Session V-6: Animal reduction through the better use of mechanistically-based translational animal disease models

Session V-6: Oral presentations

V-6-654

Computational models for predicting human toxicities

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Predicting the potential for human toxicity from a molecule structure is feasible by learning from large numbers of known compounds or understanding structure activity relationships for proteins involved in toxicity. Several examples will be presented including recent models for drug-induced liver injury (DILI), which is one of the most important reasons for drug development failure at both pre-approval and post-approval stages. In addition, the results of combined *in vitro-in silico* studies have suggested new, structurally diverse inhibitors for human transporters that may possibly cause clinically significant toxicities such as rhabdomyolysis. The versatility and potential of using such models in drug discovery may be illustrated by increasing the efficiency of screening and rapid identification of potential interactions for FDA approved drugs or new molecular entities. This may lead to insights into potential off-target effects of molecules as well potential repurposing uses.

V-6-406

A model for inter-institutional 3Rs co-operation – sharing *in vivo* research resources

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With the broad goal of reducing and replacing research animal use where possible, MedImmune entered into an inter-institutional agreement to make ferret trachea tissue available to a neighboring academic institution so as to enable them to carry out vital *in vitro* research work without having to obtain additional live animals. Over the course of 3 years, MedImmune provided the Cystic Fibrosis Research Library with over 200 ferret tracheas which enabled them to work, without further use of live animals, in a major area of unmet medical need. Key to this successful effort were communication within the laboratory animal science community and more rare but essential: the institutional recognition of the 3Rs in ethical research beyond one's own walls and the willingness to engage in a collaborative process to reduce and refine animal use where possible.

Cystic Fibrosis (CF) is a severe genetic disease that affects several organ systems in people but most destructively the pulmonary system. In CF, tracheal mucous production is abnormal, leading to inadequate removal of airway microbes. Repeated pulmonary infections then result from usually innocuous bacteria getting into the lungs. Severe scarring causes increasing impairment of respiratory capacity and often early death. Victims often succumb as children or young adults. In order to understand and then treat afflicted patients, normal and abnormal tracheal mucous production needs to be studied which cannot readily be done in people. Ferrets and pigs most closely mimic the human respiratory tract. The CF laboratory developed a system to study mucous production in the excised trachea of the ferret.

The IACUC at both institutions supported developing the tissue sharing agreement and the legal departments also engaged in the process to successfully pave the way for the tissue transfer. To date, one study has been published with others in preparation.

V-6-295

The Three Rs and the one P: Predictability

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Recent technological advances and broad initiatives to improve the predictability of animal models can significantly reduce the number of animals used in biomedical research. Factors which have historically influenced animal model development and use, such as technological advances, discoveries within a vast diversity of species, medical initiatives, and evolving public opinion, will be discussed. This will set the context for discussing how recent shifts in medical approaches toward personalized health care and technological advances will likely influence preclinical biomedical research in the future.

An example will be presented from a strategy used to compare gene profiles from healthy and diseased human populations with those of *in vivo* models in order to increase the confidence in, and predictability of, preclinical studies. Additionally, advances in molecular imaging technologies have enabled us to obtain more contextual, mechanistic information than previously possible. As a case in point, continued advancement in this area may result in the increased applications of orthotopic mouse tumor models which are believed to be more relevant to cancer in patients than the traditional subcutaneous tumor models. In short, as these approaches are applied to more closely align molecular/genetic profiles of healthy and diseased people and animal models, preclinical research efficiency will likely increase and ultimately have a positive impact on the 3Rs.

V-6-711

Development of a flow chamber test to replace animal research on arterial thrombosis and bleeding *in vivo*

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Arterial thrombosis is a main cause of death in the western world. At present, antithrombotic treatment is only partially effective, due to incomplete knowledge of the molecular determinants of this disease. Arterial thrombus formation occurs by increased activation of blood platelets and the coagulation system. On the other hand, insufficient activity of these processes leads to bleeding. Genetic approaches, where platelet or coagulant proteins are knocked down in mice, are of indispensible value in the finding of new molecular targets. However, current tests of thrombus formation and bleeding are carried out with anesthetized, living animals and are therefore cumbersome. We have developed and miniaturized flow chamber technology as an alternative for this *in vivo* testing. Herein, we perfuse blood under arterial shear rate over a spotted array of purified vessel wall proteins, and measure the buildup of a thrombus at each spot with microscopy. Application of this *in vitro* test with mouse blood shows that it is sensitive to the expression of >50 proteins (platelet signaling proteins, transcription factors and coagulation factors). Furthermore, pharmacological inhibitors and antithrombotic drugs, active *in vivo*, also suppress thrombus formation of human blood *in vitro*. This technique is now being used in many other laboratories. For the area of atherosclerosis, thrombosis and haemostasis, it provides a novel way for reduction, refinement and replacement of animal use in experimental research.

Session V-6: Poster presentations

V-6-074

Comparative study of propensity for amyloidogenesis in male and female mice

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Reactive amyloidosis is a condition that complicates a long list of chronic inflammation, chronic infectious, malignant, and hereditary disorders. In the present study, the propensity for amyloidogenesis in male and female mice on spatio-temporal pattern was evaluated. For this purpose a total of 40 male and female Swiss mice after being weighed were randomly divided into 2 treatment groups including 2 groups [10 male (Group A1) and 10 female (Group B1) each], and 2 control groups [10 male (Group A2) and 10 female (Group B2) each]. Chemical compounds included vitamin-free casein as an amyloid inducer. For induction of amyloidosis the following protocol was met: Group A1 and B1: subcutaneous injection of 0.5 ml of 12% vitamin-free casein per day, 5 days per week. Group A2 and B2: subcutaneous injection of 0.5 ml saline per day, 5 days per week. At the end of the 3rd, 5th and 7th week of the experiment three mice were randomly selected from each group and were subjected to necropsy. Liver, lung, kidney and heart samples of each animal were obtained and embedded in paraffin blocks and stained by alkaline Congo red techniques. A green birefringence under the polarized microscope was considered to be a positive criterion for the presence of amyloid, indicating development of amyloidosis. Amyloidosis scale was assigned from 0-3. The data obtained from microscopic quantitative evaluation did show significant differences between groups A1 and B1. A preferential expression of reactive amyloidosis is concluded in male, indicating sex differences in amyloidosis. The maximum and minimum amyloid density/deposition was observed in the lung and the heart of male mice, respectively.

V-6-118

Hydra as an alternative model system to study the role of dysfunctional receptor tyrosine kinases in toxicity and disease

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Receptor Tyrosine Kinases (RTKs) are key components of cell-cell signalling required to regulate cell proliferation, differentiation and apoptosis, making them potential proto-oncogenes. Mutations in RTKs have been linked to many human congenital syndromes and diseases. Functional specificity of RTKs is attained by activation of the tyrosine kinase domain following cognate ligand binding. Hydra, being a simple organism with tissue level organization and sophisticated molecular pathways, provides a powerful alternative model system to study the roles of RTKs in induction of toxicity and initiation and progression of diseases. We have carried out genome-wide screening in hydra for RTKs. The domain-based screening using Hidden Markov Models (HMMs) for RTKs in Genomescan predicted gene models of *Hydra magnipapillata* genome resulted in identification of 15 RTKs. Only 5 of these RTKs have been previously reported and a few of these have been partially characterized. These RTKs have been classified into 8 families based on domain architecture and homology. We have identified most of the RTK family members, including DDR, Eph, FGFR, IR, MuSK, PTK7, Ror and RyK in hydra. Identification of these RTKs and presence of cell types comparable to humans (musculo-epithelial cells, nerve cells and stem cells) potentially make hydra a powerful alternate model organism for the study of various aspects of RTK-related toxicity and disease promotion.

V-6-131

The justification of animal numbers – The role of sample size, precision and power analysis in assigning and justifying animal numbers

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The US Animal Welfare Act (1966, 1985) empowered every institution using animal models for research and development to have their study protocols and related support policies reviewed by its Institutional Animal Care and Use Committee (IACUC). Recently, the USDA has begun to look more closely at the justification and assignment of animal numbers, and more generally, at identifying reduction alternatives to the related study practices.

To meet immediate and potential compliance concerns, as well as those related to basic study design and animal sample size, many research investigators have turned to biostatisticians for statistical support and justification of the animal numbers used in their studies. One response has been to develop web based sample size and power analysis software tools to assist in writing the Statistics Section of the IACUC Protocol. These tools use software previously employed by the biostatistician for routine power analysis for studies using a t-test, analysis of variance, or multiple treatment comparison for their data analysis, in addition to generating a report.

An immediate consequence has been to define an empirical statistical standard for the support of animal numbers. In particular, we can now improve control over the risk of using too few animals, where variation can hide potential activity of the research drug. Similarly, we can control the risk of using too many animals, thus avoiding the unnecessary pain and distress of extra animals. Either case supports real or potential animal reduction.

V-6-159

Improved design of animal experiments

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Animal experiments are an important phase in the development of new therapeutic agents against diseases. Tests to investigate efficacy of new therapeutic agents typically have a fixed design with two control groups (diseased without treatment and healthy without treatment), and three treatment groups (low, middle and high dose of the experimental treatment).

It is common practice to randomize the animals over equally sized groups. However, since not all comparisons between groups are of equal importance to answer the research question, the choice for equal group sizes is often not the most efficient one. The optimal experimental design provides maximum information with minimum sample size. In statistical literature, optimal experimental design has been described before. However, a set of useful guidelines and tools to aid investigators in efficacy research to optimally design their own experiments is lacking. Literature addressing this issue is sparse, technical in nature and unknown.

In an example, we highlight how optimizing experimental design will lead to an increase in experimental power with the use of fewer animals. The aim of this project is to develop a set of guidelines and tools to aid investigators in efficacy research to optimally design their own experiments, potentially leading to a 10-15% decrease in the number of animals needed.

V-6-208

Reduction through statistical tools and design – impact & implementation experiences

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The Pre-Clinical Statistics Group at AstraZeneca has achieved considerable success in the area of animal reduction through close collaboration with scientists in Discovery based in UK, Sweden and USA.

A number of statistical tools and techniques are now advanced in their application (including power analysis, factorial experimental design – http://dels-old.nas.edu/ilar_n/ilarjournal/43_4/v4304Shaw.shtml, sequential design and quality monitoring) and have been providing significant impact in overall reduction of animals, and in better decision making based on available animal data. As one example, factorial experimental design provides a set of tools for rapid learning about which factors are important for an animal model. It is possible to use significantly fewer animals in comparison to more conventional approaches. A variety of methods have been used, including web-based tool delivery, scientist training and empowerment and statistical consultancy. A key element for successful implementation of these important techniques is to understand what aspects the scientist can take ownership for and what aspects require consultation with a statistician. The group has developed working processes and practices which have proved to be highly effective and have also developed strong inter-personal skills to build relationships with customers to bridge the gap between statistics and biology. The focus of the presentation will be to introduce some of these statistical concepts in a user-friendly way and to demonstrate how the techniques can be applied effectively, including quantifiable impact on animal reduction, with real examples from AstraZeneca R&D.

V-6-235

In vivo predictive efficacy

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Animal experiments are used to make decisions about which new compounds should be progressed to clinical development and ultimately used for patients. But how do we know whether a positive signal registered in an animal model will also yield a positive response in a diseased human? What are the criteria associated with a particular animal model that provide confidence in its utility as a tool for making progression decisions? More importantly, which models are we currently using for this purpose which are misleading and could be eliminated, reducing the use of animals for irrelevant work and reducing the risk (and cost) of clinical development based on a false premise? The answers to these questions are fundamental to the use of animals in research but often are not clearly understood. This work aims to understand the predictive confidence associated with models used in cardiovascular disease by comparing both clinical and pre-clinical data and establishing a set of criteria that relate to animal model performance. These criteria can then be used to consider the likely predictive utility of models for which there is currently no clinical precedent and, if required, either modify them to yield a high level of confidence or replace them with more appropriate alternatives. In this poster we present the criteria, in the form of a questionnaire, which we have used to begin to explore this area and present some preliminary data from a selection of models used in the cardiovascular disease area.

V-6-266

Evaluation of dried blood spotting – dog study

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Microsampling has been under investigation in AstraZeneca for a number of years focusing on a technique of accurate blood collection in a microtube for analysis after solvent extraction. The interest in dried blood spots (DBS) was seen as an alternative way forward in microsampling and was evaluated in a study on a compound that showed a potential paediatric indication. In this study a blood microtube method was compared to DBS with additional data collected from the conventional plasma analysis. A further study was undertaken to investigate the effect on PK by sampling from the jugular vein compared to a peripheral sample collected from the ear. The compound was validated over the range 20 to 1000 ng/ml in plasma and DBS for a 1 month toxicity study. The data for the tox study was shown to be equivalent. Correlation plots derived from the sample data combining the plasma and DBS results gave R2 values of 0.92 and 0.96 (x=y plot) for the day 1 and 28 data respectively. For the sampling site comparison study two dose levels were investigated and samples collected on DBS and as a wet blood sample in microtubes. The comparative data for the sampling sites showed good correlation with an R2 value for the plot of 0.99 (x=y plot), indicating equivalence for the two data sets across the dose ranges. No lag time was observed with a T_{max} of 6 h for both the peripheral and venous samples. The final part of the evaluation was the comparison of the wet sample and the DBS dry sample.

V-6-275

Improvements in behavioural pharmacology study design saves animal lives and cost, whilst enhancing quality of pre-clinical data

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Applying pharmacokinetic-pharmacodynamic (PKPD) modeling principles pre-clinically is essential in understanding how a drug's systemic exposure relates to the magnitude and time profile of its pharmacodynamic response in an animal model. The ultimate objective is to predict effect and duration of effect in man, and help simulate and guide design of clinical studies. Pre-clinical behavioural studies in analgesia consist of at least three different studies; efficacy (dose response), effect-duration, and tolerance development. In such studies, different sets of animals are normally used to collect PK and PD data. Historically, PKPD modeling would be applied to these existing data sets, but the precision in describing PKPD relationships is reduced since group mean values are used instead of individual animal values. The aim of this study was to improve the design of PKPD studies, more specifically, investigate if PK and PD data can be taken from the same animal.

AZ100 was orally administered daily for 5 days and tested 1, 2, 4, 7, and 24 h later on day 1, 3, and 5. PK measurements were done on day 2, and 4. Spinal Nerve Ligation pain model induced tight ligation of the L5 and L6 spinal nerve in the rat. Heat hyperalgesia was assessed using the plantar test.

PK and PD data collection from the same animals but taken on alternate days did not produce undesired stress in rats. AZ100 produced a dose- and time-dependent reduction in heat hyperalgesia. This effect is related to the plasma concentration and tolerance is not developed following repeated dosing.

This design study has proven to be very efficient; it allows for the simultaneous evaluation of effect-duration, tolerance development, and PKPD relationship, and as a result, saves time/money, and animal lives (60% reduction).

V-6-283

A bibliography on the care and use of zebrafish

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In 2010, the USDA's Animal Welfare Information Center, released a comprehensive, on-line bibliography on the use of zebrafish (Danio rerio) in multiple areas of research. This versatile vertebrate provides an alternative model organism for numerous research studies of human disease and the study of biochemistry, genetics, development, embryology, cancer, toxicology, pharmacology, physiology, hematology, ecology, cardiology and many other research areas. The publication contains more than 3,600 recent abstracts that also cover husbandry and other aspects of welfare for the fish. The transparent body of the zebrafish makes it a prime candidate for "time lapse" photography, which is very useful in the study of organ development. A recent breed of *Danio rerio* enhances the transparency to improve the study of melanomas. The fish was first recognized as a valuable, inexpensive, vertebrate research model in the 1970s when a small number of laboratories were using it in research. It can now be found as the animal of choice in 1000's of research projects worldwide. As many projects transition from warm blooded animals to the zebrafish, this bibliography will provide an excellent resource for the numerous scientific disciplines it covers and the various methods used for those experiments. Many of the records include the National Agricultural Library call numbers, but all can be easily located as the full text document with the other journal information provided.

V-6-297

Single Site Laparoscopy (SSL) abdominal access simulator – development and validation

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Design verification and validation studies for laparoscopic access systems and devices are typically performed in the porcine model. Data collected from this process is variable, subjective, expensive and limited to specialized facilities. This simulator was developed and designed with multiple objectives in mind: 1) reducing and replacing the need for live animals in the laparoscopic access new product development process, 2) creating a highly repeatable and reproducible comparative testing capability, 3) allowing market research and customer visits to occur in locations that do not allow animal testing and research and 4) ensuring a compact and portable solution was created to extend the ability to use the simulator through the sales force training and global product launch phases.

Simulator design and function was collaboratively developed through a partnership with Industrial Design, Research & Development and Preclinical Affairs. Critical steps of key laparoscopic surgical procedures were analyzed and transformed into a mechanical activity board, which was enclosed in a custom case representing an insufflated adult abdomen. The exterior was a selectable-thickness (up to 8 cm) material that was representative of the anterior body wall, subcutaneous fat and fascia. The simulator could be entered and used with any commercially available laparoscopic access devices and visualization systems.

Resource estimates and model calculations show that more than 380 large animals were not used because the SSL simulator was developed and validated. For the same amount of data generated through the use of the SSL simulator, the business impact in terms of cost savings by not using live animals is estimated conservatively in excess of $ 1.1 million dollars for this single development project. Validation of simulator design and function was performed with laparoscopic surgeons who were asked to evaluate the SSL simulator on a scale of 1-5, with 5 being "excellent".

The SSL Abdominal Access Simulator delivered on all objectives – exceptional business results were and are being obtained while striving towards higher standards in the ethical use of animals in biomedical research.

V-6-399

New use of an old animal model (wound healing in the ears of rabbits) to significantly reduce the total number of animals utilized in medical device product development

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We are developing products to address obstructive sleep apnea and for facial reconstruction. At issue is how to evaluate and screen prototype products while minimizing animal usage. Our first product in this area gained regulatory approval, but clearly demonstrated the need for a better animal model (single implant in the nasal septum of weanling rabbits). No validated *in vitro* assay or computer-model could address the questions we must answer. The intended applications dictate the need for *in vivo* product evaluation with the product located adjacent to cartilage. A single rabbit had four products implanted along the cartilage of each ear. This simple model was more than adequate for product evaluation. More importantly, we discovered we could easily evaluate the product in real-time for the first time. We "watched" dye diffuse from the implanted products, "watched" the products absorb, and could macroscopically evaluate the tissue response to the implanted products. We decreased our animal usage by 8-fold for our sleep apnea and facial reconstruction product development and have used the model elsewhere (e.g. product performance evaluation) to further reduce our animal use. The potential for this model to further reduce our overall animal use is significant. For example, *in vitro* degradation studies for many polymers are not acceptable to regulatory agencies; therefore, we generate such information using multiple study intervals. With this model, we can generate the absorption (and other) information in real-time with 1 to 2 animals. With time, this model could replace some definitive large animal studies and, hopefully, replace some of the established animal models required by regulatory agencies.

V-6-445

Mechanical characterizations of traumatic brain injury tests on mice using computer models

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Currently, *in vivo* traumatic brain injury (TBI) animal experiments are commonly used to provide insight into brain injury mechanisms as well as to test candidate therapeutic interventions. For example, controlled cortical impact (CCI) is largely used to induce brain contusions. However, different laboratories use different CCI parameters, making comparison of experimental findings very difficult. Furthermore, many animals are sacrificed during preliminary trial-and-error stages in an attempt to create the desired brain contusion severity. In this study, a computer mouse brain model was developed using advanced multi-block techniques. The mouse intracranial responses under various CCI scenarios were calculated computationally and compared favorably with published experimental results. Such computer-predicted intracranial mechanics could minimize the use of mice from unnecessary trials, and also serve as a general platform for comparison and analysis of published results from different laboratories. The developed blocking system can be further used to generate high-quality computer brain models for different transgenic mice with varying intracranial geometries. Additionally, computational analysis could be conducted before other types of experiment, such as fluid percussion and weight drop, to provide clear descriptions of regional brain injury intensity. Ultimately, incorporating current computer models (with around 100 micron spatial resolution) with molecular/cellular level modeling will allow TBI experiments to be performed entirely on computers. In the meantime, it is important to increase awareness and acceptance of such technologies so that fewer animals are sacrificed in TBI experiments.

V-6-491

Deployment of the Vitrocell *system for in vitro* toxicity assessment of aerosols and vehicular emissions at an air-liquid interface

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The Vitrocell system is an exposure device that mimics the *in vivo* conditions of the lung by allowing *in vitro* exposure of cells at an air-liquid interface. *In vitro* toxicity assessment of gaseous substances is problematic due to the fact that cultured cells need to be suspended in liquid medium or adhered to a surface that is completely covered by liquid medium. Therefore, the test article (e.g., aerosol) must be bubbled through or suspended in the medium, with neither option providing a realistic exposure scenario. Thus, the use of animal studies has been a preferred practice. The Vitrocell offers a practical alternative, in which cultured cells are grown on porous membranes and exposed, in real-time, under highly controlled experimental conditions. This has the potential for a reduction in animal-based research.

Human adenocarcinoma epithelial cell line A549 was used in all exposures. Cells were exposed to NO_2 (5 ppm and 20 ppm) and dilute diesel exhaust (1:8), as well as synthetic air controls, at a previously established flow rate of 8.3 ml/min for one hour. Cell viability was determined by examining cleavage of the tetrazolium salt WST-1 (metabolic activity) and relative ATP activity (ATPlite luciferase). All results were compared to incubator controls. Cell viability was observed to decrease with increasing concentrations of NO_2. Cells exposed to 20 ppm showed 56.1% ±9.2% less metabolic activity and cells exposed to 5 ppm exhibited a 52.7% ±7.6% reduction in metabolic activity. ATP activity was observed to be 57.0% ±5.1% less in 5 ppm NO_2 exposed cells, and 65.4% ±12.7% less in 20 ppm NO_2 exposed cells. Cells exposed to synthetic clean air were slightly less viable than incubator control (24.7% ±14.3%). Cells exposed to dilute diesel exhaust showed a significant reduction of 42.6% ±3.4% in ATP activity compared to incubator controls.

These results confirm that the Vitrocell exposure device with A549 cells is suitable for toxicity assessment of gaseous substances. Future work will include cytotoxic and genotoxic assessment of further engine exhaust exposures, representing a host of engine design, fuel formulation and after-treatment conditions. The use of primary human airway epithelial cells will be employed to assess inter-individual responses to vehicular emissions.

V-6-554

Advancing technology and the 3Rs: use of cross-over study design for pharmacological assessment in rats

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Innovation leading to development, qualification, and implementation of new technologies produces Refinements in data quality, Reductions in animal use, and better de-risking of pharmaceutical efficacy & safety. We developed a chronic cannulated rat model wherein cross-over study design is used to evaluate 2 or more compounds at weekly intervals in one cohort. Han Wistar rats (n=16) were obtained from the vendor, surgically implanted with jugular and femoral venous cannulas, for dosing and blood collection, respectively. Cannula patency was maintained through technique and use of a heparin-loc solution when not in use for several weeks. A swivel-cannula system permitted drug dosing and blood withdrawal while maintained in home caging. In the first study rats are randomized to test article 1 and control groups; a week later the groups are crossed-over to receive control and test article 2, respectively.

Dopaminergics (D) can produce unwanted endocrine side-effects in rats by affecting central prolactin release. Test articles 1) exogenous dopamine (dopamine agonist, bromocriptine), 2) endogenous dopamine (dopamine transporter, DAT inhibitors, mazindol and GBR12909) and 3) endogenous norepinephrine (NE, NE transport inhibitor, nisoxetine), and 4) peripheral-acting exogenous dopamine (dopamine agonist, carmoxirole) were evaluated (iv) for inhibition of estradiol-stimulated (E_2) prolactin release using double cannulated ovariectomized rats, incorporating the cross-over design. Rats were dosed with E_2 (0.6-20 μg/rat, iv) and test article, and blood collected every 30 min between 1.5 to 5 h, and evaluated for prolactin and luteinizing hormone (LH). Bromocriptine (1 mg/kg) inhibited the E_2-induced prolactin release, as did the DAT inhibitors mazindol (5 mg/kg) and GBR12909 (3 mg/kg). Carmoxirole (15 mg/kg) induced a rapid independent release of prolactin, and inhibited E_2-induced release. E_2-induced prolactin release was not inhibited by the central acting NE uptake inhibitor nisoxetine (10 mg/kg). Order of control of TA administration did not impact the findings. This study qualified the double cannulated ovariectomized rat model and cross-over design for evaluating the impact of central and peripheral acting compounds, suggests that the mechanism may be activated both inside and outside the blood-brain barrier with D but not NE, and demonstrates both Refinement and Reduction as a result of employing technology permitting use of the cross-over study design.

V-6-615

Comparative study of amyloidogenic potential of $AgNO_3$ and Freund's Adjuvant with that of vitamin free casein on spatio-temporal pattern of experimental amyloidosis in mice by polarized microscope

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Reactive amyloidosis is a condition that complicates a long list of chronic inflammation, chronic infectious, malignant, and hereditary disorders. In the present study, the potential effects of two amyloidogenic substances, $AgNO_3$ and Freund's Adjuvant (FA), with that of vitamin free casein on the spatio-temporal pattern of experimental amyloidosis in mice were compared. A total of 40 male Swiss mice, obtained from Pasteur Institute Tehran, were weighed and randomly divided into 4 groups: 2 treatment groups, 1 control (vitamin free casein) and 1 negative control (normal saline). At the end of the 3rd, 5th and 7th weeks of the experiment, 3 mice were randomly selected and euthanized. A spleen sample from each animal was obtained and preserved in 10% neutral buffered formalin. The samples were then processed through different stages of dehydration, clearing and impregnation, and embedded in paraffin blocks. Sections of 5 μm thickness were then cut and stained by alkaline Congo red techniques. As an indicator of developing amyloidosis, a green birefringence under the polarized microscope was considered to be a positive criterion for the presence of amyloid. For optical evaluation of amyloidogenic potential, amyloidoic areas were observed in 10 randomly selected high power fields. A light microscope equipped with polarized light optics was used to determine the birefringence intensity of the amyloid deposition in Congo red stained sections. This system was assigned to represent changes in the quantitative appearance and intensity of various microscope fields. Spleen weights and the data obtained from microscope quantitative analysis showed no significant differences between groups A (vitamin free casein) and B ($AgNO_3$) A and C (FA), and B and C. However, significant differences were observed between groups A and D, B and D, and C and D, respectively. It is concluded that two compounds, $AgNO_3$ and Freund's Adjuvant, have the same potential, as does vitamin free casein, to induce the spatio-temporal pattern of experimental amyloidosis in mice.