NSG MICE HAVE HIGH SUSCEPTIBILITY TO XENOTRANSPLANTATION OF CA-46 CELL LINE

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Burkitt's lymphoma (BL) is a rare but highly aggressive form of Non-Hodgkin lymphoma that develops in B-cells of germinal center. Therapy of this aggressive lymphoma is very difficult and prognosis is poor for most patients with progressed disease. Therefore, there is a need to develop new therapeutic approaches. In addition, we need to identify the best mouse models for the most efficient growth of xenografted tumor cells for preclinical testing. Currently, immunodeficient mouse strains like Balb/c nu/nu, NOD/SCID, RAG2, NSG, SCID/bg and others are the most frequent mouse models used for xenotransplantation of human tumors. NSG mice are unable to generate mature B-, T-, and NK cells and are deficient in many cytokine signaling pathways. Mice of the SCID/bg strain have an autosomal recessive SCID (severe combined immune deficiency) mutation, which impairs the development of both B- and T-cells. They also carry a mutation ($Lyst^{bg-J}$) that damages the development of NK cells. In this work, we evaluated whether NSG or SCID/bg mouse model serves better for propagation of CA-46 cell line derived from Burkitt´s lymphoma. Suspension cell line CA-46 was grown in RPMI medium supplemented with 10 % FBS at 37 °C. Cells were injected into NSG and SCID/bg mice by intraperitoneal and subcutaneous injection. Five million cells were administered. Tumor size was scored after 27 days. In this study we found a different pattern of engraftment in NSG and SCID/bg mice transplanted with CA-46 cells. NSG mice were characterized by enlargement of the intraperitoneal cavity and enlarged spleen (splenomegaly). The size of spleen increased more than two-times in comparison to SCID/bg mouse strain injected with CA-46 cells and control mice. It is interesting that the CA-46 cells did not engraft when injected subcutaneously in either NSG or SCID/bg mice. In conclusion, we found that the NSG mouse model has high susceptibility for propagation of Burkitt´s lymphoma cell line CA-46. Animal experiments were approved by the Institutional Ethic Committee and by the national competence authority – State Veterinary and Food Administration of the Slovak Republic (Project Registration No. 3512/2023-220), in compliance with the Directive 2010/63/EU and the Regulation 377/2012 on the protection of animals used for scientific purposes.

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THE INFLUENCE OF SELECTIVE VOLTAGE-GATED SODIUM CHANNELS $NA_V1.7$ BLOCKER ON COUGH REFLEX IN A MODEL OF ALLERGIC RHINITIS

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Allergic rhinitis (AR) may contribute to excessive and irritable coughing associated with airway inflammation. Different inflammatory mediators overstimulate upper and lower airway C-fibres increasing cough reflex sensitivity. Regardless of the stimulation of different receptors, voltage-gated sodium channels (Na_Vs) are essential for action potentials initiation and conduction, so they become targets for potential therapeutic use. Our previous study has shown a significant decrease in citric acid-induced cough by $Na_V1.7$ blocker pre-treatment in healthy guinea pigs. This study aimed to analyse the effect of $Na_V1.7$ blocker PF-05089771 on cough reflex in guinea pigs in a model of chronic allergic rhinitis. Dunkin Hartley guinea pigs sensitized by intraperitoneal ovalbumin (OVA, 100 µg) were intranasally challenged with OVA in 7-day intervals for 6 weeks with monitoring symptoms of AR. The cough was induced by inhalation of citric acid aerosol (0.4 M) according to protocol. The tested OVA-inhibitor group was pre-treated with an inhaled $Na_V1.7$ blocker (PF-05089771, 100 µM, 10 min) directly before tussigen inhalation (0.4 M citric acid containing either PF-05089771 or vehicle, 5 min) and compared to OVA group. Chronic AR significantly increased the cough reflex in the early phase of 0.4 M citric acid application compared to the control group (11.5±2.11 vs. 5.9±1.20, $P<0.05$, $n=9$ for each group) which corresponds with manifested symptoms such as increased sneezing, nasal rubbing and other eye and nose symptoms. Pre-treatment with the $Na_V1.7$ blocker (PF-05089771, 100 µM) resulted in significant inhibition of the cough reflex by ≈ 75 % (2.7±0.75 vs. 11.5±2.11, $P<0.0001$) without changes in the respiratory rate. Our results support the concept that targeting $Na_V1.7$ is a rational strategy for effective treatment of pathological cough associated with allergic airway disease.

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NEW TRENDS IN ANESTHESIOLOGY OF SMALL LABORATORY RODENTS

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New trends in the anesthesiology of small laboratory rodents represent a critical area of headway in preclinical research. These trends involve various aspects, including developing novel anesthetic protocols, optimizing existing methods, and exploring alternative attitudes to enhance the safety and efficacy of anesthesia in animal models. One crucial trend involves the pursuit of agents that offer rapid induction and recovery times while ensuring precise control over general anesthesia depth. The main aim of such an anesthetic agent is to minimize the stress experienced by animals during experimental procedures and trigger research outcomes together with its minimal cardiovascular and respiratory depressant effects to maintain physiological stability during anesthesia. Advancements in inhalation anesthesia systems have also emerged as a significant trend in small laboratory rodent anesthesia. These systems enable the delivery of volatile inhalational agents in a controlled manner, allowing for precise titration of anesthesia depth and helping the transition between different stages of anesthesia. Moreover, inhalation anesthesia offers advantages in terms of metabolic stability and reduced risk of severe adverse effects compared to other routes of administration. Another emerging trend is the exploration of multimodal anesthesia approaches, which combine multiple agents or techniques to achieve synergistic effects and enhance the overall anesthetic procedure. By combining different approaches, researchers can acquire sufficient and experimentally demanding anesthesia while minimizing side effects in animals and assessing data. Furthermore, there is an increasing interest in the development of non-invasive monitoring techniques to assess the depth of anesthesia and monitor physiological parameters as a source of additional experiment information during procedures. This monitoring equipment, including optical temperature sensors, oximetry, or pneumatic pillow sensors, offers invaluable real-time feedback on the animal's physiological condition, enabling prompt adjustments to anesthesia protocols as required.In conclusion, these trends reflect ongoing efforts to refine and optimize anesthesia protocols for small laboratory rodents, ensuring the welfare of laboratory animals while maintaining scientific, precise, and reproducible preclinical data.

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MONITORING OF MYCOBACTERIUM TUBERCULOSIS INFECTION IN MICE

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We developed two animal models of experimental tuberculosis in mice. The BALB/c and the highly susceptible CH3 animals were inoculated with Euro-American and Asian lines of M. tuberculosis. Upon tuning the radiation sterilization, 2.5 kGy was selected as an appropriate irradiation dose, which provided both fully sterilized but mechanically intact lung tissues. The aim of the next experiment is to demonstrate the presence of siderophores in mice with ongoing tuberculosis and to further analytically characterize these without necessary working in BSL3 lab.

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THE SHEEP AS AN ANIMAL MODEL IN CARDIAC ELECTROPHYSIOLOGY

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Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide, with cardiac arrhythmias being a significant contributor. Radiofrequency ablation (RFA) is the established treatment modality, however, irreversible electroporation (IRE, also known as pulsed field ablation [PFA]) has emerged as a promising alternative. Pre-clinical animal model testing is crucial for evaluating novel therapies. Pigs are commonly used for cardiac electrophysiology studies, but they are highly susceptible to ventricular fibrillation (VF), limiting their suitability. Sheep represent a potential alternative due to their lower VF incidence. Our study aimed to assess the feasibility of utilizing sheep as a large animal model for testing a novel human IRE generator designed for cardiac arrhythmia treatment. Six female Suffolk crossbred sheep (weight: 50-60 kg) underwent IRE ablation using the newly developed generator. Considering their ruminant nature, specific adjustments were made to anesthesia and procedural protocols. Femoral artery and vein access, followed by catheter insertion into the heart, mirrored established pig model techniques. Compared to 50-60 kg pigs, sheep hearts were smaller and were generally more narrow. Notably, the incidence of VF was significantly lower in sheep compared to the expected rates in pigs. All animals completed the experiment without complications. Our findings demonstrate the suitability of sheep as a large animal model for cardiac electrophysiology research. This alternative model offers advantages due to the reduced risk of VF compared to the pig model.

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ANIMAL MODELS IN EVALUATING THE ANTI-TUMOR EFFECT OF NEW ORGANOTIN COMPOUNDS

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Breast cancer is the most frequently diagnosed cancer worldwide. Organometallic compounds (cisplatin, oxaliplatin) have been used in cancer therapy for decades. However, their use is limited by their toxicity, poor pharmacokinetics and drug resistance development. In a continuing effort to identify new organometallic compounds as alternative therapies, tri-organotin compounds have been previously shown to induce apoptosis in various tumour cell lines. Here, we investigated the effect of tributyltin trichloroacetate (Cl_3CO), tributyltin trifluoromethane sulfonate (F_3SO_3) and N,N-dimethyldithiocarbamate triphenyltin (PhS_2) on a panel of breast cancer cell lines both *in vitro* and *in vivo*. The inhibition of cell proliferation was measured using a CellTiter-Glo cell viability assay. All compounds inhibited the proliferation in a dose-dependent manner, with IC_{50} between 0.09-0.33 μM. The most significant impact exerted PhS_2 with IC_{50} between 0.09-0.21 μM. IC_{50} for cisplatin was 2.4-52.3 μM. Induction of apoptosis was demonstrated by Annexin V assay and by increased activity of caspases 3 and 7. An *in vivo* test of acute toxicity was performed to determine the safety of organotin compounds according to OECD 423/2001 guideline. BALB/c mice were intraperitoneally treated with studied compounds and cisplatin. After 14 days of experiment, no toxicity or mortality was observed in the treated animals. Furthermore, histopathological analysis indicated no hepatotoxic or nephrotoxic effects in treated animals compared to the untreated control group. In the test of subacute toxicity, BALB/c mice were treated with Cl_3CO and F_3SO_3 at a dosage of 55 mg/kg, 25 mg/kg and 10 mg/kg, and PhS_2 at a dosage of 55 mg/kg, 25 mg/kg, 10 mg/kg and 5 mg/kg daily for a total of 28 days. There were no changes in body weight, gross appearance, physical abnormalities, or behaviour changes in the group treated with Cl_3CO and F_3SO_3. The PhS_2 showed toxicity at dosages of 55 mg/kg and 25 mg/kg (apathy and death of 2 animals). The maximal non-toxic concentrations were used to test the compounds anti-tumour activity. BALB/c mice bearing orthotopic allografts induced by 4T1 cells and SCID/bg mice with MDA-MB-231 orthotopic xenografts were treated twice a week for four weeks. The selection of the mouse strain influenced the sensitivity to the compounds. We observed adverse effects (stooped posture, breast swelling) in SCID/bg mice treated with F_3SO_3 on the 25th day after the start of treatment. All tri-organotin compounds exhibited efficacy in reducing tumour volume by 20% and cisplatin by 55 % compared to the control group. The inter-strain variations in the sensitivity to drugs should be considered in preclinical experiments.

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CLINICAL SCORING OF SYMPTOMS OF CHEMICALLY INDUCED COLITIS IN EXPERIMENTAL RATS DURING COLONIZATION OF THE GUT PROTIST BLASTOCYSTIS

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Blastocystis is a common intestinal protist in humans and animals, but its impact on health and the microbiome is unclear. Although it is sometimes associated with gastrointestinal problems, it often occurs in healthy, asymptomatic individuals. The conflicting views on the role of *Blastocystis* in health and disease underscore the significant gaps in our understanding of its interactions with the gut microbiome and host

colonization. In our study, we investigated the effect of *Blastocystis* ST3 colonization on the immune system and the gut bacteriome, alone and in combination with colitis induced by DNBS (58 g/l in 50 % EtOH). We focus on the clinical scoring of the health status of the experimental rats before and after colitis induction during *Blastocystis* colonization. Female Wistar rats (SPF, 13 weeks old, sourced from Envigo RMS B.V.) were inoculated with *Blastocystis* ST3. Subsequently, colitis was induced at three weeks (short-term exposure) and at three months (long-term exposure) following colonization. We assessed the intensity of inflammation by analyzing cytokine gene expression, and through clinical, as well as by macroscopic, and microscopic observations of the colon. We also monitored weight loss, stool consistency, hematochezia, and post-dissection colon inflammation. Additional symptoms such as apathy and a dull coat were recorded qualitatively. Our results show that short-term colonization with *Blastocystis* ST3 has no effect on intestinal inflammation, while long-term exposure appears to improve recovery from colitis in rats. Specifically, we observed a significant reduction in inflammatory markers (TNFα, IL-1β) and an improvement in clinical score in rats with long-term colonization two days after colitis induction. In contrast, short-term colonization showed no changes in clinical or macroscopic assessments of the gut. Interestingly, although long-term colonization initially resulted in poorer health indicators first day after colitis induction, such as greater weight loss, intestinal shortening, and more severe intestinal inflammation than in control animals, these conditions improved markedly the next day. The rats then showed significant weight gain, less hematochezia and intestinal inflammation, and greater gut length. Overall, our results suggest that long-term colonization with *Blastocystis* ST3 may have a protective effect against intestinal inflammation by promoting faster recovery. These results could potentially also apply to humans, who also experience long-term colonization with *Blastocystis*. For a detailed analysis of the gut bacterial microbiome, see our publication in Billy et al. (2021).

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3RS CENTRE CZECH REPUBLIC AT THE NATIONAL INSTITUTE OF PUBLIC HEALTH IN PRAGUE

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The Directive 2010/63/EU is implemented in the Czech Republic in the Act No. 359/2012, amending Act No. 246/1992, on the protection of animals against cruelty. The harmonized legislation covers the protection of animals used for scientific purposes and for toxicological testing within the principles of 3Rs, i.e. reduction of animals in tests, limitation of their suffering by refinement of procedures and replacement of animal testing by alternative toxicological methods. The 3Rs Centre Czech Republic is a public establishment seated at a governmental institution, the National Institute of Public Health in Prague (NIPH). The 3Rs Centre is actively promoting the 3Rs principles and contributing to the fulfillment of 3Rs strategy. Currently, it functions as a multidisciplinary network comprising National Reference Centres (NRC), National Reference Laboratories (NRL) and the Unit for Alternative Toxicological Methods at the Centre of Toxicology and Health Safety, at NIPH. Thanks to the active multidisciplinary approach, advanced technologies and equipment, the 3Rs Centre covers all three principles of the 3Rs strategy. To cover the Replacement principle, the NRL for Experimental Immunotoxicology focuses on scientific research, development, validation and standardization of New Approach Methodologies (NAMs), such as in vitro methods based on 3D tissues and cell lines. The Unit for Alternative Toxicological Methods implements validated NAMs and provides professional testing of chemicals, cosmetics, consumer products and medical devices in compliance with ISO standards, accreditation and GLP quality systems. The dissemination of the 3Rs concept is realized by active participation at national and international events, organized by e.g. EUSAAT, ESTIV, SETOX (Slovak Toxicology Society), PROKOS (Association of producers, importers and distributors of cosmetics and their ingredients), CLASA (Czech Laboratory Animal Science Association), etc. Members of the 3Rs Centre are involved in international societies such as EUSAAT, ESTIV, serve as experts for OECD, ECHA and EURL-ECVAM. Members of the 3Rs Centre participate in authorization of experimental projects in cooperation with the animal welfare committee at the Ministry of Health, ensuring that the approved projects fulfill the 3R principles required by the Directive 2010/63/EU. With regard to scientific quality/translatability, the Ministry of Agriculture of the Czech Republic has nominated NIPH as the contact point to provide advice on the regulatory relevance and suitability of alternative approaches proposed for validation (PARERE) and the NRL for Experimental Immunotoxicology as a specialized laboratory to the network of qualified laboratories participating in validation of NAMs (EU-NETVAL).

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EFFICACY OF NEW-GENERATION TAXANES SB-T-121605 AND SB-T-121606 IN RESISTANT OVARIAN CARCINOMA MODEL

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Conventional taxanes (paclitaxel, docetaxel or nab-paclitaxel) are widely used anticancer drugs for treatment of various types of solid tumors including ovarian carcinomas. Unfortunately, one of the main problems with conventional taxanes is the occurrence of multidrug resistance (MDR). As a possible solution to overcome MDR, new-generation experimental taxanes (Stony Brook Taxanes; SB-T) have been developed, which exhibit promising activity against various resistant tumor models. The aim of our study is to investigate the *in vitro* potency and *in vivo* antitumor activity of clinically used paclitaxel (PTX) and SB-Ts, i.e., second-generation taxanes (SB-T-1214, SB-T-1216) and third-generation taxanes (SB-T-121402, SB-T-121605, and SB-T-121606) in PTX-resistant ovarian carcinoma cells (NCI/ADR-RES and SKOV3/PTX-RES). In NCI/ADR-RES resistant *in vitro* model, the potency of the new SB-Ts was up to 50-times higher than PTX. SB-T-121605 and SB-T-121606 induced cell cycle arrest in the G2/M phase much more effectively, as compared to PTX. Incorporation of SB-T-121605 and SB-T-121606 into treatment regimens containing PTX were effective in suppressing tumor growth *in vivo* in mice NCI/ADR-RES cell-derived xenografts at low doses (≤3 mg/kg), where their adverse effects were negligible. In SKOV3/PTX-RES *in vitro* model, the efficacy of the new SB-Ts was even 360-times higher than PTX. The most efficacious taxane, SB-T-121606, successfully reduced tumor growth *in vivo* in SKOV3/RES xenografts at low dose (1 mg/kg) when combined with 9 mg/kg of PTX. In conclusion, the third-generation Stony Brook taxanes, SB-T-121605 and SB-T-121606, are highly promising candidates for further studies, that could potentially lead to the development of novel cancer therapeutics effective against conventional taxane-resistant tumors.

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BIOTECHNOLOGICALLY RECONSTRUCTED HUMAN TISSUE MODELS USED AS REPLACEMENTS OF IN VIVO TESTS

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The potential of substances (chemicals, cosmetics, ingredients, and formulations) to cause effects such as corrosion or irritation to skin and eye is a concern of toxicologists in their assessment of possible worker and consumer safety issues. Moreover, national and international regulatory agencies require that substances are labelled with regard to their toxicity potential to skin or eye. To prevent the unnecessary use of animals for the above mentioned purposes, EU as well as US regulations recommend the use of alternative tests methods "whenever appropriate and feasible". Since reconstructed human tissue (RhT) models closely mimic native tissues, they can be used for reliable estimation of hazard and risk related to human health. Tests with RhT models for topical toxicity testing are cost-effective and deliver faster and more reproducible results than many of the traditional *in vivo* assays. Their characteristics can be precisely controlled by established Quality Assurance procedures to ensure long-term reproducibility, which is important in the regulatory toxicology. RhT-based assays for skin and eye irritation/corrosion and phototoxicity testing are validated and regulatory accepted by the OECD – as TG 431, 439, 492, and 498. A number of *in vitro* RhT-based methods have completed pre-validation testing (genotoxicity) or are ready to enter the pre-validation process in the near future. They enable testing without an excessive need for laboratory animals, which is of great importance for REACH as well as for the EU cosmetic legislation. This presentation will describe currently available RhT-based assays for toxicity testing. Approaches to the development, validation, and implementation of these assays into regulatory systems and testing strategies will be discussed.

DIFFERENCES BETWEEN POTENTIAL SKIN AND RESPIRATORY SENSITIZERS TESTED BY NON-ANIMAL METHODS

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Sensitization is a specific immune response induced by a sensitizer, a substance which after repeated contact with skin or respiratory system elicits hypersensitivity and allergic responses leading up to contact dermatitis, asthma or other adverse systemic reactions. The suggested Adverse Outcome Pathways (AOP) for respiratory and skin sensitization share some of the molecular events and mechanisms of action. Recently, a battery of non-animal *in vitro* and *in chemico* methods for assessment of skin sensitization potential has been adopted as OECD guidelines, however, no defined approaches for evaluation of respiratory sensitization potential have been developed yet and current classification relies solely on (Q)SAR *in silico* modelling and non-regulated inhalation tests on animals. The prerequisite for reliable hazard identification of respiratory sensitizers includes identification of best exposure-response parameters and tools for distinction between irritants and skin and/or respiratory sensitizers. This pilot study was focused on investigation of differences and similarities between skin and respiratory sensitizers, which might lead to the definition of relevant endpoints and testing strategies. A group of known positive and negative respiratory sensitizers was tested by 2 of 3 alternative methods validated for skin sensitization, namely *in chemico* method DPRA (Direct Peptide Reactivity Assay, OECD TG 442C) and *in vitro* method LuSens (ARE-Nrf2 luciferase test method, OECD TG 442D). The substances were selected on the basis of previous results reported in literature and data available in the ECHA database of chemicals. The group of 6 positive chemicals consisted of toluene diisocyanate, hexamethylene diisocyanate, ammonium hexachloroplatinate, diphenylmethane diisocyanate, trimellitic anhydride, and phthalic anhydride. The group of 4 negative chemicals included Thiuram (tetramethgylthiuram), isoeugenol, eugenol, and 2,4-dinitrochlorbenzene, which are often used as positive controls in the *in vitro* skin sensitizations tests. According to the recently published results, DPRA seems to be a promising method for the evaluation of the first key event (haptenation) for both skin and respiratory sensitization and might distinguish between respiratory and skin sensitizers in their reaction with peptides containing lysine or cystein. This hypothesis was confirmed in our study, as 5 of 6 tested positive respiratory sensitizers had affinity to both cysteine and lysine peptides, while the negative respiratory sensitizers reacted only with cysteine. As expected, results from LuSens (based on activation of keratinocytes) did not correspond with respiratory results in most cases, which indicates that the second key event in each type of sensitization differs in respective molecular and cellular responses. The results show the sensitizing potential of each chemical in both tests and provide recommended non-cytotoxic concentrations for future *in vitro* testing. This study will further aim at other possible endpoints and key events in the process of sensitization (e.g. dendritic cells activation tested by h-CLAT, cytokine evaluation using 3D airway epithelial models) in order to contribute to the knowledge on the specific respiratory hypersensitivity hazard.

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ENRICHMENT IN SCIENCE: IMPROVING THE ENVIRONMENT AND THE QUALITY OF RESULTS

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For many years, unenriched housing was the standard, but in the last 20 years or so there has been a remarkable increase in the provision of enriched conditions for animals used in research. Therefore, from a scientific and ethical point of view, the welfare of laboratory animals should be an important concern for veterinarians, animal facilities and researchers. Recently, there has been criticism of the conventional housing of laboratory rodents, which induces abnormal behaviour, poor living conditions, physiological and immunological abnormalities that call into question the validity and reliability of many animal experiments. Environmental enrichment can prevent abnormalities and improve animal welfare. However, there are concerns that it may also undermine standardisation and thus reduce the accuracy and repeatability of animal experiments. However, there are concerns that it may also interfere with standardisation, thereby reducing the accuracy and repeatability of animal experiments. But animal welfare can be improved by environmental enrichment without compromising standardisation. There are several things to consider before introducing enrichment. It must be verified that enrichment will not adversely affect the results of experiments in which the animals or their offspring may be used. For example, if the research involves monitoring the gut microbiota, enriched diets and treats that could affect it will not be given. In order to avoid differences between the control and experimental groups, the same enrichment elements should be given to both groups, unless otherwise specified. On the other hand, enrichment can be used in an experiment intentionally. New insights into its effects on brain structure and function have led directly to biomedical research into the possible therapeutic effects of enrichment on neurodegenerative diseases (e.g. Huntington's and Alzheimer's disease), aging and recovery from stroke and other forms of brain damage. Enrichment can lead to: increased neurogenesis, increased capillary development, formation and changes in synapse efficiency, and changes in neurotransmitter activity. Animals raised in enriched environments often show improved memory, learning and behaviour, which may be related to changes in structures such as the cerebral cortex, hippocampus and thalamus. An example of the purposeful use of enrichment will be given in a mouse line targeting the gene ($Grin2a^{N615S}$). This line is a model organism for a specific group of mutations found in the M2 loop of the NMDA receptor ion channel that functionally manifest as reduced sensitivity to Mg^{2+} block, leading to excessive activation of the receptor leading to deleterious effects on neuronal function. This group of GRDs (GRIN related disorders) is most commonly manifested by intellectual disability, hypotonia and speech/language disorders. In mice, the mutation is manifested by impaired locomotion, anxiety, impulsive behaviour, impaired cognitive memory and also induces audiogenic seizures.

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DEMONSTRATION OF ANIMAL MODELS OF INFECTION USING LABORATORY MOUSE AND LABORATORY RAT

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The animal facility of the Institute of Molecular and Translational Medicine of Palacky University (IMTM) provides preclinical *in vivo* efficacy models for different diseases, including those caused by infectious agents. At IMTM, we used to work with topical infection animal models: Full-thickness skin model; Skin abscess infection model; Myositis-induced model of infection; Lung infection model; and Stereotactic brain infection model. Skin and muscle infection models are essential for new skin and deep wound infection treatment strategies. Injury or surgery often leads to persistent chronic infections, which are challenging to treat due to multi-drug resistance and the formation of biofilms. Infections in the lung leading to pneumonia constitute a significant cause of death in the young, elderly, immunocompromised, and cystic fibrosis patients. The development of multi-drug resistant strains of microorganisms makes effective treatment complicated. We use stereotactic surgery to create rodent brain infection models. The reason for creating these models is the research of brain infection therapies focused on developing and progressing related pathophysiological changes, including inflammation and loss of adequate response or function to stimulus. Animal models of infections are very delicate. The animals used are infected, so a comprehensive and detailed plan for animal studies is required to avoid unnecessary suffering of laboratory animals and to follow the principles of the 3Rs (replacement, reduction, and refinement). Efforts to replace animals in experimental procedures and studies that could cause discomfort, pain, and suffering are correct and justified. However, no alternative method can now fully replace the use of animals in the studies that follow a host response to the infection caused by applied agents and tested therapy. The search was conducted on the Tracking System for Alternative Methods Towards Regulatory Acceptance (TSAR) website, managed by the Joint Research Centre (European Commission). Animal models have proven useful to design and minimize the risk of subsequent clinical trials on antimicrobial drugs. The well-standardized models, such as the mouse thigh infection model, have led to the formulation of indices and targets that determine optimal drug exposures. These results can be translated from the *in vivo* model to humans because the antimicrobial target is the microorganism, not the host. An animal model may be used to predict a novel treatment toxicity, pharmacokinetics, and effectiveness before it progresses to patient studies. The reduction of experimental animals is caused by *in vitro* antimicrobial testing. *In vitro* testing provides a selection of active compounds that can be used in the next step of an *in vivo* study. It is possible to reduce the number of animals used further by better planning procedures, determining the optimum sample size, and responding flexibly throughout the experimental design based on the data obtained (Bayesian updating). Gentle handling and strictly defined and controlled procedures with animals will further ensure accurate results with a low degree of variation in the same experimental group.

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GNOTOBIOTIC PIGLET MODEL OF IMMUNOCOMPROMISED HOSTS

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Immunocompromised hosts, such as preterm infants and elders, are susceptible to enteric infections. Synthetic microbiota can increase their resistance to pathogens and pathobionts. Pattern recognition receptors sense molecular motifs related to pathogens and tissue damage. Thus, various ligands can trigger inflammatory reactions and sepsis. The hysterectomy-derived microbiologically defined (gnotobiotic) piglets allow the simulation of states occurring in immunocompromised hosts. TLR4 plays a crucial role in infections by Gram-negative bacteria and the development of necrotizing enterocolitis. Intestinal histology, tight junction proteins claudin-1 and occludin expression, TLR4 and its coreceptors MD-2 and CD14, and adaptor proteins MyD88 and TRIF trigger an inflammatory reaction. Local and systemic levels of inflammatory mediators IL-6, IL-8, IL-10, IL-12/23 p40, TNF-alpha, and HMGB1 belong to inflammatory biomarkers that can describe the severity of the reaction and its expected consequences to the host. Regulation of inflammatory signaling by probiotics and defined microbiota can be one of the ways to regulate the relationship between the sensing of dangerous molecular patterns and the detrimental effect of unregulated inflammatory response.

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PRETERM GNOTOBIOTIC PIGLET AND PRIMARY MICROBIOTA

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Preterm infants are delivered before 37 weeks of pregnancy. They have underdeveloped organ systems, e.g., lungs and intestine, and show low colonization resistance to enteric infections. Colonizing their intestinal tract with primary microbiota is essential and can bring benefits in their following life. Suitable animal models are needed to verify the safety of the microbiota for immunocompromised preterm infants. The work aimed to develop and characterize a gnotobiotic piglet model of preterm infants. The preterm germ-free piglet ileum showed decreased lamina propria cellularity, reduced villous height, thinner and less distinct stratification of tissue layers, and increased tight junction protein claudin-1 expression compared to its term counterparts. No apparent differences in pattern recognition receptor expressions were found, but the preterm intestine expressed higher constitutive IL-6, IL-8, IL-10, IL-12/23p40, and TNF-alpha levels. The preterm piglets were mono-colonized with probiotic *Lacticaseibacillus rhamnosus* GG or *Bifidobacterium animalis* subsp. *lactis* BB-12 early after hysterectomy. The bacteria did not translocate or induce significant production of inflammatory cytokines. The preterm gnotobiotic piglet is an animal model that allows verification of the suitability and safety of bacteria for preparing the synthetic microbiota. It will allow optimization of the composition of a primary microbiota intended for colonizing the intestinal tract of preterm infants.

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IN VITRO AND IN VIVO PRECLINICAL STUDIES ON NEW TAXANES AS POTENTIAL CHEMOTHERAPEUTICS FOR RESISTANT SOLID TUMORS

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Intrinsic and acquired tumor resistance limits clinical use of conventional chemotherapeutics, including taxanes, in cancer treatment. Using extensive structure-activity relationship (SAR) studies of taxanes, the laboratory of Prof. I. Ojima developed taxane derivatives called Stony Brook Taxanes (SB-Ts) with potential to overcome paclitaxel resistence. The aim of our research was to evaluate the activity of SB-Ts in cancer cell lines *in vitro*, analyze the efficacy and systemic toxicity of the most successful agents in preclinical models (CDX and PDX) *in vivo*, and recommend the most effective derivatives for the next phase of clinical studies. At first, the viability, proliferation, apoptosis, and uptake of SB-Ts in paclitaxel-sensitive and paclitaxel-resistant tumor cell lines were investigated *in vitro*. SB-T taxanes demonstrated 50 to 1000-times higher cytotoxicity than paclitaxel and significantly higher accumulation in resistant tumor cells. For the most potent taxanes, SB-T-121605 and SB-T-121606, their antitumor efficacy and systemic toxicity was then examined *in vivo* using mouse cell-derived xenograft (CDX) and patient-derived xenograft (PDX) models. The incorporation of SB-T-121605 and SB-T-121606 into the regimens containing paclitaxel was effective in suppressing tumor growth in ovarian carcinoma resistant CDX mouse models at low doses (≤3 mg/kg), where their adverse effects were minimal. The same combination was also highly efficacious in PDX models of highly aggressive subtypes of pancreatic carcinoma. Furthermore, we also use PDX models for the establishment of patient-derived organoids (PDXOs). In conclusion, taxanes (i.e., SB-T-121605 and SB-T-121606) are efficacious in experimental treatment of resistant and highly aggressive solid tumors. Furthermore, the established PDX or PDXO models serve us not only for the search for optimal therapeutic regimens but also for future discovery of signaling pathways behind the drug resistance.

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