorih Centra za funkcijsko genomiko in biočipe (CFGBC) se nahaja vrhunska oprema za raziskave funkcijske genomike. Študije metabolomike izvajamo v tesnem večletnem sodelovanju s prof. dr. Ingemarjem Bjorkhemom (Inštitut Karolinska, Stockholm, Švedska), kar nam je omogočilo razviti učinkovito analitsko metodo plinske kromatografije, sklopljene z masno spektrometrijo, primerno za kvantitativno določevanje enajstih strukturno podobnih sterolnih intermediatov poskvalenskega dela biosinteze holesterola. Analitska metoda je omogočila vse nadaljnje študije biosinteze holesterola: cirkadiani (dnevno-nočni) ritem, vpliv CREM, pomen CYP51 ter vpliv različne prehrane pri miših. Najpomembnejše dognanje naših raziskav predstavlja vpliv spola na biosintezo holesterola. Večina študij uporablja pri svojih poskusih samo samce, vendar vključitev samic omogoča neposredno primerjavo med spoloma.

Transgenic animal models for studies of cholesterol synthesis

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Cholesterol is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. Therefore, the cholesterol synthesis is regulated at various levels and understanding this process is a key factor in human medicine, since cholesterol is often mentioned in relation to cardiovascular diseases. Cholesterol synthesis constitutes of two phases, the isoprenoid phase which is very well studied and the subsequent post-squalene phase for which precise sequence of reactions remains undetermined. Our research group is primarily focused on the research of post-squalene cholesterol synthesis. The use of transgenic animals for studying this kind of complex metabolic pathways and to understand the overall mosaic of cholesterol synthesis is essential. In collaboration with prof. Dr. Gregor Majdič and prof. Dr. Simon Horvat we obtained *Crem* (cAMP response element modulator) knock-out and *Cyp51* (lanosterol-14α-demethylase) knock-out mice, respectively. CREM is a transcription factor which regulates transcription of genes by binding to corresponding CRE-promoter regions of DNA. For example *Cyp51* contains CRE-promoter regions. Lanosterol, the first sterol intermediate of cholesterol synthesis with cyclic steroid ring structure, is converted to follicular fluid meiosis-activating sterol by enzyme CYP51. The Centre for Functional Genomics and Bio-Chips (CFGBC) is equipped with state-of-the art equipment which enables high-throughput functional genomic studies. Metabolomics studies are being carried out in collaboration with prof. Dr. Ingemar Bjorkhem (Karolinska Institute, Stockholm, Sweden). This fruitful collaboration resulted in development of a powerful gas chromatographic/mass spectrometric method allowing quantitative analysis of 11 structurally similar post-squalene cholesterol synthesis intermediates. The analytical method enabled all further studies of cholesterol synthesis: circadian (day-night) rhythm, the impact of CREM, the influence of CYP51 and effect of different diet in mice. We observed that gender has the most significant impact on cholesterol synthesis. Most studies use only male mice, but experiments including females enable direct gender comparisons.

Metode za raziskovanje delovanja možganov pri laboratorijskih glodavcih

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Predstavili bomo kratek pregled zaživljenjskih raziskovalnih pristopov, s katerimi pri poskusnih živalih lahko poglabljamo razumevanje motenj v delovanju živčevja in razvijamo nove terapevtske pristope. Prikazali bomo primer uporabe poskusnih živali v raziskovanju možganske funkcije med anestezijo.

Mnoge pogoste motnje v delovanju možganov lahko posnemamo z različnimi okvarami možganov laboratorijskih podgan in na njih spremljamo strukturne in funkcijske spremembe med okrevanjem. Trajne ali prehodne okvare, ki so značilne za humane nevrološke ali psihiatrične motnje (npr. okvara substance nigre pri parkinsonizmu, amigdal pri shizofreniji) povzročimo s fizikalnimi ali kemijskimi postopki. Ustrezni vedenjski testi omogočajo oceno in spremljanje funkcijske oškodovanosti (npr. testi motorične ali kognitivne funkcije) po okvari in med okrevanjem. Analiza okvarjenega in z njim povezanih področij v možganih *in vivo* (npr. s slikovnimi tehnikami kot je magnetno resonančno slikanje) in po smrti (npr. histološko in imunokemijsko) odpirajo vpogled v nevralne korelate vedenja. Ti pristopi omogočajo raziskovanje postopkov za zmanjšanje funkcijske oškodovanosti (npr. celični presadki, učinkovine ipd.).

Z merjenjem vitalnih znakov na poskusni živali med splošno anestezijo (preko namestitve tipal za 2-kanalno merjenje elektroencefalograma (EEG), 1-kanalno merjenje elektrokardiograma (EKG), intrarektalno merjenje temperature, neinvazivno merjenje krvnega tlaka, merjenje gibov prsnega koša, merjenje pO_2 v arterijski krvi) lahko ocenimo globino anestezije in spremljamo njeno spreminjanje pri uporabi različnih splošnih anestetikov. Ta ocena sloni na izračunu stopnje sinhronizacije aktivnosti možganov, respiratornega sistema in srčnožilnega sistema, ki se statistično pomembno spremeni pri prehodu med globoko in plitvo anestezijo. Sprememba je splošne narave in se pojavi ne glede na vrsto uporabljenega splošnega anestetika.

In vivo methods for the study of brain function in laboratory rodents

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A brief overview of *in vivo* brain research methods will be presented to illustrate how animal studies contribute to understanding brain disorders and to development of treatment strategies. An example will be given how animal research provides insight into brain function during anesthesia.

Different brain lesions are used for simulating prevalent brain disorders and for studying structural and functional brain recovery in the rat. Reversible or permanent brain lesions, known to be associated with human neurological and psychiatric disorders (e.g., substantia nigra in parkinsonism, amygdala in schizophrenia) are induced by physical or chemical means. Specific behavioral tests enable quantification and monitoring of functional damage (e.g., motor and cognitive function tests) after injury and during recovery. In addition, analysis of the lesioned and relevant other brain regions *in vivo* (e.g., imaging techniques like magnetic resonance imaging) and post mortem (e.g., histology and immunochemistry) offer insight into neural correlates of behavior. These approaches enable testing of candidate interventions for reduction of functional impairment (e.g., cell transplants, pharmacological substances etc.).

The rat model can also be used to study the changing level of unconsciousness caused by different anaesthetics during general anaesthesia. Associated with the state of anaesthesia, there are characteristic changes in both cardio-respiratory and cerebral oscillator parameters and couplings varying with depth of anaesthesia. Simultaneous recordings of electrocardiogram (ECG), respiration and electroencephalogram (EEG) during general anaesthesia enable tracking and separation between deep and shallow anaesthesia with the phase dynamics approach by extracting the instantaneous frequencies of heart beat, respiration and brain-waves with the Morlet mother wavelet. This approach reveals two distinct phases of anaesthesia independent of the anaesthetic used.

Model enostranske ekscitotoksične poškodbe striatuma, kot model za preučevanje snovi s potencialnimi nevroprotektivnimi učinki

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Ekscitotoksična poškodba možganov, ki nastane zaradi prekomernega sproščanja endogenega ekscitatornega živčnega prenašalca glutamata, ima pomembno vlogo pri patofizioloških procesih, npr. po možganski kapi (cerebralna ishemija in reperfuzija), pri epileptičnih napadih, travmatskih poškodbah možganov ter pri Parkinsonovi, Alzheimerjevi in Huntingtonovi bolezni. Preučevanje mehanizmov ekscitotoksičnosti bi zato lahko privedlo do učinkovitejšega zdravljenja zgoraj naštetih bolezni.

V prispevku bo prikazan model unilateralne ekscitotoksične poškodbe striatuma s stereotaktično injekcijo agonista glutamatnih receptorjev ter uporaba nekaterih laboratorijske metode, ki lahko pripomorejo pri odkrivanju novih potencialnih snovi z nevroprotektivnimi učinki.

Akutni učinek ekscitotoksina se pokaže s hemiepileptogeno možgansko aktivnostjo. Možganska področja, ki se aktivirajo zaradi neposrednega delovanja ekscitotoksina, oziroma posredno, zaradi pretiranega sproščanja endogenega glutamata, lahko prikažemo z *in vitro* metodami, ki omogočajo vizualizacijo povečanega izražanja genov zgodnjega odgovora (npr. z in situ hibridizacijo mRNA oziroma imunohistokemijo proteinov zgodnjega odgovora). S slikanjem z magnetno resonanco lahko zasledujemo razvoj edema v epileptogenih možganskih področjih.

V obdobju dveh tednov se pojavi atrofija striatuma, ki jo lahko vrednotimo z *in vivo* s slikanjem z NMR. Funkcionalno neravnovesje med hemisferama pa lahko ovrednotimo tudi z analizo ipsilateralnega kroženja podgan, ki ga povzročimo z agonisti dopaminskih receptorjev. Spremembe metabolne aktivnosti v poškodovanih področjih lahko ocenimo *in vitro*, z denzitometrično analizo histoloških preparatov obarvanih na aktivnost citokrom c oksidaze. Atrofijo striatuma lahko ocenimo z morfometrično analizo možganskih rezin obarvanih s histološkimi ali histokemičnimi postopki, ki pokažejo povečanje velikosti lateralnih ventriklov oziroma skrčenje striatuma na poškodovani strani.

Vse zgoraj opisane metode omogočajo (semi)kvantitativno analizo. Prednosti unilateralnega modela pri ocenjevanju nevroprotektivnih učinkov novih snovi so možnost primerjave nevrodegenerativnih sprememb s stanjem v intaktni hemisferi ter relativno manjši vpliv na dobro počutje podgan.

Rat model of unilateral excitotoxic striatal injury for the study of drugs with potential neuroprotective effects

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Excitotoxic damage to the brain caused by excessive release of endogenous excitatory nervous neurotransmitter glutamate plays an important role in pathophysiological processes, such as stroke (cerebral ischemia and reperfusion), epileptic seizures, traumatic injuries of the brain, Parkinson's, Alzheimer's and Huntington's disease. The study of the mechanisms of excitotoxicity could thus lead to more effective treatments for the above diseases.

This presentation will describe a model of unilateral ekscitotoxic striatal injury induced by stereotaxic injection of agonist of glutamate receptors and some of the laboratory methods that may help to reveal new drugs with neuroprotective potential.

Acute effect of excitotoxin is revealed by the occurrence of hemiepileptogenic brain activity. Brain areas that are activated due to the direct action of excitotoxin, or indirectly, as a result of the excessive release of endogenous glutamate during seizure activity, may be visualized by increased gene expression of early response genes (by *in situ* hybridization and immunohistochemistry of their mRNAs and/or proteins). The development of edema within epileptogenic brain regions could be assessed with magnetic resonance imaging (MRI).

Striatal atrophy can be assessed morphometrically, by the increase in the size of the lateral ventricles/shrinkage of the striatum on the damaged side. Brain sections stained by histological or histochemical methods could be used for this purpose. Striatal atrophy could also be visualized by MRI. Functional imbalance between hemispheres can be evaluated by quantifying ipsilateral turning behavior induced by agonists of dopamine receptors. Changes of metabolic activity in the injured areas can be evaluated by densitometric analysis of brain sections processed histochemically for cytochrome-c-oxidase activity.

All mentioned methods allow (semi)quantitative analysis of neurodegenerative changes. The advantages of unilateral model are its ability to display neurodegenerative changes in comparison with the situation in the intact brain hemisphere and relatively smaller impact on the welfare of experimental rats.

Nevrobiološke raziskave na laboratorijskih glodavcih

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V prispevku bodo predstavljeni modeli odvisnosti od drog in nekatere nevrobiološke raziskave bolezni perifernega živčevja in mišic na laboratorijskih glodavcih, ki jih že dolga leta izvajamo na Inštitutu za patološko fiziologijo Medicinske fakultete Univerze v Ljubljani.
1. Uporaba živalskih modelov pri raziskavah nevrobioloških mehanizmov odvisnosti od psihomotoričnih stimulansov je pomembna za razvoj novih metod zdravljenja. Za raziskovanje nastanka, vzdrževanja, odtegnitvenega sindroma in recidiva zasvojenosti z omenjenimi drogami se uporabljajo modeli: ”samojemanje drog”, ”možganska samostimulacija z električnim tokom” in ”preferenca prostora”. V prispevku bodo predstavljeni nekateri postopki in protokoli modela ”samoinjiciranje kokaina”, ki jih uporabljamo pri proučevanju učinkovin za zaviranje recidiva zasvojenosti v obdobju prisilne abstinence.
2. Regeneracijo poškodovanih aksonov in kolateralno brstenje nepoškodovanih aksonov v perifernih živcih proučujemo z modelom podganjega ishiadičnega živca, pri čemer eno izmed njegovih vej stisnemo, prerežemo in zašijemo proksimalni in distalni konec na način konec s koncem ali prerežemo in distalni konec od strani prišijemo na sosednji nepoškodovan živec. Po opravljenih različnih vrstah posegov ugotavljamo obseg obnove senzoričnih živčnih vlaken s funkcionalnima testoma vščipa kože in živca, elektrofiziologijo in histomorfometrijo regenerirajočega se živca in z retrogradnim barvanjem nevronov v ganglijih dorzalnih hrbtenjačnih korenin.
3. Tudi mišice so tkivo, ki kaže izrazito plastičnost, saj se njihove značilnosti prilagajajo funkciji. Za raziskovanje plastičnosti in proučevanje regeneracije skeletne mišice pri podgani uporabljamo naslednje postopke: trajna ali prehodna denervacija mišice, oživčenje mišice s tujim živcem po živčni anastomozi med lastnim in tujim živcem,indirektno električno draženje mišice s fazičnim ali toničnim vzorcem impulzov, ishemično-toksično uničenje mišice in regeneracija, navzkrižna transplantacija in regeneracija med hitro in počasno mišico (hitra mišica po uničenju transplantirana na kite počasne mišice, regeneracija in oživčenje z živcem počasne mišice) ter kombinacije teh postopkov.

Neurobiological research on laboratory rodents

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This paper will present some of the rodent models of drug addiction and of the regeneration of peripheral nerves and muscles that have been in use for many years at the Institute of Pathophysiology of Medical Faculty of Ljubljana University.

1. A great deal of interest has focused on the development and application of animal models of addiction that are relevant to drug-addicted humans. With the aim to get better insight in neurobiological mechanisms of the initiation, maintenance, withdrawal and relapse of drug- addiction, animal models of ”place preference”, ”brain self-stimulation with electrical current” and ”drug self –administration”, have been developed. This paper will present some of the procedures and protocols of cocaine self-administration experiments in rats, which are used for the study of potential anti-craving medication during the period of enforced abstinence.

2. Regeneration of injured axons and collateral sprouting of uninjured axons in peripheral nerves are studied using the model of rat’s sciatic nerve in which one of its branches is either crushed or transected and its distal and proximal stumps are coapted in end-to-end fashion or transected and its distal stump is coapted to the adjacent uninjured nerve in end-to-side fashion. After different treatment options are applied, the extent of sensory nerve fibers recovery is evaluated by functional skin and nerve pinch tests, electrophysiology and histomorphometry of the regenerated nerve and by using retrograde labeling of the neurons in the dorsal-root ganglia.

3. Muscle fibers also display extensive plasticity through which their properties adapt to their function. We use the following procedures to investigate muscle plasticity and their regeneration in rats: permanent and transient muscle denervation, innervation of muscles by a foreign nerve achieved by end-to-end nerve anastomosis, indirect electrical muscle stimulation by phasic or tonic impulse patterns, ischemic-toxic muscle injury to elicit muscle regeneration, cross-transplantation of slow and fast muscles followed by their regeneration, and different combinations of these procedures.

Uporaba laboratorijskih živali v onkologiji

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Uporaba laboratorijskih živali ima na področju raziskovanja raka velik pomen. Uporaba laboratorijskih živali v onkologiji poteka v raziskavah na področjih karcinogeneze (ugotavljanje bioloških mehanizmov, ki vodijo v nastanek raka), ugotavljanju potencialnih karcinogenov v okolju in testiranju novih terapij na eksperimentalnih tumorskih modelih, ki bi vodila v ozdravitev ali kontrolo maligne bolezni pri ljudeh in živalih. Kljub razvoju mnogih testov *in vitro* ter molekularnih in biokemičnih testov so poskusi na živalih še vedno potrebni pred začetkom kliničnih študij. Te študije obsegajo potrditev aktivnosti terapije na določenem tumorskem modelu, dajo vpogled v metabolizem zdravila in njegovo toksičnost za normalna tkiva ter služijo kot osnova za določitev maksimalne tolerantne doze v kliničnem poskusu faze I.

Na Oddelku za eksperimentalno onkologijo Onkološkega inštituta Ljubljana imamo več kot 30-letne izkušnje pri delu s tumorskimi modeli na laboratorijskih živalih, predvsem miših. Onkološki inštitut Ljubljana je uporabniška organizacija za delo s poskusnimi živalmi vpisana v register pri Ministrstvu za kemtijstvo in okolje. Onkološki inštitut Ljubljana ima poleg tega tudi dovoljenje za delo z gensko spremenjenimi organizmi v 2. varnostnem razredu in dovoljenje za delo z zaprtimi viri ionizirajočega sevanja. Delo s poskusnimi živalmi na Onkološkem inštitutu Ljubljana se izvaja izključno na Oddelku za eksperimentalno onkologijo, kjer imamo v vseh prostorih, kjer se nahajajo živali sterilne pogoje, ter kontrolirano temperaturo, vlago in svetlobo. Pri našem raziskovalnem delu, ki je predvsem usmerjeno v raziskovanje novih in kombiniranih pristopov za zdravljenje raka, uporabljamo različne vrste mišjih in humanih trajnih tumorskih modelov, ki so singenski določenim linijam miši. Največ uporabljamo naslednje linije miši: inbridirane imunsko odzivne C57BL/6, BALB/c in A/J linije ter imunsko zavrte SCID miši. Med pomembnejšo raziskovalno opremo, ki jo imamo na Oddelku za eksperimetnalno onkologijo za delo z živalmi, prištevamo obsevalno aparaturo za lokalno obsevanje tumorjev ali obsevanje celotnega telesa, fluorescentno lupo za neinvazivno spremljanje fluorescence tkiv ali tumorjev v daljšem časovnem obdobju na isti živali, ter elektroporator za transdermalni vnos učinkovin v različna tkiva. Pri delu uporabljamo standardne teste za ovrednotenje protitumorskega učinka terapij, kot so zaostanek v rasti in test lokalne kontrole rasti tumorjev. Specializirali smo se tudi za preučevanje terapij, ki ciljajo tumorsko žilje z razvojem metode dorzalnega okna, kjer lahko v *in vivo* pogojih spremljamo učinke terapij na normalno ali tumorsko žilje. V sodelovanju z drugimi oddelki na inštitutu in drugimi inštitucijami pa izvajamo tudi molekularno-biološke, citološke in histološke analize.

Namen in cilji Oddelka za eksperimentalno onkologijo bodo tudi v prihodnje, kot so bili že dosedaj, na področju dela z laboratorijskimi živalmi, slediti načelu 3R in zagotoviti čim boljše prostorske in materialne pogoje, ter visoko kakovostno usposobljen kader za delo s poskusnimi živalmi v onkologiji, kjer je zaradi posebnosti dela potrebno še dodatno posebno opazovanje, spremljanje in skrb za živali, predvsem takrat, ko pride do razvoja bolezni. Posebno pozornost zato posvečamo načrtovanju poskusov (pilotne študije, nasvet statistika, učenje dela z živalmi in pravilna dokumentacija), napovedovanju in prepoznavanju stranskih učinkov, določevanju stopnje bolečine in stresa, poznavanju biologije tumorjev, humanemu izboru postopkov, stalnemu pregledovanju živali, dokumentaciji in objavljanju.

The use of laboratory animals in oncology

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Animal testing is of great importance for the cancer research. The use of laboratory animals in oncology is carried out in the exploration of carcinogenesis (study of the biological mechanisms that lead to cancer), identification of potential carcinogenic agents in the environment and testing of new therapies on the experimental tumor models. Even though many *in vitro* and also molecular and biochemical test were developed, animal testing is still needed for the launch of a clinical trials. These studies involve the conformation of the therapy efficacy on a specific tumor model; they give the insight into the drug metabolism and its normal tissue toxicity and serve as the basis for the determination of maximum tolerated dose in the phase I clinical trial.

The Department of Experimental Oncology at the Institute of Oncology Ljubljana has more than 30 years of experience in working with the tumor models in the laboratory animals, predominantly mice. Institute of Oncology Ljubljana is a user organization for the work with experimental animals registered at the Slovenian Ministry of agriculture and the environment. Institute also has the authorization for the work with genetically modified organisms in the biosafety level II and the authorisation/licence for the work with closed ionising radiation sources. The work with laboratory animals at the Institute is executed exclusively at the Department of experimental oncology, in the animal laboratories with sterile conditions and controlled temperature, humidity and lightning. In our research work, which is mainly orientated in the exploration of new and combined cancer treatment approaches, different mouse and human tumor models syngeneic to specific mouse strains are used. Mouse strains that are mainly used at our department are: inbred immunocompetent C57Bl/6, Balb/c and A/J, and immunocompromised SCID mice. Some of the most important research equipment includes: X-ray unit for local tumor or whole body irradiation, fluorescent stereomicroscope for non-invasive follow up of tissue or tumor fluorescence over longer periods of time on the same animal, and electroporator for transdermal delivery of the different agents. For the evaluation of antitumor effectiveness of the therapy standard test are used like tumor growth delay and local tumor control assay. We have also specialised for the vascular-targeting therapies using the dorsal window chamber technique that enables *in vivo* monitoring of the therapy effects on the normal and tumor vasculature. In the collaboration with other department at the Institute and other institutions we also perform molecular-biological, cytological and histological analysis.

Objectives of the Department of experimental oncology in the field of laboratory animals work in oncology will stay the same as they were, that is to follow the 3Rs principle and to ensure as good as possible space and material conditions, and highly qualified staff for the work with experimental animals in oncology, where work specifics demand specific animal observation, monitoring and care. Special attention is therefore paid to the planning of the experiments (pilot studies, statistical counselling, staff training, and appropriate documentation), prediction and recognition of the side effects, determination of pain and stress degree, knowledge of tumor biology, choice of humane procedures, and constant survey of animals, documentation and publications.

Brez eksperimentalnih živali ne bi bilo sodobnih diagnostičnih reagentov in mnogih znanstvenih odkritij

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Protitelesa so pomemben proizvod imunskega sistema in nastanejo kot odziv posameznika na tujo snov - antigen. Struktura protitelesnih molekul je bifunkcionalna: na N-terminalnem koncu nosijo zapis za specifičnost (v t.i. CDR domenah), na C-koncu pa za efektorske funkcije, ki so pomembne za odstranitev ali nevtralizacijo antigena. Poleg pomembne vloge v obrambi pred povzročitelji bolezni so protitelesa postala nenadomestljivo orodje v osnovnih, uporabnih in kliničnih raziskavah ter v diagnostiki in v terapiji, še posebej v preteklih treh desetletjih, ko so poliklonska protitelesa začela zamenjevati monoklonska protitelesa, za pripravo katerih sta G. Koehler in C. Milstein prejela Nobelovo nagrado za medicino.

Monoklonska protitelesa se od poliklonskih protiteles ločijo v svojih lastnostih ter v možnosti proizvodnje, saj se vežejo le na eno antigensko determinanto oz. epitop, imajo enako afiniteto in jih lahko pripravimo teoretično v neomejenih količinah. Proizvajajo jih celične linije hibridomov.

Ključne pri pripravi monoklonskih protiteles, na katerih temelji sodobna diagnostika v humani in veterinarski medicini, pa tudi vrsta bioloških zdravil, ki so osnova modernih načinov zdravljenja, so bile eksperimentalne živali, največkrat miške BALB/c. Z dobro načrtovano imunizacijo živali z izbranimi antigeni in premišljenim načinom selekcije namreč lahko pridobimo specifična, stabilna in visoko afinitetna monoklonska protitelesa željenih razredov (IgG oz. IgM).

Na Zavodu Republike Slovenije za transfuzijsko medicino smo v preteklih 20 letih pripravili vrsto monoklonskih protiteles, ki so uporabna v znanstvene in v diagnostične namene, nekatera pa predstavljajo tudi potencialna zdravila. Predstavljena bosta dva primera:

1.) priprava monoklonskih protiteles proti antigenom krvnoskupinskega sistema AB0, kjer smo pripravili mednarodno priznana lastna monoklonska protitelesa, razvili diagnostične reagente, jih registrirali in postavili proizvodnjo skladno z dobro proizvodno prakso ter

2.) priprava monoklonskih protiteles proti prionom, ki so uporabna v znanstvene, diagnostične in/ali terapevtske namene.

Experimental animals were prerequisite for the development of modern diagnostic reagents and many scientific discoveries

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Antibodies represent an important part of the immune system and are produced in the immune response against a foreign substance - the antigen. The structure of the antibody molecule is bifunctional: antigen binds to the CDR regions at the N-terminal part, whereas the efector functions for the elimination and neutralisation of the antigen are at the C-terminal. Although the natural role of the antibodies is a defend against infectious diseases, they became an indispensible tool in basic and clinical research, diagnostics and therapy, especially in the last three decades, when polyclonal antibodies were substituted with monoclonal antibodies for whose invention G. Koehler and C. Milstein were awarded with the Nobel prize in medicine.

Monoclonal antibodies differ from polyclonal antibodies in their properties and in the way of production. They bind to the same epitope with the same affinity and can be produced by hybridoma cell lines in unlimited quantities.

Experimental animals (in most cases BALB/c mice) were crucial for the production of monoclonal antibodies againts a number of the antigens and which are the basis of a modern human and veterinary diagnostics, as well as for a number of biological drugs. It is possible to produce specific, stable and high affinity monoclonal antibodies of IgG or IgM class after the immunisation of animals with well-defined antigen and carefully chosen screening procedure.

In the last 20 years, monoclonal antibodies of variable specificities were prepared at the Blood Transfusion Centre of Slovenia. They can be used as a research or diagnostic tool, whereas some of them are drug candidates. Monoclonal antibodies against the antigens of the bloog group system AB0 (development, production according to GMP, registration) and prions (research, diagnostics and therapy) will be presented.

Od glukoze-odvisni dogodki v Langerhansovih otočkih znotraj svežih tkivnih rezin mišjega pankreasa

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Glukoza je fiziološko najpomembnejši sekretagog za izločanje hormona inzulina iz celic beta Langerhansovih otočkov. Po sprejetem modelu se glukoza v celicah beta presnovi v ATP, ki zapre od ATP-odvisne kalijeve kanale in depolarizira celično membrano v kateri se odprejo napetostno-občutljivi kalcijevi kanali. Aktivnost the kanalov povzroči dvig citosolne koncentracije prostih kalcijevih ionov, ki sprožijo uravnavano eksocitozo sekretornih granul, ki vsebujejo hormone inzulin. Omenjeni model sklopitve med presnovo in sekrecijo v celicah beta pa še vedno vsebuje pomembne nejasnosti in ni vedno v enostavno združljiv z izsledki *in vivo* študij. Od pričetka moderne fluorimetrije, so številne *in vitro* študije, izvedene skoraj izključno na izoliranih otočkih miši, preučevale vpliv glukoze na membranski potencial in raven znotrajceličnega kalcija v celicah beta. Tehnične omejitve so botrovale, da so bile proučevane zgolj celice v zunanjih plasteh otočka. Poglavitne nejasnosti, ki so ostale se nanašajo na tipe oscilacij znotrajceličnega kalcija, prisotnost valovanja znotrajceličnega kalcija, ravni sinhronosti med celicami in zvezo med električno aktivnostjo in oscilacijami znotrajceličnega kalcija. Naš namen je bil pojasniti te nejasnosti z uporabo in situ preparat sveže tkivne rezine mišjega pankreasa z neinvazivnim fluorescenčnim označevanjem ravni znotrajceličnega kalcija in fluorescenčnega označevanja membranskega potenciala. Fluorescenčni signal pa smo z izvirnim sočasnim opazovanjem številnih celic tudi v globljih plasteh otočkov spremljali s konfokalnim vrstičnim mikroskopom. Naši rezultati so pokazali, da so stabilne in hitre oscilacije v ravni znotrajceličnega kalcija in membranskega potenciala najpogostejši tip odziva v sveži tkivni rezini. Nadalje smo odkrili, da je valovanje znotrajceličnega kalcija mehanicistični substrat za sinhronizacijo oscilacij. Uporaba opisanega poskusnega pristopa nam bo, kot pomembna metoda izbire, v prihodnje omogočila najti odgovore na pomembna vprašanja o fiziologiji celic beta.

Glucose-stimulated events in islets of Langerhans from acute mouse pancreas tissue slices

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The physiologically most important secretagogue of insulin releasing beta cells in the islets of Langerhans is plasma glucose. According to a consensus model glucose is metabolized to ATP, which closes ATP sensitive potassium channels and depolarizes the cell plasma membrane to open voltage-sensitive calcium channels. The activity of these channels causes a raise in cytosolic activity of calcium ions to trigger regulated exocytosis of insulin-containing secretory granules. However, this attractive model of metabolism-secretion coupling in beta cells still suffers important limitations and is not always easily reconcilable with findings *in vivo*. Since the advent of modern fluorimetry, numerous *in vitro* studies employing almost exclusively isolated mouse islets have investigated the effects of glucose on membrane potential and intracellular calcium in beta cells. Due to technical shortcomings, insights of these studies were inherently limited to a rather small subpopulation of the cells in the outermost layers of the islet. The main controversies regard the types of intracellular calcium oscillations, presence of calcium waves, the level of synchronized activity and relation between the electrical activity and calcium oscillations. We set out to combine the *in situ* acute mouse pancreas tissue slice preparation with noninvasive fluorescent calcium labeling and fluorescent labeling with voltage-sensitive dyes and subsequent confocal laser scanning microscopy to shed new light on the existing controversies utilizing an innovative approach enabling the characterization of responses in many cells from all layers of islets. Our experiments reproducibly showed stable fast calcium and membrane potential oscillations as the predominant type of response in acute tissue slices. Furthermore, we found evidence that calcium waves are the mechanistic substrate for synchronization of oscillations. Our experimental approach, as a method of choice has a significant potential to help answer important questions related to the physiology of the beta cells.

Uporaba laboratorijskih miši v nevroendokrinoloških in vedenjskih raziskavah

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Nevroendokrinologija in vedenjska nevroendokrinologija sta pomembni področji nevroznanosti, ki skušata ugotoviti, kako med seboj sodelujeta endokrini in živčni sistem in kako skupaj uravnavata številna instinktivna obnašanja. Vedenjskih raziskav ni mogoče izvajati z računalniškimi modeli ali z metodami *in vitro*, zato so pri tovrstnih raziskavah nujno potrebne poskusne živali. Največkrat se uporablja laboratorijske miši in podgane, za določene specializirane raziskave pa se uporablja tudi druge laboratorijske živali kot so npr. hrčki, morski prašički ali voluharice. V preteklih desetletjih so bili razviti in standardizirani številni testi, ki naj bi vsaj delno posnemali človeško vedenje in nam omogočajo raziskave o medsebojnem vplivu hormonalnega in živčnega sistema na določene oblike obnašanja. V Centru za genomiko živali uporabljamo številne takšne vedenjske teste, ki naj bi vsaj deloma ustrezali in posnemali določene duševna obolenja oziroma težave pri ljudeh kot so testi za anksioznemu podobno obnašanje, testi za depresivnemu podobno obnašanje, testi za avtističnemu podobno obnašanje in drugi. Poleg tega uporabljamo teste, pri katerih se raziskuje osnovne instinktivne vedenjske vzorce kot so testi spolnega obnašanja, testi starševskega obnašanja, testi socialnega obnašanja, testi nasilnega obnašanja in podobno. V centru za genomiko živali tudi rutinsko uporabljamo različne metode za ugotavljanje izraženosti genov ter v zadnjem času metode za ugotavljanje metilacije DNK. Poleg testov obnašanja v Centru za genomiko trenutno uvajamo metodo utišanja genov v možganih z uporabo zaviralnih RNK molekul, ki jih v možgane uvedemo s pomočjo stereotaksičnega injiciranja v točno določene dele možganov/možganska jedra. Osrednji namen naših raziskav je boljše razumevanje, kako geni in hormoni vplivajo na določena obnašanja ter predvsem, kako geni in hormoni različno delujejo med spoloma in urejajo različne vzorce obnašanja ter razlike v pojavnosti duševnih bolezni med spoloma.

Laboratory mice in neuroendocrinological and behavioural studies

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Neuroendocrinology and behavioural neuroendocrinology are important fields of neurosciences that aim to establish how genes and hormones interact, and together regulate many innate behaviours. Behavioural studies could not be performed by computer models or by *in vitro* systems and therefore, use of experimental animals is necessary in such studies. Most often used species are rats and mice, although, especially in studies of some specialized behaviours, other species such as hamsters, guinea pigs and voles are occasionally used. During the last decades, many different tests were developed that aim to modulate certain human behaviours and made possible studies of interactions between genes and endocrine system in the regulation of different behaviours. In the Centre for Animal Genomics, many tests are routinely used such as tests for anxiety like behaviours, tests for depressive like behaviours, tests for autistic like behaviours and others. We are also using different tests for studying basic behavioural patterns such as sexual behaviours, maternal and paternal behaviours, aggressive behaviours, social behaviours and others. In the Centre for Anima Genomics we are routinely using different methods for studying gene expression and more recently, methods for studying DNA methylation. Currently, we are introducing a method of gene silencing using siRNA molecules, combined with stereotaxic brain injections that enable us to inject siRNA and silence gene expression in specific brain nuclei. The major aim of all our studies is to better understand how genes and hormones interact in the regulation of certain innate behaviours and especially, how genes and hormones act differently between the sexes and therefore, differentially regulate behavioural patterns and incidence of certain psychiatric disorders between males and females.

Študij vloge proteaz in uravnavanja njihove aktivnosti v živalskih modelih

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Proteaze regulirajo številne ključne fiziološke procese, kot imunski odziv, celični cikel, celično smrt, celjenje ran, prebavo hrane in recikliranje proteinov in organel. Njihovo delovanje je strogo regulirano, neravnovesje njihovih aktivnosti pa je ključnega pomena za razvoj številnih patologij, kot so kardiovaskularne bolezni, vnetje, rak in nevrodegenerativne bolezni, zaradi česar so proteaze pomembne tarče za zdravila. Med proteazami imajo pomembno mesto tudi cisteinski katepsini, ki so predmet naših raziskav. Katepsini imajo ključno vlogo v številnih procesih, vključno z intracelularno razgradnjo proteinov in imunskim odzivom, obenem pa so povezane s številnimi boleznimi, kot so rak, osteoporoza, revmatoidni artritis, artroza in ateroskleroza. Da bi razumeli njihovo fiziološko vlogo pri teh procesih in raziskali njihov potencial za zdravljenje in diagnostiko bolezni, uporabljamo različne pristope vključno z živalskimi modeli. To obsega proteomsko identifikacijo fizioloških substratov kar lahko vodi do identifikacije in validacije novih biomarkerjev ter bioloških signalnih poti v normalnih in patoloških procesih, razvoj sond za spremljanje aktivnosti, ki omogočajo *in vivo* validacijo potencialnih zdravil in so lahko zelo pomembne pri neinvazivnih optičnih diagnostičnih metodah, razvoj sistemov za ciljano dostavo zdravil in diagnostiko ter študije fiziološke regulacije katepsinov s pomočjo genetske manipulacije njihovih regulatornih inhibitorjev v mišjem modelu raka.

Animal models as a tool for understanding the role of proteases and their regulation

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Proteases control a great variety of physiological processes that are critical for life, including the immune response, cell cycle, cell death, wound healing, food digestion and protein and organelle recycling. Their action is strictly controlled and imbalances in their activities have been found to be critical in a number of pathologies, such as cardiovascular diseases, inflammation, cancer, and neurodegenerative diseases, thereby suggesting proteases as suitable and valuable drug targets. Among the proteases an important role have also cysteine cathepsins, a subject of studies in our laboratory. They have a critical role in a number of processes, including intracellular protein turnover and immune response, and are implicated in a number of diseases, including cancer, osteoporosis, rheumatoid arthritis and osteoarthritis, and atherosclerosis. In order to understand their physiological role in these processes and explore their theraputic and diagnostic potential in disease, we are combining numerous approaches, involving the use of animal models. These include proteomic identification of their physiological substrates, which may lead to identification and validation of novel biomarkers, development of activity-based probes, which allow *in vivo* validation of drug candidates and can be of major use in noninvasive *in vivo* imaging for diagnostic purposes, development of targeted delivery systems for diagnostic and therapeutic purposes, as well as studies of physiological regulation of cathepsins involving genetic manipulation of their regulatory inhibitors in a mouse cancer model.

Testiranje varnosti, učinkovitosti in kakovosti zdravil za humano in veterinarsko uporabo

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V Sloveniji testiranje varnosti in dajanje zdravil v promet urejata Zakon o zdravilih in Zakon o kemikalijah. Oba zakona sta usklajena z evropskimi smernicami, ki urejajo področje razvoja in proizvodnje farmacevtskih učinkovin in zdravil za humano in veterinarsko uporabo.

Za razvoj generičnega zdravila je zahtevanih precej manj testov kot jih mora med razvojem narediti originator. Za oba pa je predpisano obvezno minimalno število testov in vrsta teh testov. V predklinični fazi razvoja se testiranje zdravil za človeka ne razlikuje bistveno od testiranja zdravil za živali. Predklinično testiranje se vedno začne z *in vitro* metodami, največkrat na celičnih kulturah. Postopoma se dodajajo *in vivo* metode, na izbrani vrsti glodavcev. Za testiranje generičnega zdravila na splošno velja, da je obvezno testiranje le na tistih točkah razvoja, kjer se generik razlikuje od originatorja in kadar ni na razpolago zadosti že objavljenih podatkov. Za generika veliko število testov pomeni ponavljanje testov in nepotrebno trošenje časa, denarja in testnih sistemov.

Kontrola kakovosti zdravila v proizvodnji in zdravila v prometu mora potekati skladno s smernicami, ki veljajo v času, ko generik prihaja na trg. Osnovna testiranja določajo farmakopeje in agencije za registracijo zdravil v posameznih državah (kot na primer EMA, FDA in/ali posamezne države).

Testiranje varnosti, učinkovitosti in kakovosti se izvaja le z uporabo dovoljenih metod in na predpisan način, ki ga določata OECD (Organisation for Economic Co-operation and Development) in ICH (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use). V smernicah so definirani celotni postopki in metode, predpisan je testni sistem, določeni so pogoji za izvajanje.

Metode se izvedejo po principih dobre laboratorijske prakse DLP v certificiranem laboratorij in z osebjem, ki je primerno izobraženo in izurjeno za izvajanje metod, kar omogoča primerljivost eksperimentalnih rezultatov.

Testing the safety, efficacy and quality of medicinal products for human and veterinary use

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The testing of safety, efficacy and quality of medicinal products are in Slovenia regulated by the Law on Medicinal Products and Chemicals Act. Both laws are in line with EU guidelines governing the development and production of active pharmaceutical ingredients and final medicinal products for human and veterinary use.

For the development of generic drug there is significantly lower number of tests required than for the originator product. The mandatory minimum number of tests and a type of tests are defined for both. In the pre-clinical stage of development testing of medicines for humans does not differ significantly from the testing of veterinary medicinal products. Preclinical testing always begins with *in vitro* methods, mostly in cell cultures and is continued by gradually adding *in vivo* methods, usually performed on the rodent species. In general, testing in generic development is mandatory only at those points in the development, where the generic differs from the originator and where the experimental data is not publicly available. Performing too many tests means repetition of tests and unnecessary waste of time, money and test systems.

Quality control of the production line and of already marketed products must take place in accordance with the guidelines that apply when a generic comes to market. The basic test set is defined by Pharmacopoeia and agencies for the registration of medicines in individual countries (like EMA, FDA and / or country-specific).

Testing of safety, efficacy and quality must be carried out only with the use of granted methods and in the manner, defined by the OECD (Organization for Economic Co-operation and Development) and ICH (International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use) guidelines. These guidelines define the procedure, method, test system and the conditions required to perform the tests.

The methods are implemented and performed in compliance with the principles of Good Laboratory Practice in a certified laboratory with adequately educated and trained staff which allows comparability of experimental results.

Študije genetike debelosti s poligenimi mišjimi modeli ter razvoj transgenih modelov za preučevanje biosinteze holesterola

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Debelost je posledica delovanja številnih genov ter okoljskih vplivov in predstavlja rizičen dejavnik za razvoj kroničnih bolezni kot so sladkorna, srčno žilne in rakave bolezni. Oblike morbidne debelosti z enostavnim Mendelskim načinom dedovanja so redke. Zato se osredotočamo na iskanje genov za pogosto večgensko obliko debelosti z uporabo poligenih mišjih modelov. Odkrili smo več genomskih odsekov (angl., quantitative trait loci (QTL)) z učinkom na debelost in izbrali dve področji na mišjem kromosomu 15, Fob3a in Fob3b2 za pozicijsko kloniranje. Pristop za identifikacijo vzročnih genov za debelost vključuje genetsko kartiranje po celem genomu, lokalno kartiranje z uporabo F2 križanj kongenih linij, analizo haplotipov, primerjalno genomiko med vrstami, diferencialno analizo izražanja z mikromrežami, funkcijske teste in študije pri debelih oziroma vitkih ljudeh. Za QTL Fob3a smo pokazali, da je gen Deptor odgovoren za povišano adipoznost. Za QTL Fob3b2 analiziramo glavnega kandidata, ki pri povišanem izražanju v maščevju povzroča zmanjšanje zamaščevanja. Novi geni za debelost oziroma vitkost lahko predstavljajo nove terapevtske tarče za zdravljenje debelosti in z njo povezane metabolne bolezni.
Metode transgeneze pri živalih predstavljajo pomembno orodje za temeljne in aplikativne študije v bioznanostih in biotehnologiji. Razvili smo transgene modele za encim lanosterol 14α-demetilazo (gen Cyp51), ki regulira enega ključnih korakov v biosintezi holesterola. Uporabili smo tehnologijo Cre/loxP, da smo vstavili v intron 2 and 4 mesta loxP, ki v kombinaciji z izražanjem rekombinaze CRE omogoča izrez in inaktivacijo gena Cyp51 v poljubnem tkivu ali časovnem obdobju. Najprej smo razvili model z izničenim genom Cyp51 v vseh celicah, ki je kazal morfološke značilnosti humanega sindroma Antley-Bixler in v homozigotnem stanju povzročil embrionalno smrtnost 15-ti dan razvoja. Da bi preučili učinek blokade biosinteze holesterola med spermatogenezo, smo razvili model s pogojnim izničenjem Cyp51 gena samo v moških spolnih celicah. Predstavljeni bodo rezultati fenotipske karakterizacije tega modela med mejozo in reprodukcijo.

Genetic analyses of obesity using polygenic mouse models and development of transgenic lines for cholesterol biosynthesis functional studies

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Obesity results from the action of numerous genes and interaction with »obesogenic« environment presenting a risk factor for diabetes, cardiovascular diseases and cancer. Forms of obesity with simple Mendelian inheritance of large effects are rare. We focus attention to searching for genes of the more common polygenic form of obesity in polygenic mouse models. We identified several quantitative trait loci (QTL) in the genome and selected two regions on mouse Chr15, Fob3a and Fob3b2 for positional cloning. Our approach to identify causal obesity genes consists of genome-wide genetic mapping, regional fine mapping in F2 crosses of congenic lines, interval-specific haplotyping, between species comparative mapping, differential expression by microarrays, functional tests, as well as confirmatory studies in obese or lean humans. For Fob3a QTL, we show that Deptor is causal for the increased adiposity. For Fob3b2 QTL, the highest priority candidate gene was identified that exhibits gain-of-function lean phenotypic effect and adipose tissue specificity. Novel obesity/leanness genes identified in our model may represent new therapeutic targets for the treatment of obesity and metabolic disorders.

Transgenic methods in animals present important tools in basic as well as applied biosciences and biotechnology. We developed transgenic models for lanosterol 14α-demethylase (Cyp51 gene) that controls one of the key steps in cholesterol biosynthesis. Cre/loxP-based conditional transgenic technology was used to introduce two loxP sites into intron 2 and 4, which in combination with CRE expression, enables excision and inactivation of Cyp51 in a temporal or tissue specific manner. We first developed a full knockout model that exhibited several prenatal Antley-Bixler (ABS) syndrome features leading to lethality at day 15 of embryonic development due to heart failure. To examine the effect that Cyp51 inactivation on spermatogenesis we generated male germ-cell specific Cyp51 knockout. Results on the phenotypic effects on meiosis and fertility will be presented.

Prehranske raziskave na živalih na Biotehniški fakulteti

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Na Katedri za prehrano preučujemo različne vidike prehrane živali in na živalskih modelih tudi prehrano ljudi. Vrsto let že preučujemo učinke in hranilno vrednost krme in krmnih dodatkov (prebavljivost, vpliv na prirejo živali, kakovost in hranilno vrednost proizvodov živalskega izvora), delovanje antinutritivnih snovi krme (toksinov …), kakovost, delovanje in potrebe po nekaterih hranljivih snoveh v prehrani živali in ljudi (maščobne kisline, minerali, antioksidanti, semi esencialna hranila), pomen živalskih proizvodov v prehrani ljudi. Preučujemo tudi, kakšne so možnosti s spremembami v prehrani živali spremeniti hranilno vrednost živalskih proizvodov, da postanejo funkcionalna živila (obogatitev z esencialnimi maščobnimi kislinami, mikrominerali kot Se, Zn, I …).

Za raziskave na živalih uporabljamo posebne kletke za *in vivo* ugotavljanje prebavljivosti in bilance HS pri miših, podganah, prašičih, kuncih in perutnini. Imamo fistulirane ovne, ki so potrebni za *in vitro* merjenje prebavljivosti pri prežvekovalcih, hlev za talno rejo piščancev in hlev za rejo kuncev. Dobro sodelujemo s prakso, kar nam omogoča opravljati prehranske poskuse v praktičnih pogojih. Imamo klavnico za študij vplivov prehrane na zdravje ter prehransko vrednost in kakovost živalskih proizvodov.

V kemijskem laboratoriju lahko izvajamo klasične analize krme, hrane, živalskih tkiv in drugih vzorcev. Na plinskem kromatografu določamo MK: od hlapnih MK do dolgoverižnih večkrat nenasičenih MK, tudi cis- in trans- izomere in izomere konjugirane linolne kisline, na tekočinskem kromatografu (HPLC) določamo nekatere vitamine in produkte oksidacije maščob, kot je malondialdehid. Določamo stopnjo poškodb DNA (kometni test, 8-hidroksi-deoksi gvanozin). Z atomsko absorpcijsko spektroskopijo (AAS) analiziramo makro in mikro minerale, tudi v kombinaciji s hidridno tehniko (Se), za določanje P, TBARS in peroksidov uporabljamo molekulsko spektrometrijo v UV in vidnem delu spektra. Določamo *in vitro* antioksidativno kapaciteto v maščobah in v vodi topnih antioksidantov (Photochem) v različnih vzorcih rastlinskih in živalskih tkiv. Uporabljamo *in vitro* sisteme za simulacijo prebave pri prežvekovalcih in kuncih (Daisy, plinski test).

Animal nutrition research in Biotechnical faculty

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At the Chair of Nutrition various aspects of animal and on animal models also human nutrition are investigated. The members are working since many years on the effects and nutritive value of feedstuffs and feed additives (digestibility, influence on animal productivity, quality and nutritive value of animal products), function of antinutritive substances of feed (toxins), quality, function and requirements of some nutritive substances in animal and human nutrition (fatty acids, minerals, antioxidants, semi essential nutrients), importance of animal products in human nutrition. Possibilities of animal nutrition to change the nutritive value of animal products in a way that they become functional food are studied (enrichment with essential fatty acids, micro minerals like Se, Zn, I…).

In the purpose of the research, our group has special cages for *in vivo* digestibility and balance studies on mice, rats, pigs, rabbits and poultry, fistulated animals (the ram), stable for the floor breeding of chickens, stable for rabbit breeding, good cooperation with practice enables to perform feeding trials under practical conditions. There is also a slaughter house.

The following analysis are performed in our lab: classical proximate analyses of feed, food, animal tissue and other samples, GC (fatty acid analyses: from SCFA to LC-PUFA, also cis- and trans-, CLA isomers), HPLC (some vitamins, products of fat oxidation like malondialdehyde), the rate of DNA damage (Comet assay, 8-hydroxy-deoxy guanosin), AAS used for macro and micro minerals analyses, also combined with hydride generation technique (Se). For the determination of P, TBARS and peroxides is used molecular spectrometry in the UV and visible spectrum. *In vitro* antioxidant capacity in fats and in water-soluble antioxidants in different samples of plant and animal tissues can be determined by Photochem. We have applied the *in vitro* system for the simulation of digestion of ruminants and rabbits (Daisy, gas test).

Uporaba laboratorijskih živali za proučevanje varnosti in mehanizmov delovanja probiotičnih bakterij

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Probiotiki so živi mikroorganizmi, ki gostitelju prinašajo koristne zdravstvene učinke, če so vnešeni v zadostnih količinah. Probiotiki so že dolgo v uporabi v funkcionalnih živilih in prehranskih dopolnilih. Glede na nedavne zavrnitve zdravstvenih trditev za probiotike s strani EFSA, zaradi pomanjkanja zadostnih dokazov njihove učinkovitosti, postajajo ustrezne raziskave varnosti in učinkovitosti probiotikov esencialnega pomena za njihov nadaljnji razvoj in uporabo. Živalski modeli predstavljajo dragoceno orodje za študij interakcij med bakterijami in gostitelji. Navadno so živalski preskusi vmesni korak med raziskavami *in vitro* in kliničnimi raziskavami na ljudeh. Ker so probiotiki vse bolj prisotni tudi v zdravilih brez recepta in ker naj bi probiotiki nove generacije utrli pot tudi uporabi v terapevtske namene, so zahteve za njihovo testiranje vedno strožje in se približujejo tistim za zdravila. V prispevku bo predstavljenih nekaj raziskav probiotičnih bakterij na laboratorijskih živalih (miši in podgane), ki jih izvajajo študenti in raziskovalci Katedre za mlekarstvo ter Inštituta za mlekarstvo in probiotike, Biotehniške fakultete. Večina raziskav je namenjenih dokazovanju varnosti in proučevanju mehanizmov delovanja lastnega seva Lb. gasseri K7. V *in vivo* študiji na miših C57BL/6J smo ugotavljali, kako dobro Lb. gasseri K7 (Rif^r) preživi prehod skozi prebavila, ali jih je sposoben začasno naseliti in ali je varen za uporabo. Nekaj miši smo tudi inficirali z enterohemoragično *E. coli* O157:H7 ter ugotavljali morebitno zaščitno vlogo seva K7 pri infekciji ter vpliv na transkriptom krvnih celic miši. Vpliv uživanja probiotikov na mikrobioto mlečne žleze in možnost endogenega prenosa probiotikov iz črevesja v mlečno žlezo proučujemo na modelu brejih miši linije FVB/N, pri čemer kot modelna probiotika uporabljamo dobro proučeni Lb. rhamnosus GG in lastni sev K7. V prehranskem poskusu na podganah proučujemo vpliv kefirana in kefirnih zrn na sestavo črevesne mikrobiote podgan ter na dve tipični motnji metabolnega sindroma, višji nivo serumskega holesterola in trigliceridov ter oksidacijski stres.

Use of laboratory animals for the study of safety and mechanisms of action of probiotic bacteria

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Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Probiotics have long been in use in functional foods and dietary supplements. In the light of recent refusal of health claims for probiotic by EFSA due to the lack of proven effects, the appropriate safety and efficacy studies of probiotics are becoming essential for their further development and application. Animal models represent a valuable tool for studies of interactions between bacteria and the host. Usually animal trials are an intermediate step between *in vitro* studies and human clinical studies. Since probiotics are increasingly present in OTC drugs and next generation probiotics are believed to be increasingly used also for therapeutic purposes, the requirements for their testing are becoming tougher, approaching those for pharmaceuticals. In the present work some studies of probiotic bacteria in laboratory animals (mice and rats) carried out by the students and researchers of a Chair of Dairy Science and Institute of Dairy Science and Probiotics of Biotechnical faculty will be presented. Most research is aimed at demonstrating the safety and study of the mechanism of action of our own strain Lb. gasseri K7. In *in vivo* study on C57BL/6J mice we studied the survival of Lb. gasseri K7 (Rifr) in the gastrointestinal tract, its ability for transient colonization and safety. Some mice were also infected with enterohemorrhagic *E. coli* O157:H7 in order to assess the potential protective effect of Lb. gasseri K7 against infection and the effects on the blood cell transcriptome. Effect of probiotics' consumption on the microbiota of mammary gland and the possibility of endogenous transfer of probiotics from the gut into mammary gland is currently studied in pregnant mice model line FVB/N, using well known Lb. rhamnosus GG and our own Lb. gasseri K7 strain as model probiotics. In the nutritional experiment in rats we study the effects of kefiran and kefir grains on the composition of the intestinal microbiota in rats and on two typical metabolic syndrome disorders, i.e. higher levels of serum cholesterol and triglycerides and oxidative stress.

Proučevanje imunskih procesov pri perutnini ter priprava monoklonalnih protiteles mišjega in kokošjega izvora

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Proučevanje imunskih mehanizmov pri perutnini je v naši skupini nadaljevanje dolgoletne prakse diagnosticiranja mikoplazemskih okužb, predvsem Mycoplasma synoviae (MS) in Mycoplasma gallisepticum (MG) ter razvijanja novih metod in testov za to dejavnost. Kot laboratorijske živali so v ospredju kokoši, kjer proučujemo učinke aviarnih mikoplazem in/ali virusov na njihove imunske in tkivne celice. V dosedanjih *in vivo* poskusih smo kokoši in embrie okuževali z različnimi pripravki in analizirali imunski odziv, drugi sklop poskusov pa je bil izveden na tkivih iz naravno okuženih ali na primarnih celičnih kulturah, pridobljenih iz tkiv zdravih živali. Opisali smo mehanizme nastanka avtoimunskih procesov, ki jih sproža MS in definirali glavne signalne poti naravnega in pridobljenega imunskega odziva na MS. Pri tem smo prvi opisali mehanizem apoptoze hondrocitov, sprožen z MS, mehanizem nastanka avtoantigenov in definirali ter opisali ligand za TLR 15. Primerjalno smo analizirali imunski odziv na posamezne okužbe z MS, *E. coli* in NDV (La Sota) in na hkratno okužbo z MS/NDV La Sota ter na podlagi izraženih genov in proteinov opisali učinke hkratnih okužb, ki so pomembni iz vidika vakcinacijskih postopkov. Prvi smo opisali tudi mnoge imunogene in encime (nevraminidazno, nukleazno, proteazno) MS in MG, ki bistveno vplivajo na potek imunske obrambe in razvoj bolezni.

Dejavnost pridobivanja mišjih monoklonskh protiteles (mAb) teče na Oddelku za zootehniko od leta 1990, ko je bil ustanovljen Laboratorij za imunologijo in celične kulture. Mišja mAb pripravljamo rutinsko za lastne raziskovalne potrebe in za različne naročnike iz raziskovalne in gospodarske sfere. Vzporedno smo razvili tudi tehnologijo pridobivanja kokošjih mAbs (ch mAb), ki so predvsem aktualna v primerih, ko naj bi bile tarče mAb evolucijsko visoko ohranjeni sesalski proteini, ki so za miške slabi imunogeni. IgY imajo tudi nekaj drugih prednosti (ne reagira s sesalskimi IgG in RF, ne aktivira komplementa), zaradi česar je povpraševanje po ch mAb vedno večje.

Study of immune processes in poultry and production of mouse and chicken monoclonal antibodies

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Long practice and experiences of diagnosing mycoplasma infections, especially Mycoplasma synoviae (MS) and Mycoplasma gallisepticum (MG) were upgraded to studies of immune mechanisms in poultry. In our group the effects of avian mycoplasmas and / or viruses on chicken immune and tissue cells are studied therefore as laboratory animals hens are at the forefront. In *in vivo* experiments chickens and embryos were experimentally infected with different preparations and the immune response was analyzed. A second set of experiments was performed on tissues from naturally infected birds or on primary cell cultures derived from tissues of healthy animals. We described the main signaling pathways of innate and acquired immune response to MS and the mechanism of MS induced autoimmunity. MS induced apoptosis in chondrocytes and autoantigens appearance were demonstrated and the ligand for TLR 15 was defined for the first time. On the basis of expressed genes and proteins we described the effects of infections with MS, *E. coli* and NDV (La Sota) and concurrent infections with MS / NDV La Sota, which are important from the perspective of vaccination procedures. Many immunogenic proteins, including enzymes (neuraminidase, nuclease, protease) from MS and MG were described, which significantly affect the course of immune defense and disease development.

Production of mouse monoclonal antibodies (mAb) started in our department in 1990, when the Laboratory for Immunology and cell cultures was established. Mouse mAbs are produced routinely for various clients from research and economic spheres. In parallel, we have developed a technology of chicken mAbs (ch mAb), which are mainly relevant in the cases when antigen that should have been targeted by mAb is an evolutionary highly conserved mammalian protein, which is poorly immunogenic for mouse. IgY also have some other advantages (does not react with mammalian IgG and RF, does not activate complement), which increases the demand for ch mAb.

Pedagoško raziskovalni center za perutninarstvo – genski viri in infrastruktura

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Zgodovina in tradicija selekcije perutnine v Sloveniji sega v šestdeseta leta 20. stoletja, ko je bil osnovan Pedagoško Raziskovalni Center (PRC) za perutninarstvo. Na tem centru se redi štiri pasme kokoši lahkega (nesnega) in eno pasmo kokoši težkega (mesnega) tipa. V toku cele vrste generacij je pasme izoblikovala selekcija na točno določene lastnosti. Trenutno se selekcija osredotoča na nesnost in maso jajc pri pasmah kokoši lahkega tipa ter na doseženo desettedensko telesno maso pri pasmi kokoši težkega tipa. Končni proizvodi dvopasemskih križanj so tri nesnice jajc z rjavo barvo jajčne lupine: Prelux-R (avtoseks križanka - izkoriščanje dominantnega gena za srebrno barvo perja), Prelux Č (avtoseks križanka - izkoriščanje dominantnega gena za grahasto barvo perja) in Prelux-G (določanje spola en dan starim piščancem na osnovi hitrosti operjanja – izkoriščanje dominantnega gena za pozno operjanje). Vsi programi križanj temeljijo na pridobivanju avtoseks piščancev, katerim lahko že ob izvalitvi določimo spol na temelju barve puha ali hitrosti operjanja. Ker se v selekcijskem programu veliko pozornosti posveča odpornosti, prilagodljivosti in robustnosti, so kokoši primerne zlasti za sonaravne oblike reje (npr. pašno rejo). Poleg treh križank lahkega tipa se na PRC za perutninarstvo redi še jerebičasta štajerska kokoš kot edina slovenska avtohtona pasma kokoši ter slovenska pozno operjena kokoš kot edina slovenska tradicionalna pasma kokoši težkega tipa. Zaradi boljšega razumevanja kvantitativnih lastnosti, ki so ključnega pomena pri selekciji perutnine, je bil koncem sedemdesetih let 20. stoletja začet dvosmerni selekcijski poskus na telesno maso piščancev pri osmih tednih starosti. Dvosmerno selekcionirani liniji kot tudi populacija križancev med tema dvema linijama so se izkazali kot odlični modeli za študij dolgotrajnih učinkov selekcije na rast ter za študij v drugih disciplinah biologije (npr. kartiranje kvantitativnih lokusov).

Educational and research centre for poultry breeding – genetic resources and infrastructure

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The history and tradition of poultry selection and research in Slovenia dates from the mid nineteen-sixties when the Educational and Research Centre (ERC) for poultry breeding was founded. At ERC four layer-type chicken breeds and one meat-type chicken breed are maintained. Breeds have been developed for specific traits through many generations of selection. Currently, selection strategy is focusing on high egg production and higher egg weight in egg-type breeds and on higher body weight at 10 weeks of age in meat-type breed. Based on two-way crosses selection programme includes production of the following three laying strains of brown eggs: Prelux-R (coloursexed through silver-red S/s alleles of Silver gene), Prelux Č (coloursexed through barred/nonbarred B/b alleles of Bar gene) and Prelux-G (feathersexed through slow/fast feathering K/k alleles of K gene). All of the parent stock programmes use autosexing for easy segregation of day old hen and cockerel chicks. Since the strategy of the selection programme is to support the adaptability, flexibility and robustness of the birds, they are especially suitable for rearing in alternative (e.g. free range) rearing systems. Beside three brown egg layer hybrids ERC for poultry breeding offers a partridge-like Styrian hen as the only Slovenian autochthonous hen breed and Slovenian late feathering hen as the only Slovenian meat-type traditional hen breed. To better understand quantitative traits which are of great interest to the poultry breeders a divergent selection experiment for body weight at 8 weeks of age was started in late 1970's. Divergently selected lines and intercross between lines proved to be an excellent models for the study of long-term effects of selection for growth and for studies in other disciplines of biology (e.g. mapping of quantitative trait loci).

Poskusi na konjih kot proizvodnih, ljubiteljskih in terapevtskih živalih

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V prejšnjem stoletju je zatonu konjereje botrovala industrializacija. Posledično je bil konj kot objekt za proučevanje nezanimiv ravno v času razvoja raziskav na laboratorijskih živalih. V tem obdobju je pridobil nove funkcije, kot so: športnik, hišni ljubljenček, žival za popestritev prostega časa, sodelavec pri terapiji in pomočnik pri izobraževanju. Njegova vloga v funkciji delovne živali in prireje mesa pa se je zmanjšala. V zadnjem obdobju se zanimanje za raziskave na konjih povečuje, zaradi posebnosti uporabe konja v primerjavi z drugimi vrstami domačih živali ter velikega kroga uporabnikov, ki ne izhajajo iz podeželja. Pomembna razlika pri uporabi konj glede na druge vrste domačih živali je v kategoriji ljudi, ki dela z živalmi. Ocenjuje se, da je v dnevnem stiku s konji le 20% 'proizvajalcev - rejcev' in kar 80% 'konzumentov – uporabnikov'. Pri proizvodnih domačih živalih so v dnevnem stiku z živalmi le 'proizvajalci - rejci'. Pri tem nastajajo številne omejitve za izvedbo klasičnih poskusov pri katerih je potrebno zagotoviti enak tretma živali pred poskusom, naključen izbor živali za poskus, različen tretma živali v času poskusa in preprečiti morebitni vpliv poskusa na žival, ko je ta že zaključen. Pričakuje se, da bodo konji v poskusih sodelovali vse pogosteje, vendar bo potrebno upoštevati etične in etološke standarde, ki še niso usklajeni med 'proizvajalci' in 'konzumenti'.

Horses in experiments as productive, pet and therapeutic animals

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In the previous century, the development of horse industry decline due to industrialization development. Consequently, the horse like an object for study became uninteresting just during the development of researches on laboratory animals. However, at that time horses acquired new roles, such as athlete, pet, leisure animal, therapy assistant and education assistant. Its role in the function of draft animal as well as animal for meat production decreased. In recent years, the interest in researches on horses increased, due to its specific use compared to other agricultural animals as well as a large number of users who do not come from rural areas. The important difference between horses and other species is the daily contact between person and animal. The proportion of person – horse daily contact is estimated on only 20% of 'producers - farmers' and 80% of 'consumers - users'. In the daily contact with agricultural animals are only 'producers - farmers'. Considered all of above mentioned, a number of limitations for the implementation of the classic experiments where is needed to ensure equal treatment prior to the experiment, a random selection of animals for the experiment, different treatment of animals during the experiment and to avoid the potential impact on the animal after it has been completed. It is expected that the horses will be included in the trials more often. However, the ethological and ethical standards have to be considered in the future. Unfortunately, they are not harmonized between 'producers' and 'consumers at the moment.

Posterji

Poster presentations

Lokus *Fob3b2*, ki povzroča vitkost pri miših, kaže na signifikantne interakcije med geni, spolom in dieto

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Ena najnevarnejših kroničnih bolezni današnjega časa je debelost, saj povečuje predispozicijo za nastanek sladkorne bolezni, bolezni srca in ožilja, raka, itd. Tudi pri domačih živalih predstavlja nezaželeno komponento, kajti povezujejo jo s slabim priraščanjem in konverzijo krme. Debelost, kot tudi vitkost, sta kompleksni lastnosti, na kateri vplivajo številni geni in okolje. Za genetsko mapiranje kvantitativnih lokusov (QTL) in preučevanje njihovih vplivov predstavljajo dober genetski material poligeni živalski modeli. Nove kandidatne gene, ki povzročajo variabilnost pri razvoju debelosti ali vitkosti, lahko odkrivamo z uporabo različnih kongenih inbridiranih mišjih linij. Učinek QTL-a *Fob3b2* smo v raziskavi preučevali na kongeni mišji liniji 15FHM-M2, ki se od osnovne debele linije razlikuje samo v ~ 2 Mbp dolgem segmentu na 15. kromosomu. Na F2 populaciji, ki je bila razvita s križanjem heterozigotnih osebkov za segment *Fob3b2*, smo preučevali interakcije med genotipi in dieto. Živali so bile razdeljene na dve skupini, prva polovica je bila krmljena s krmo z nizko vsebnostjo maščob (LFD), druga pa z visoko vsebnostjo maščob (HFD). Živali so bile tehtane pri 3., 5., 6., 8., 10., 12., 14. ter nazadnje pri 16. tednih, ko smo odvzeli in stehtali različne maščobne depoje. Preliminarna analiza telesnih tež in mas maščobnih depojev je pokazala statistično značilne razlike ($p < 0.0001$) med genotipi FF in LL pri F2 populaciji samic, krmljeni s HFD, kar nakazuje na možne interakcije genov s spolom. Pričakujemo, da bomo znotraj lokusa *Fob3b2* potrdili obstoj kandidatnega gena za vitkost. Nova dognanja bodo pomemben prispevek k razvoju novih terapij za boj proti debelosti, kot tudi nov potencial za selekcijo na manj zamaščene domače živali.

Fob3b2 locus causing leanness in mice displays significant gene-diet and gene-sex interactions

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Obesity is one of the major risk factor for a number of chronic diseases (diabetes, cardiovascular diseases, increased blood pressure, cancer, etc.). Slow growth and poor food conversion in farm animals due to excessive fat deposition, are mostly undesirable both for consumers and economy of production. Because obesity as well as leanness are complex traits, influenced by a large number of genes and environment, polygenic animal models represent genetic resource suitable for genetic mapping of quantitative trait locus (QTL) and for studies of QTL effects. New candidate genes that cause variability in the development of obesity or leanness can be identified through the use of different crosses and congenic inbred mouse lines. For studying the effects of a QTL *Fob3b2* we used a newly developed congenic line, which differ from the background Fat (F) line (mice selected for higher percentage of body fat) only for ~ 2 Mbp segment of mouse chromosome 15. To examine genotype-diet interactions we developed an F2 cross for one congenic line (15FHM-M2). Half of the segregated F2 population was fed with high fat diet (HFD) and the other half with the calorie matched low fat diet (LFD). Animals were weighed at 3., 5., 6., 8., 10., 12., 14. and finally at 16. weeks, when abdominal (ABD), mesenteric (MES), gonadal (GON) and femoral (FEM) fat pads and plasma were collected. Differences between the FF (homozygotes for fat alleles) and LL (homozygotes for Lean alleles) genotypes in body mass and fat data in preliminary analyses were statistically significant ($p < 0.0001$) in F2s fed with HFD but not LFD. Additionally, significant effect for most traits was obtained in females suggesting a gene-sex interaction.We expect that this study will provide further support for existence of an important causal leanness gene located within the *Fob3b2* interval potentially applicable for novel therapies in humans or diagnostics of farm animals with a genetic potential for lean growth.

Vpliv palmove maščobe in lanenega olja na oksidacijski stres in ekspresijo jetrnih genov pri piščancih pitancih

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V raziskavi smo preučevali vpliv krmljenja palmove maščobe in lanenega, ki imata različno maščobnokislinsko sestavo, na presnovo maščob pri piščancih pitancih. Dvajset piščancev smo razdelili v dve skupini. Skupina PALM (N=10) je prejemala 5 % palmove maščobe, ki je dober vir nasičenih maščobnih kislin, skupina LIN (N=10) pa enako količino lanenega olja, ki vsebuje velik delež večkrat nenasičenih maščobnih kislin. Ker je laneno olje podvrženo oksidaciji, smo v plazmi in jetrih določili koncentracijo malondialdehida, ki je marker za prepoznavanje oksidacije nenasičenih maščobnih kislin v organizmu, in vitamina E, ki je naravni antioksidant. V plazmi smo izmerili še koncentracijo trigliceridov in holesterola, v jetrih pa določili njihovo maščobnokislinsko sestavo. Iz jeter smo izolirali RNA in s pomočjo analize mikromrež določili ekspresijo genov. Rezultate smo potrdili s PCR v realnem času. Oksidacija maščob je bila večja v skupini LIN, vsebnost vitamina E pa je bila večja v skupini, ki je prejemala palmovo maščobo. Razlik v koncentraciji trigliceridov in holesterola ni bilo. Maščobnokislinska sestava v jetrih je odražala maščobnokislinsko sestavo krme, ki so jo piščanci prejeli. Kar zadeva ekspresijo genov, so se največje razlike pokazale pri genih, ki so vpleteni v presnovo maščob in holesterola, različno je bilo izraženih tudi nekaj genov, ki so vpleteni v regulacijo oksidacijskega stresa.

The effect of palm and linseed oil on oxidative stress and liver gene expression in broiler chickens

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The study was conducted in order to investigate the effect of different sources of fat on lipid metabolism in broiler chickens. Twenty chickens were equally divided in two groups: chickens in group PALM (N=10) received 5 % of palm oil as a source of saturated fatty acids, while chickens in group LIN (N=10) received the same amount of highly unsaturated linseed oil. Linseed oil is prone to oxidation, for this purpose malondialdehyde as a marker of lipid oxidation and vitamin E, which is a natural antioxidant, in plasma and liver were measured. Additionally, plasma triglyceride and cholesterol concentrations were analysed and the liver fatty acid composition was determined. The RNA was isolated from chicken liver and microarray analysis was performed to elucidate the expression of genes. The results were confirmed using qRT-PCR. Lipid oxidation measured as MDA concentration was higher in a linseed oil fed group and vitamin E concentrations were higher in group PALM. No differences in triglyceride and cholesterol concentrations were observed. Liver fatty acid composition reflected the fatty acid composition of the diets. As considers the expression of genes, major differences were regarding genes involved in lipid and cholesterol metabolism. Some genes involved in regulation of oxidative stress were also differentially expressed.

Koencim Q10, prisoten v krmni mešanici z visoko vsebnostjo maščob, vpliva na zmanjšanje simptomov debelosti le pri vitki liniji poligenega mišjega modela

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Ena izmed pogostejših kroničnih bolezni pri ljudeh in tudi pogostejša težava v reji proizvodnih živali je debelost, ki je povzročena s strani številnih genetskih in okoljskih dejavnikov. Prehranske študije z različnimi prehranskimi dodatki so pokazale, da se lahko simptomi, ki povzročajo debelost, zmanjšajo. Metabolni sindrom je posledica nekaterih bolezenskih stanj, med drugim debelosti, inzulinske rezistence, povečanje pritiska in povečanje tvorbe lipidov v krvi. S koencimom Q10 (CoQ10) so v prejšnjih raziskavah dokazali pozitivne antioksidativne učinke. Neraziskana pa je njegova vloga kot prehranski dodatek, ki bi lahko vplivala na različno ekspresijo genov sodelujočih pri metabolnem sindromu. Miši so najbolj primeren živalski model za študije debelosti pri ljudeh in živalih za rejo. V raziskavi je bila uporabljena vodotopna oblika CoQ10, da bi ocenili ali vpliva na fenotipske parametre in ekspresijo genov, opazovanih na poligenem mišjem modelu (debela (F) in vitka (L) selekcijski mišji liniji). Sintetično pripravljena krmna mešanica z visoko vsebnostjo maščob je omogočila objektivno oceno vpliva dodatka CoQ10. Znotraj posamezne linje sta bili določeni dve prehranski skupini: ena krmljena s krmno mešanico z visoko vsebnostjo maščob (HF), druga s krmno mešanico HF z dodatkom CoQ10 (HF-Q). Kontrolna skupina je ostala na standardni krmni mešanici Altromin 1324. Po 14 tednih na standardni krmni mešanici so miši prešle na eksperimentalno krmno mešanico. Žrtvovane so bile pri starosti 26-28 tednov. Odvzeti so bili vzorci krvi ter po anatomski sekciji živali še jetra in maščobni depoji (epididimalni, abdominalni, fermoralni in mezentrialni). Določeni so bili parametri plazme (holesterol, glukoza, trigliceridi, HDL- in LDL- holesterol). Statistično značilne razlike v maščobnih depojih so bile pri vitki liniji miši, krmljeni s krmno mešanico HF-Q. Rezultati analize na mikročipih so razkrili obsežen seznam genov, kar je omogočilo ovrednotiti fenotipske podatke.

Coenzyme Q10 supplemented to high fat diet exhibited obesity resistant effect in the Lean but not Fat polygenic mouse model

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One of the major chronic diseases in human health and animal production is obesity, which is controlled by several genetic and environmental factors. Long-term dietary intervention studies with different food additives established that obesity symptoms can be attenuated. Metabolic syndrome is a series of conditions such as obesity, insulin resistance, hypertension and dyslipidemia. Positive effects of Coenzyme Q10 (CoQ10) have been established previously in different studies, but mainly for its beneficial antioxidative characteristics. However, its effect as a food additive to alter specific gene expressions in connection with metabolic syndrome is still not well understood. Mice have proved to be useful models for understanding obesity in humans and farm animals. We used water-soluble form of CoQ10 in order to determine its effect on phenotype parameters and gene expression in polygenic mouse models (Fat (F) and Lean (L) selection mouse lines). Our own composed synthetic purified high-fat diet (HF) enabled us to objectively measure effects of water soluble CoQ10. One group was fed HF diet (HF), the other with HF diet supplemented with water-soluble CoQ10 (HF-Q), whereas the control group with commercial food mixture Altromin 1324. Mice were switched to experimental diet after being 14 weeks on the standard diet. They were sacrificed at the age of 26-28 weeks. Blood samples liver tissue and fat depots (epididymal, abdominal, femoral and mesenteric) were collected. Plasma parameters (cholesterol, glucose, triglycerides, HDL- and LDL-cholesterol) were determined. We observed significantly reduced fat accretion in the lean mouse line on the HF diet supplemented with CoQ10. Results of microarray analysis of gene expression revealed comprehensive list of genes that helped us to explain phenotypic data.

Živalski modeli pri študiju 7TM receptorjev uravnavanih s strani virusov ali kodiranih v virusnem genomu Epstein-Barr virusa

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Herpesvirusne infekcije so zelo razširjene, virus pa je v obliki latentne infekcije sposoben vztrajati v organizmu vse življenje. Epstein-Barr virus (EBV), gama-herpesvirus, ima ~50-90-odstotno prevalenco v človeški populaciji in je povezan s transformacijo celic in nastankom raka. Specifično zdravilo proti z infekciji povzročeni z EBV ali razvoju bolezni ne obstaja. Receptorji s sedmimi transmembranskimi (7TM) območji, imenovani tudi receptorji, sklopljeni s proteini G (GPCR), so vključeni v patologijo bolezni povzročenih z omenjenimi virusi tako, da so uravnavani s strani virusov ali pa kodirani v virusnem genomu. Receptor za Epstein-Barr virus tip 2 (EBI2) je receptor 7TM, uravnan s strani virusov. Njegova aktivnost se poveča za več kot 200-krat po vstopu EBV v celico. Izraža se v limfocitih B v limfnih vozličkih. Nanj se vežejo in ga aktivirajo oksisteroli, 7α,25-dihydrocholesterol (7α,25-OHC) z največjo potenco (1,2). V odprtem bralnem okvirju genoma EBV se nahaja receptor 7TM BILF1. BILF1 je močno konstitutivno aktiven receptor, ki sproži aktivacijo $G\alpha_i$ in lahko zavre aktivacijo CREB, povzročeno s forskolinom, preko $G\alpha_i$ in je občutljiv na pertusis toksin (3).

Da bi ugotovili vlogo receptorjev, ki so uravnavani s strani virusov (EBI2) ali pa kodirani v virusnem genomu (BILF1) smo uporabili dva živalska modela. Gole (nude) miške so bile uporabljene z namenom, da bi ugotovili ali BILF1 vlogo pri nastanku tumorjev *in vivo*. Da bi pokazali vlogo EBI2 pri boleznih, povzročenih z EBV, še posebej rakastih obolenj, je bil pripravljen na Univerzi v Kopenhagnu, Danska transgenski mišji model z čezmerno izraženim genom za EBI2, pod kontrolo IgH promotorja (cilja limfocite B in T). Pri golih miškah smo pokazali, da BILF1 sodeluje pri nastanku tumorjev v 90-odstotkih (4). Pri starejših transgenih miškah smo opazili povečano proliferacijo celic B (1,2). Dobljeni rezultati kažejo, da imata BILF1 in EBI2, ko se izražata med infekcijo z EBV, vlogo pri malignih obolenjih povzročenih z EBV. Rezultati so pomembni iz vidika razvoja specifičnega zdravljenja za rakasta obolenja, povzročena z EBV v stanju imunodeficience organizma.

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Animal models in studying Epstein-Barr virus regulated or encoded 7TM receptors

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Herpesvirus infections are widespread, persist through lifetime and are usually balanced in a subtle interplay with the host immune system. Epstein-Barr virus (EBV), gamma-herpesvirus, has ~50-90% prevalence in human population and is associated with cell transformation and cancer development. No specific anti-virus treatment exists today against EBV-mediated diseases. Seven transmembrane (7TM) receptors (also known as G protein-coupled receptors, GPCRs), are involved in the interplay between virus and immune system either by being regulated by the virus, or by being encoded in the virus genome. EBV-induced Epstein-Barr virus-induced receptor 2 (EBI2) is a 7TM receptor, induced >200 fold upon EBV cell-entry. It is localized in B cells in the lymphoid follicle and is bound and activated by oxysterols, most potently by 7α,25-dihydrocholesterol (7α,25-OHC) (1,2). EBV open reading frame BILF1 encodes a 7TM receptor BILF1. BILF1 is highly constitutively active 7TM receptor, activating $G\alpha_i$ and it is able to inhibit forskolin-triggered CREB-activation via $G\alpha_i$ in a pertussis toxin-sensitive manner (3).

Two animal models have been used to define role of 7TM receptors regulated by the EBV (EBI2) or being encoded in the EBV genome (BILF1). Nude mice were used to determine whether BILF1 mediates tumor formation *in vivo*. To describe the role of EBI2 in EBV-mediated diseases, especially cancers, transgenic mouse model with over-expression of human EBI-2 under the control of an IgH promoter (targets B- and T-cells) was established in University of Copenhagen, Denmark. In nude mice, BILF1 promoted tumor formation in 90% of cases (4). In transgenic mice, increased proliferation of B-cells were observed (1,2). Data suggest that BILF1 and EBI2, when expressed during EBV infection, could be involved in the pathogenesis of EBV associated malignancies. This is important in relation to develop specific treatment for EBV cancers in the state of organism immunodeficiency.

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Pomen kontrole kakovosti dela v laboratoriju za zanesljivost rezultatov poskusa

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Pri izvajanju poskusov na laboratorijskih živalih proučujemo različne vplive na njihovo zdravstveno stanje. Pri poskusih je pomembno upoštevati načelo 3R zato se poskusi izvajajo v čimbolj definiranih in kontroliranih pogojih po točno določenih protokolih. Doslednost upoštevanja le-teh omogoča, da je poskus uspešno izveden in zaključen ter da se uporabi minimalno število živali. V prispevku želimo predstaviti potek kvalitetnega dela v kliničnem laboratoriju, njegov pomen in možnost vpliva na rezultate analiz.

Pri živalih v poskusu običajno spremljamo oz. merimo kvalitativne in kvantitativne variable pred, med in na koncu poskusa. V ta namen se pošilja vzorce v različne laboratorije. Za pridobitev zanesljivih rezultatov laboratorijskih preiskav je pomembno, da pošljemo vzorce v laboratorij z ustrezno kontrolo kakovosti dela. Med kvantitativnimi variablami, ki jih spremljamo, so tudi hematološke in biokemijske analize krvnih vzorcev pri katerih moramo, če želimo, da so rezultati analiz zanesljivi, upoštevati določena pravila. Za ta namen moramo v laboratoriju nadzirati in kontrolirati številne dejavnike, ki bi lahko vplivali na rezultate analiz, jih nadzirati in kontrolirati. Tako je potrebno zagotoviti ustrezno ravnanje s preiskovanim materialom in ga, glede na vrsto analize, tudi primerno pripraviti in shranjevati. Izvajati moramo redno dnevno kalibracijo in/ali dnevno uporabo kontrolnih krvnih vzorcev za preverjanje delovanja aparatur in reagentov. Pomembno je tudi sodelovanje v medlaboratorijskih kontrolah (domačih in mednarodnih), kar zagotavlja primerljivost rezultatov z drugimi laboratoriji. Za kakovost izvedbe analiz moramo ustrezno nadzorovati in beležiti potek analiz, ki se izvajajo po določenih predpisanih operacijskih postopkih, zagotoviti sledljivost vzorca in uporabljenih reagentov itd. S takim delom lahko laboratorij zagotovi kakovost dela in s tem zanesljivost v izvajanju analiz in zaupanje v dobljene rezultate.

Še tako skrbno načrtovan in izveden poskus ne bo dal ustreznih rezultatov, če ne bo enaka skrb posvečena izbiri laboratorija in izvedbi laboratorijskih preiskav.

The importance of laboratory quality control for reliability of results of the experiment

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With performing experiments on laboratory animals we research different influences on their health status. The 3R principle should be followed and for this reason the experiments are executed in strictly defined and controlled conditions exactly following the protocols. Consistent consideration of them enables successful execution of experiment and minimal number of laboratory animals used. The aim of our contribution is to highlight the quality work in clinical laboratory, its importance and possibility to influence the results of analyses.

Different qualitative and quantitative variables are usually monitored or measured before, during and at the end of experiment. For this purpose the samples are sent to different laboratories. To get reliable results of laboratory analyses is important to send the samples to the laboratory with adequate quality control. Quantitative variables measured in experiments are also haematological and biochemical analyses of blood samples where we have to consider defined rules to get reliable results. In laboratory we have to monitor and control various factors which can possibly influence the results of analyses. Suitable handling and regarding the type of analysis, appropriate preparing and storing of investigated material must be assured. Regular daily calibration and/or daily use of blood control samples must be performed to check the working of machines and reagents. For the laboratory is very important to be included in interlaboratory control (national, international) what enables comparability of results with other laboratories. To assure the quality of analyses we must appropriately control and register the course of analyses which are performed following defined operating procedures, assure traceability of samples and reagents etc. Considering all this the laboratory can assure quality of work and consequently the reliability of analyses and obtained results.

Even very carefully planed and executed experiment can not give appropriate results if the same care would not be devoted to the selection of laboratory and performance of laboratory analyses.