MRF 2013

18-19 November 2013
In Rome

Programme and abstracts
Participant list
Minipig in non-clinical testing (since 1993)

**Routes of administration**
- Oral (by gavage or capsules)
- Intravenous (bolus or infusion)
- Subcutaneous
- Intramuscular
- Dermal
- Ocular
- Intra-articular

**Local tolerance studies**

**Wound healing studies**

**Pharmacokinetic studies**

**Systemic toxicity**

**Neonatal and juvenile toxicity and kinetics**
Dear MRF attendee

Welcome to the seventh annual meeting of the Minipig Research Forum in Europe. We are very pleased to welcoming you to this meeting and to Rome. The Hotel Boscolo Exedra will create nice surroundings for this meeting where we are more participants than ever.

The scientific programme is based on large molecules/biopharmaceuticals, CNS and GI-Tract. A group of excellent speakers have prepared informative presentations and we hope that you will enjoy them. The programme includes lunch-time workshops and we hope that you will all participate so that we can have some good discussions.

Once again we welcome you to this meeting and we look forward to spending two days accompanied by so many minipig users.

- The MRF Steering Committee

In this booklet you will find the programme, abstracts for the presentations and a participant list.

The sponsors of this year’s MRF meeting are:

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<td>08:30-08:45</td>
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<td>Gaps, Challenges and Opportunities for the minipig in nonclinical</td>
<td>Flavio Crameri, F.Hoffmann-La Roche, CH</td>
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<td>09:25-10:05</td>
<td>'Why use minipigs in development of biological medicinal</td>
<td>James McBlane, MHRA, UK</td>
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<td>products? - perspectives of a regulator &amp; some case studies'</td>
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<td>10:05-10:35</td>
<td>Minipig genome data aid species selection in pharmaceutical</td>
<td>Peter Woollard, GlaxoSmithKline, UK</td>
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<td>Case study: the pig as a preclinical model for heart regenerative</td>
<td>Rocco De Siena, Medestea, IT</td>
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<td>11:20-11:50</td>
<td>Case study: Gene therapy and DNA vaccine testing in the minipig</td>
<td>Zuhal Dincer, Novartis, CH</td>
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<td>Immunogenicity of biologics in Göttingen Minipigs</td>
<td>Geertje van Mierlo, TNO Triskelion, NL</td>
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<td>12:15-14:15</td>
<td>Buffet Lunch &amp; discussion groups/workshops</td>
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<td>Workshop participants will grab dinner and go to workshop rooms.</td>
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<td>introduction (max 10min) to the topic and their own experience</td>
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<td>followed by a round-table discussion. A rapporteur will prepare</td>
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<td><strong>Workshop 1:</strong> Minipigs in safety and efficacy testing of large</td>
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<td><strong>Workshop 3:</strong> Use of minipigs in gastrointestinal research</td>
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<td>14:15-14:30</td>
<td>Case study: Potential of excipients to cause pseudoallergic</td>
<td>Matthias Festag, F.Hoffmann-La Roche, CH</td>
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<td>reactions in the minipig</td>
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<td>14:30-15:10</td>
<td>Complement-mediated reactions (pseudo-allergy) to drug carrier</td>
<td>Janos Szebeni, Semmelweis University, HU</td>
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<td>nanosystems and other medicines - a pig model</td>
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<td>15:10-15:25</td>
<td>Poster Presentations</td>
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<td>16:00-16:25</td>
<td>In vivo studies and modeling to elucidate physiological</td>
<td>Claudia Suenderhauf, F.Hoffmann-La Roche, CH</td>
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<td>determinants of absorption</td>
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<td>16:25-17:00</td>
<td>Nausea and emetic reflex in the minipig: legend and reality</td>
<td>Stéphane Milano, WIL Research, F</td>
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<td>Open Q&amp;A</td>
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<td>08:45-09:25</td>
<td>Minipig and pig behaviour</td>
<td>Elise Gieling</td>
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<td>09:25-09:40</td>
<td>The use of positive reinforcement in Göttingen Minipigs</td>
<td>Peter Glerup, CiToxLAB Scantox, DK</td>
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<td>09:45-10:00</td>
<td>Functional observational battery testing in minipigs, Part I</td>
<td>Ken Kearney, WIL Research, USA</td>
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<td>Functional observational battery testing in minipigs, Part II</td>
<td>Ken Kearney, WIL Research, USA</td>
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<td>Carotid electrical stimulation and cerebral perfusion monitoring by invasive Laser-Doppler probe in the swine model</td>
<td>Yaron Assaf, Samson NeuroSciences, IL</td>
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<td>11:20-11:35</td>
<td>Use of minipigs in anti-cancer therapies – Evaluation of the toxicity of cyclophosphamide, methotrexate and doxorubicin</td>
<td>Andrea Grassetti, RTC, IT</td>
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<td>Combined PET/microdialysis studies in minipig for PET tracer validation</td>
<td>Annie Landau, University of Aarhus, DK</td>
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<td>12:05-12:35</td>
<td>MPTP and other minipig models of Parkinson’s Disease</td>
<td>Aage Kristian Olsen Alstrup, Uni of Aarhus, DK</td>
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<td>Evaluation/Feedback</td>
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Abstract 1

Speaker: Flavio Crameri, F. Hoffmann-La Roche

Gaps, Challenges and Opportunities for the Minipig in Nonclinical Safety Testing of Biopharmaceuticals

Flavio Crameri and Sven Kronenberg
F. Hoffmann-La Roche Ltd., Non-Clinical Safety, CH-4070 Basel, Switzerland

The ICH S6 (R1) guideline states that safety evaluation programs should include the use of relevant species in which the test material is pharmacologically active due to the expression of the receptor or an epitope (in the case of monoclonal antibodies, mAbs). For this reason, the non-human primate (NHP) and especially the cynomolgus monkey is still the species of choice for nonclinical safety testing of mAbs, and biopharmaceuticals in general. The use of NHPs for safety testing, however, raises ethical concerns and meets increasing public resistance. There is, therefore a need to identify suitable alternative non-rodent species. In safety assessment of small molecule based pharmaceuticals, the minipig is gaining acceptance as potential alternative non-rodent species, but for biopharmaceuticals, in particular mAbs; it is still not the case. This is likely due to the lack of experience with this species and the perceived associated scientific and regulatory risk in using a “new” species for this purpose. One unavoidable draw-back in using the minipig for testing of biopharmaceuticals is the relatively high body weight and requirement for considerably higher amounts of costly test material when compared to the NHP. This can represent a particular challenge during earlier phases of development before up scaling and advanced manufacturing processes are in place.

Apart from this intrinsic challenge, progress has been made to increase our knowledge of key aspects relevant for the use of minipigs in nonclinical safety testing with mAbs. The minipig genome has been sequenced and this allows for initial assessment of potential cross-reactivity of a given human mAb with the porcine target. Relevant cross-reactivity is a prerequisite for selection of any safety species. The definitive species selection, however, is based on binding (affinity) assays and this often requires the cloning and expression of the recombinant porcine target protein, if not expressed on blood cells. In addition, appropriate in vitro assays using porcine cells need to be established to assess functionality (potency) of the mAb in the minipig.

Using microarray profiling, it has been shown that tissue gene expression profiles in minipig and humans are highly similar and this further supports the translational value of the minipig model. Pathological and histopathological background data are available and hematology, coagulation as well as clinical chemistry parameters are well established in the minipig and can be assessed using automated standard methods.

The porcine immune system has been well studied, however, little effort has been dedicated to the evaluation of its relevance and translatability to humans. Some differences (e.g. lymph node structure and IgG subclasses) to human are known, but do not appear to have biological impact with possible exception of higher \( \gamma \) T-cell counts as well as the unclear role and effector function of FcR. There are no data regarding potential cytokine release (whole blood assay) in minipigs upon treatment with human mAbs. Many marker tools for immunophenotyping in the minipig are available; however, there are no distinct markers for pig NK cells. Immunogenicity testing with Kineret\textsuperscript{a}, a recombinant human IL-1\textsubscript{R} antagonist, in the minipig indicates a similar response to human proteins as in NHP.

Dedicated safety pharmacology studies are usually not done for biopharmaceuticals and observation of vital signs is included in the repeat-dose studies. The minipig is a well-recognized and valuable species in cardiovascular safety pharmacology testing of small molecules and there is no reason to assume that for biopharmaceuticals this would not be the case. Non-invasive jacketed telemetry methods are available. However, assessment of other vital signs such as respiratory function as well as functional CNS observations in the minipig repeat dose studies would need to be established and validated.

Testing for reproductive and developmental toxicity is also part of the safety assessment of biopharmaceuticals.
Potential effects on fertility are usually evaluated in the repeat-dose NHP studies by assessing female estrus cycle and by macroscopic as well as histopathological evaluation of female and male reproductive organs, and this can be implemented also in the minipig. Assessment of pre-/postnatal developmental toxicity in the minipig appears to represent a potential challenge due to the epitheliochorial type placenta that prevents placental transfer of endogenous antibodies in contrast to humans and NHP. Furthermore, FcRn expression has not been detected in the minipig placenta and there are neither data on placental transfer of therapeutic human IgG in the minipig, nor on other non-placental transfer mechanisms as described for primates.

Based on published data, the minipig appears to be a translatable model of IV pharmacokinetics of mAbs with similar properties as NHP. For SC absorption of mAbs, the minipig appears to be a superior model as compared to e.g. NHP or rodents.

In order for the minipig to gain acceptance as alternative non-rat species for safety testing of mAbs, the identified gaps (e.g., characterization of effector function, placental transfer of human IgGs) should be addressed and appropriate validation studies should be performed.
Abstract 2
Speaker: James McBlane, MHRA

Why use minipigs in development of biological medicinal products? - perspectives of a regulator & some case studies

In drug development, there is an expectation that preclinical safety testing should be done in a rodent and a non-rodent. Often this has meant use of mice/rats and dogs for safety pharmacology and general toxicity testing and rabbits for reproductive toxicity testing. However, for many biological products, lack of the primary pharmacodynamic effects in rodents has led to reliance on use of a single species, often cynomolgus monkeys. This talk shall consider use of (mini)pigs with a particular emphasis on biological products and will include case studies where swine were successfully used in drug development.

Abstract 3
Speaker: Peter Woollard, GlaxoSmithKline

Minipig genome data aid species selection in pharmaceutical discovery and development

Peter Woollard, Computational Biology, GlaxoSmithKline

Drug attrition continues to be a challenge in pharmaceutical discovery and development. Safety signals are a major cause of early attrition. Knowing the differences in gene homologues between model organisms and human helps understanding of the clinical context of safety signals. Appropriate animal model species selection is also essential for later regulatory toxicology studies. We recently sequenced the genomes of the Sus scrofa Göttingen minipig and the Canis familiaris beagle. This talk will focus on properties of the minipig genome and what questions the data can help us answer. The minipig has orthologues of only 19 of the 23 human ADME associated cytochrome P450s. The minipig shows different gene duplications to human in the Phase II enzymes UTG1 and UTG2 (UDP glucuronosyltransferase) families. Comparisons of the homologues of human drug target genes, showed multiple differences to the minipig. Interpretation of the minipig genome would improve with more omics and other data.

Abstract 4
Speaker: Isabella Andreini, RTC
On behalf of Rocco De Siena, Medestea

Case study: The pig as a preclinical model for heart regenerative therapy
Abstract 5

Speaker: Zuhal Dincer, Novartis

Safety assessment of Particle-Mediated Epidermal Delivery (PMED) device for DNA vaccines in pig and mouse

Zuhal Dincer, Novartis Institute of BioMedical Research, PreClinical Safety, Novartis Pharma AG, Basel, Switzerland

DNA vaccination is direct injection of a gene(s) coding for a specific antigenic protein(s) into a living host, resulting in the direct production of such antigen(s) within the vaccine recipient; this triggers an appropriate immune response against the delivered antigen. Such vaccination can provide long-term humoral and cellular immunity against various targets e.g. infectious agents, tumours or autoimmune diseases. Developing such methods of immunisation has advantages in terms of simplicity, adaptability and cost.

Particle-Mediated Epidermal Delivery (PMED) is a technology created for delivering DNA vaccines to animals and human. The technology relies on the delivery of DNA coated gold particles into the epidermis, where the DNA can be processed to antigen by resident cells (i.e. keratinocytes). Langerhans cells (the antigen presenting cells in the skin) take the antigen, delivers to lymph nodes through lymphatics and presents it to MHC class II molecules to stimulate CD4+ and CD8+ T cells. PMED devices function with gas pressure-ejected cassettes loaded with different payloads of gold, resulting in varying immune responses.

The aim in the preclinical safety assessment of PMED Device for DNA vaccines is to evaluate local and systemic tolerability of appropriate or inappropriate distribution of delivered DNA, and expressed gene product for potential adverse effects (inflammatory responses, hypersensitivity or autoimmunity) associated with single or repeated applications of antigen/adjuvant by PMED.

This presentation details the experimental variables in pathological assessment including relevant animal models for local tolerability (pig) and immunogenicity (mouse), the influence of PMED pressure and gold load on the degree of skin changes and their extent and nature at different time points including recovery/reversibility and the impact of single or multiple applications. The experimental variables concern also antigen/adjuvant combinations, age of animals, and presence of local or systemic gold particles.

References:

Pilling et al (2002). The assessment of local tolerance, acute toxicity, and DNA biodistribution following Particle-Mediated Delivery of a DNA vaccine to minipigs. Tox Path, 30 (3): 298-305.
Abstract 6

Speaker: Geertje van Mierlo, TNO Triskelion

Immunogenicity of biologics in Göttingen Minipigs


Immunogenicity is a major issue of concern for biologics used for treatment of several human diseases. Non-human primates (NHP) are often considered to be the most relevant test species for the prediction of immunogenicity in humans. Because the immune system of the minipig is functionally similar to that of other mammalian species, we investigated their potential of predicting immunogenicity, using three biologics which are already used in patients.

We performed two studies in which the immunogenicity in minipigs was measured and compared with available data on immunogenicity in NHP and humans. In the first study, we evaluated the immunogenicity of the recombinant human IL-1 receptor antagonist anakinra (Kineret) by treating minipigs (n=3 per sex) with 0.5 mg/kg or 5.0 mg/kg anakinra for 29 consecutive days by subcutaneous (SC) injections. In the second study, we evaluated two TNF inhibitors i.e. adalimumab (Humira) and infliximab (Remicade), the latter being not biologically active in the minipig. The minipigs (n=4) were injected 5 times SC with either adalimumab (dose levels of 0.1; 1.0 or 5.0 mg/kg) or infliximab (5 mg/kg) with 2-week intervals between the injections. On several time points, blood samples for anti-drug antibody (ADA) determinations and pharmacokinetic (PK) analysis were collected.

No signs of toxicity of any of the treatments were observed. The T1/2 of anakinrwas comparable after the first and the last injection. No remarkable sex-differences were observed for most PK parameters. All anakinra-treated minipigs showed induction of ADA from day 14 onwards, which were not considered to be neutralizing. In NHP also almost all animals develop ADA, with comparable Tmax and T1/2 to that found in minipigs.

Adalimumab elicited an ADA response in 11 out of 12 minipigs, which is comparable to the findings in NHP. As in humans, induction of ADA in minipigs was correlated with decreased plasma levels of adalimumab. However, the half-life of (functional) adalimumab in minipigs was much lower than that observed in NHP and humans.

Infliximab did not induce a clinically relevant ADA response, as the clearance was comparable after first and last dosing. Although further research is required, we ascribe this surprising lack of immunogenicity of infliximab in minipigs to the lack of biological activity of this compound in this species.

In conclusion: Immunogenicity results obtained in the minipig are overall comparable with those obtained in NHP, showing the potency of the minipigs for safety assessment for human biopharmaceuticals. However, the choice of the test species should be made well-based and, as for all toxicity studies, the biological activity of the test substance should be taken into consideration.
Abstract 7

Speaker: Matthias Festag, F. Hoffmann-La Roche

Potential of selected excipients to cause pseudoallergic reactions in the minipig

Authors: M Festag, F Christen, B Jacobsen and C Ploix

It is well established that polysorbates, surfactants widely used in drug formulations for non-clinical safety studies, cause pseudoallergic reactions when administered intravenously (i.v.) to dogs. These reactions may include the acute release of histamine, erythema, oedema, and decrease in arterial blood pressure and may result ultimately in the death of the animal. While immune mediated responses require multiple contacts with the allergen, the pseudoallergic reactions occur after already a first, single exposure. The pathogenesis of these reactions is not fully revealed but may be related to the activation of Complement. Non-IgE mediated hypersensitivity reactions as e.g. hypo- and hypertension, respiratory and cutaneous effects were also seen after i.v. administration of pharmaceuticals containing Poloxamer 188 as an excipient. Yet, there is virtually no information publically available whether such non-IgE mediated, pseudoallergic reactions due to polysorbates also occur in minipigs.

In an investigative study three male and three female minipigs were administered a slow bolus of 0.1% polysorbate 20, 0.1% polysorbate 80 or 0.1% Poloxamer 188 intravenously to determine potential pseudoallergic reactions. At different time points blood was sampled for investigations of histamine, thromboxane B2 and IgE levels as well as determination of Complement activation. In addition, multiple clinical chemistry, coagulation and haematology parameters were determined. During the in life period clinical observations, body temperature, peripheral oxygen saturation and heart rate were monitored. Findings from this study will be discussed.
Abstract 8

Speaker: Janos Szebeni, Semmelweis University

Safety assessment of nanomedicines in pigs:
A need and potential new use of minipigs in this role

Janos Szebeni, Nanomedicine Research and Education Center,
Semmelweis University & SeroScience Ltd, Budapest, Hungary

Nanomedicines, such as liposomal drugs, and biopharmaceuticals are increasingly used in the treatment of cancer and other severe, chronic, incurable diseases. One problem with these novel, state-of-the art drugs is that their i.v. infusion triggers in a relatively high percentage of patients a hypersensitivity syndrome that cannot be predicted by conventional laboratory tests and which can be fatal is a small, but not negligible percentage of individuals. Because the phenomenon has been shown to be a consequence of immune (complement system, C) activation, it has been referred as C activation-related pseudoallergy (CARPA). CARPA thus represent a safety problem in the rise of modern pharmacotherapy, whose testing has been recently included among the recommended assays for clinical application of second generation (generic) liposomes by the EMA. Importantly from the focus of the present workshop, the most sensitive animal species in which CARPA has been tested and studied to date is the pig, which develops severe cardiopulmonary distress following i.v. administration of a small amount of liposomes and other nanoparticles that are known to be reactogenic in humans. This anaphylactoid reaction in pigs closely mimics the human reaction both in terms of symptoms and trigger dose, and it is highly reproducible and quantifiable. Nevertheless, despite the high sensitivity of pigs for CARPA, they do require a relatively high amount of test drug for regulatory safety tests, particularly if dose escalation is needed. For this reason the potential use of minipigs for the testing of CARPA is recommended for exploration. The lecture will address many details of porcine CARPA, including the development of tachyphylaxis and likely role of pulmonary intravascular macrophages (PIM cells) in its pathomechanism.
Your partner for non-clinical drug R&D in Minipigs

Aurigon has extensive expertise in the use of Minipig models

- **Complex pharmacology studies**  
  e.g. PK/PD studies in diabetic Minipigs

- **Toxicological studies using standard application routes**  
  e.g. po, iv, dermal (intact & abraded skin) and infusion

- **Innovative administration routes such as**  
  - transbuccal  
  - in situ in the urinary bladder sphincter under surgery

- **Testing of new drug delivery devices**  
  - nanoparticle-coated stripes  
  - needle-free vaccination devices

- **Radiolabeled DMPK**

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Abstract 9

Speaker: Claudia Suenderhaft, F. Hoffmann-La Roche

In vivo studies and modeling to elucidate physiological determinants of absorption

Claudia Suenderhaft, MD/PhD, M&S Scientist, NCS PL and Modeling & Simulation, F. Hoffmann-La Roche Ltd., Pharmaceuticals Division, CH-4070 Basel, Switzerland

Onset and rate of gastric emptying are important determinants of drug absorption after oral dosing. Therefore, robust estimates of these parameters are needed in physiologically based absorption models to predict reliably plasma concentration time profiles. For human and some other laboratory animals, reasonable parameterization of gastric emptying has been established. However gastric emptying is less well characterized in minipigs, a large animal model rapidly gaining importance in pharmaceutical research.

A pharmacokinetic crossover study using different dosage forms of paracetamol in Göttingen minipigs was conducted as well as a deconvolution analysis was performed to determine the absorption kinetics. Estimated lag times and first order gastric emptying parameters were incorporated in a previously published PBPK model of the minipig and simulations verified. Postmortem assessments of minipig stomachs were made after an overnight fast. In conclusion, fasted gastric transit times in minipig were longer than those observed in humans. This might be caused prolonged food retention in minipigs. Improved starving protocols may be necessary to obtain a fasted state in the species.

Abstract 10

Speaker: Stéphane Milano, WIL Research

Nausea and emetic reflex in the minipig: legend and reality

S. Milano, C.S.O. Europe
WIL Research Europe, Lyon, France

Food intake is a risky behaviour leading to the exposure of the body to possible food-poisoning, including viral and bacterial infection, allergies, food intolerance or chemicals. Smell and taste, the gatekeepers of the alimentary tract, are not always effective in detecting the quality of the ingesta, and nausea and vomiting, as additional safeguards for dealing with poisoning, play a large role in subsequent levels of defence. Emesis, along with diarrhoea, helps to preserve the gastrointestinal tract from dangerous ingested toxins. The vomiting response is present in many species, appearing in most vertebrates. It is not uncommon to hear that minipigs are much less prone to emesis than dogs. Drawing upon the knowledge of the anatomy and the physiology of the emetic reflex and by means of concrete examples, this lecture will revisit this assertion.
Abstract 11
Speaker: Elise Gieling, CAH Vilentum Applied University

The pig as a model animal for studying cognition and neurobehavioral disorders

Elise T. Gieling
CAH Vilentum Applied University, Almere, the Netherlands

In experimental animal research, a short phylogenetic distance, i.e., high resemblance between the model species and the species to be modelled is expected to increase the relevance and generalizability of results obtained in the model species. The (mini)pig shows multiple advantageous characteristics that have led to an increase in the use of this species in studies modelling human medical issues, including neurobehavioral (dys)functions. For example, pigs possess a well-developed, large brain. Their cerebral cortex, unlike that of mice or rats, has cerebral convolutions (gyri and sulci) similar to the human neocortex. It is expected that appropriately chosen pig models will yield results of high translational value. However, this claim still needs to be substantiated by research, and although growing, the area of pig research is still in its infancy. This presentation provides an overview of the pig as a model species for studying cognitive dysfunctions and neurobehavioral disorders and their treatment. To aid the selection of appropriate tasks, several basic criteria for pig cognition tests will be discussed. These criteria together with knowledge about pig-specific sensorimotor abilities and behaviour is necessary to evaluate the merits, drawbacks, and limitations of the different types of tests used to date. The pros and cons of various cognitive/neurobehavioral tests will be discussed briefly, as an aid to researchers considering the use of pigs as model animal species in biomedical research. The use of appropriate tasks will facilitate the collection of reliable and valid data on pig cognition.

One of the first cognitive tests in pigs; the “multiple choice method” (Yerkes et al., 1915)
Minipigs in (bio)pharmaceutical safety testing

The minipig is considered a useful non-rodent species for testing the safety of (bio)pharmaceuticals. Human parallels in many features of its anatomy, physiology, biochemistry and behavioural patterns along with its suitability for different types of study make the minipig a good model for pre-clinical safety testing. Given this, the minipig can replace dogs and non-human primates in several applications in pre-clinical safety testing, such as general toxicity (including immunotoxicity / immunogenicity), reproductive toxicity, juvenile developmental and safety pharmacology studies. The close similarity to humans (especially for the cardiovascular system, the skin, the digestive tract, the urogenital system and drug metabolism) means that studies using minipigs will result in better safety prediction for humans when the minipig is the most appropriate species for evaluating a particular product.

TNO Triskelion has extensive experience with different types of standard (immune) toxicity studies in minipigs (e.g. Ehlebaedt Gottingen minipigs) according to international guidelines and in compliance with GLP.

Standard Toxicity Studies (STS) in minipigs
- Different exposure routes: oral, i.m., s.c., i.d., dermal
- Adverse effects can be monitored by all routine endpoints in toxicity studies according international guidelines.
- Pharmacokinetics (PK), pharmacodynamics (PD) and metabolism can be included.

Immunotoxicity testing in minipigs\(^1,2\)
- Screening for potential immunotoxicity by studying Immuno Pathology endpoints.
- Functional assays to assess potential effects on acquired immunity, e.g. TDAR, DTH reaction, and ex vivo lymphocyte proliferation, and non-specific immunity like Natural Killer (NK) cell activity.

Immunogenicity testing of biopharmaceuticals in minipigs\(^3,4\)
- Analysing the formation of anti-drug antibodies (ADAs).
- Determining the neutralising capacity of the anti-drug antibodies (bridging ELISA, cell-based assay).
- Analysing the pharmacokinetics (PK) of the biopharmaceutical.

Juvenile (immune)development in minipigs\(^5,6\)
TNO Triskelion offers experience with juvenile (immune) developmental studies in minipigs from birth to six months of age focused on general development parameters, (immune) pathology parameters and functional signs of (immune) development over time.

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Abstract 12

Speaker: Peter Glerup, CiToxLAB Scantox

The use of positive reinforcement in Göttingen minipigs

Peter Glerup, DVM, MSc
CiToxLAB Scantox, Denmark

Various experimental procedures, such as dosing, weighing, physical examinations etc are usual parameters included in non-clinical safety and efficacy studies using laboratory animals. The majority of these procedures are un-natural and is often associated with discomfort for the animals. Therefore, in most cases the animals must be restrained during the procedures in order to complete them successfully. However, this causes stress to the animals, which may have potential impacts on the study results. In addition, technical staff may have to work in ergonomic un-desired positions during the restrain of large animal species, such as the Göttingen minipig.

At our laboratory we have therefore aimed to find a method to facilitate various procedures, eliminating stress experienced by the animals and at the same time optimising the working conditions for our technical staff.

Positive reinforcement is a process of training an animal using a marker for a behaviour that will earn positive reinforcement. The method has been used for many years training pet and zoo animals, but has only been used to a very limited extent in laboratory animal species.

We therefore chose to implement this method in a repeat dose toxicity study with intranasal dosing 10 times daily for one week in Göttingen minipigs. A total of three animals were included in the study and each animal was trained for approximately 30 minutes per day during 14 days. Training was performed by a dedicated team of trainers, using clickers as a marker for the desired behaviour and GLP-certified dietary pellets as rewards. The principle of “shaping” was used in the training sessions, gradually transforming specific behaviours into the desired behaviour. First, the sound of the clicker was associated with the reward, followed by the acceptance of approximation of the trainer to the animal. This was followed by further successive training steps until the complete desired behaviour was reached. The completely trained animal voluntarily came forward, stepped onto a box (to elevate the animal), accepted approximation of the intranasal device and the subsequent dosing in one nostril in a “freeze” position.

All animals learned the complete behaviour prior to study start, although differences in the ease of learning were noticed between the animals. The study was completed successfully using positive reinforcement throughout the study, increasing animal welfare and working conditions significantly. We consider positive reinforcement a great potential in relation to other experimental procedures in the Göttingen minipig as well as in other laboratory animal species.
Abstract 13
Speaker: Ken Kearney, WIL Research

Functional observational battery testing in minipigs

All new chemical entities must be evaluated for potentially undesirable effects on the cardiovascular (CV), respiratory, and central nervous systems (CNS) as directed by the ICHS7A guidelines. While the ICHS7B guidelines mandate that assessment of ventricular repolarization must be conducted in a large animal model, there is no such guidance set forth for species selection for evaluation of CNS and/or respiratory evaluations. These assessments are commonly conducted in the rodent; however this may not always be the most applicable species. It is the opinion that a more scientifically robust risk assessment can be established when comparing CV, respiratory, and CNS evaluations conducted in the same animal model. The use of a single species mitigates variability in response time and/or duration of pharmacological action, thus allowing for more direct comparisons between CNS, CV, and respiratory assessments.

The Göttingen minipig presents advantages as an alternative non-rodent model for toxicology and safety pharmacology studies due to its numerous similarities to humans in the anatomy of organs (such as the skin, heart or brain), as well as the function of all major physiological systems (Bode, 2010). In addition to similarities between the minipig and human cardiovascular systems, a large database of knowledge exists on the swine brain. Such similarities allow for extensive use as a model in neuroscience. Therefore, the minipig is increasingly accepted for regulatory toxicology requiring neurobehavioral studies. In general, the minipig is a pharmacological model that is more similar in function to humans for both cardiovascular and neurological testing and is recommended in cases where either species-specific toxicity is encountered in non-rodent models, emesis is prohibitive (especially in dogs), or the metabolical differences between species are significant.

In the present study, we evaluated the feasibility of the minipig model for neurological and cardiovascular evaluation in support of preclinical studies. In particular, we developed a Functional Observational Battery (FOB) consisting of neurological evaluations and behavioral assessments of normal physiological functions. Furthermore, we evaluated the parameters included in the FOB; validated the procedures; and demonstrated the inter-observer reliability following the administration of neuro modulating chemicals (d-amphetamine and clonidine).
Abstract 14
Speaker: Yaron Assaf, Samson NeuroSciences

Carotid electrical stimulation and cerebral perfusion monitoring by invasive Laser-Doppler probe in the swine model.

Yaron Assaf  MD, Lori Fein  PhD - Samson NeuroSciences, Israel.
A collaboration with Lahav CRO, Israel & RTC, Rome, Italy

This work demonstrates preliminary data showing that electrical stimulation of the carotid chemoreceptor afferent nerves, by a designated electro-stimulation apparatus, induces instantaneous and sustainable cerebral perfusion (CP) augmentation in both naive and SAH induced animal models. Moreover, direct measurement of constricted cerebral vessel diameter in SAH-related vasospasm swine model demonstrated a prolonged increase in vessel diameter in major cerebral arteries, which had a residual effect even after end of stimulation.

Background: Reverse of vasospasm and increase of cerebral blood flow is a major therapeutic aim in Subarachnoid Hemorrhage (SAH) -related vasospasm. A swine model of cerebral blood flow augmentation by carotid body electrical stimulation is presented. Peripheral chemoreceptors are located in the carotid bodies, and provide afferent input to the cardiovascular center in the medulla via branch of the glossopharyngeal (CN IX). Outputs from the medulla flow along efferent fibers of the autonomic nervous system to regulate respiratory activity, cardiovascular function and cerebral blood vessel diameter. This work presents the technique of inducing SAH in a large animal model, the carotid electrical stimulation and the detecting and recording of cerebral perfusion (CP) by Laser-Doppler micro probes. A promising data is showing that electrical stimulation of the carotid chemoreceptor afferent nerves, by a designated electro-stimulation apparatus, induces instantaneous and sustainable cerebral perfusion (CP) augmentation in both naive and SAH induced animal models. 

Materials and Methods: A I group of six (6) swine underwent double intrathecal arterial blood injection, in order to induce a SAH model. The animals get short term carotid electro stimulation treatment after 12±2 days, where they exhibited minor to moderate vasospasm in different major circle of Willis arteries. Post stimulation angiographic measurement of cerebral arteries diameters was done. Additional group of five (5) naive swine underwent electrical stimulation of the carotid bifurcation for 8 hours, of whom 4 hours were of spontaneous breathing and additional 4 hours under mechanical ventilation. These animals awake, recover and sacrifice after 14 days follow up. The last group of five (5) swine get continues electrical stimulation for 24 hours. All three groups stimulate by biphasic current under certain electrical parameters and regimes. Cerebral perfusion detected by bilateral Laser-Doppler probes fixated just on top of the Dura. The study held at the lab of Lahav CRO, Lahav, Israel that was the suppliers of animals as well. Histopathology perform by Research Toxicology Center (RTC), Rome, Italy.

Results: Both models of SAH induced in swine, and of carotid stimulation and monitoring by Laser Doppler probe was found to be feasible and reliable. The SAH model animals had shown discernible arterial dilatation, of 21-40% in the anterior portion of circle of Willis, both middle cerebral and basilar arteries. Notably, the vasodilatation has a residual effect which last up to the last time point tested (120 minutes post stimulation). Generally, during stimulation the vasodilatation was correlative to CP enhancements, concomitantly to minor changes in blood pressure (BP) and heart rate (HR). Prolong stimulation, stimulation under spontaneous breathing and recovery after this treatment were found feasible and safe.

Conclusion: This study demonstrates a swine model for novel technique of cerebral vasodilation and CP elevation induced by electrical stimulation of the carotid bifurcation. The model details, advantage and limitations are described.
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Abstract 15
Speaker: Andrea Grassetti, RTC

Evaluation of the toxicity of Cyclophosphamide, Methotrexate and Doxorubicin in minipigs

In recent years the minipigs are gathering increasing success as a predictive animal model in non-clinical studies. In the present work, we want to examine and prove the applicability of this model in the toxicological evaluation of anticancers, as alternative to dogs, the most widely used model, so far, in the evaluation of anticancers. For this purpose 3 progenitors in cancer therapy were used: cyclophosphamide, methotrexate and doxorubicin.

The subacute toxicity of cyclophosphamide, methotrexate and doxorubicin was investigated after 2 (cyclophosphamide and doxorubicin) or 4 (methotrexate) intravenous treatment cycles (each cycle consisting of a single dose followed by a 3 week treatment free period) in the Göttingen minipig. The mentioned drugs were administered intravenously to 2 males and 2 females at dose levels of 18 mg/kg (500 mg/m²) for cyclophosphamide; 2 and 4 mg/kg (50 and 100 mg/m²) for doxorubicin and 6, 12, 18 and 25 mg/kg (175, 350, 500 and 700 mg/m²) for methotrexate, respectively. The starting doses for each drug and the intervals of the cycles were selected according to the dose regimens recommended for cancer indications in humans (Goldman & Gilman’s: The Pharmacological Basis of Therapeutics, eleventh edition). During the experimental period, clinical signs, mortality, body weights, food consumption, ECG, ophthalmoscopy, urinalysis, haematology and serum biochemistry were assessed. Gross findings and organ weight evaluation were carried out at the end of the treatment cycles. Toxicokinetic analysis and histopathology of the main tissues are in progress.

The clinical data showed that cyclophosphamide and doxorubicin induced some effects on bone marrow (i.e. mielosuppression) and haematological profile (e.g. leukopenia) similar to those reported in humans, while methotrexate, at the dose tested, did not induce relevant clinical abnormalities.

According to our experience (data not published), minipigs appeared to have a greater ability, as compared to dogs, to adapt to the effects caused by anticancer with less severe symptoms and therefore with a behaviour more similar to that observed in humans.

A further study for the evaluation of chronic toxicity of doxorubicin, including cardiotoxicity assessment, has been planned in the next months.
Abstract 16

Speaker: Annie Landau, University of Aarhus

Combined PET/microdialysis studies in minipig for PET tracer validation

Anne M. Landau, Aage K.O. Alstrup, Gregers Wegener, Steen Jakobsen, Anna C. Schacht, Jan Jacobsen, Arne Mørk, Jens Christian Sørensen, Doris J Doudet

Dysfunction of the noradrenaline (NA) system is implicated in neuropsychiatric disorders. However, the exact role of NA in the pathogenesis and treatments of these disorders remains unclear. In vivo evaluation of NA transmission in health and disease is an important but difficult endeavor. In tracer doses, \(^{11}\text{C}\)yohimbine is a selective antagonist of the alpha2 adrenoceptors and may potentially be useful to assess NA function. Gottingen minipigs were anesthetized and positioned in a stereotaxic headholder. A high resolution CT was performed in a Siemens PET/CT and microdialysis probes were stereotaxically placed in thalamus, striatum and cortex. Samples were collected every 10 minutes throughout the course of the experiment. After a 2-3 hour equilibrium period, three 90 min \(^{11}\text{C}\)yohimbine PET scans were acquired: the baseline scan was followed by a pharmacological intervention (amphetamine (1-10 mg/kg) or nisoxetine (1mg/kg)), and scans were conducted at 30 and 150 min after challenge. Microdialysis samples were analyzed with HPLC for NA. Yohimbine volume of distribution was obtained in the thalamus, striatum and several cortical regions. Amphetamine caused an immediate release of NA, which resulted in an immediate and sustained decrease in yohimbine binding. Nisoxetine, which blocks the NA transporter but does not directly induce NA release, caused a slow accumulation of NA leading to a progressive increase in competition with the tracer, resulting in a progressive decrease in yohimbine binding. Thus, yohimbine appears to be sensitive to acute changes in the concentration of extracellular/synaptic NA and therefore may be useful in the study of neuropsychiatric disorders.

Boehringer Ingelheim
Abstract 17

Speaker: Aage Kristian Olsen Alstrup, University of Aarhus

MPTP and other minipig models of Parkinson’s disease

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Background
Parkinson’s disease is a degenerative disorder of the central nervous system. The characteristic motor symptoms result from the death of the dopamine producing neurons in the substantia nigra, which is located in the midbrain. However, the cause of cell death is unknown. A crucial neuropathological hallmark of Parkinson’s disease is the proteinaceous inclusions termed Lewy bodies. Animal models play a major role in research in Parkinson’s Disease. We are presenting three minipig models of Parkinson’s disease in which cell death is induced by three different methods. The first model is well established, while the two others are currently under development.

MPTP model
A classic model of Parkinson’s disease model is based on the systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in laboratory animals. After crossing blood-brain barrier, MPTP is transformed by Monoamine Oxidase B (MAO-B) into its active metabolite 1-methyl-4-phenylpyridinium ion (MPP+), which is then transported into dopaminergic neurons, where it blocks mitochondrial activity. The pig is a useful model, as its brain contains MAO-B, which is not the case of all laboratory animal species. The MPTP minipig model is associated with alterations in both behavior and neurotransmission similar to human patients with Parkinson’s disease. However, none of the species tested have developed Lewy bodies. Therefore, alternative minipig models are needed.

The proteasome inhibition model
The ubiquitin proteasome system is the main intracellular pathway for protein degradation, and its dysfunction has been implicated in the pathophysiology of Parkinson’s disease. A Parkinson’s disease model is under development by injection of the proteasome inhibitor, lactacystin, into minipig brains. On a weekly basis, the inhibitor is injected into a subcutaneous injection port connected to cisterna magna of the brain. The preliminary results suggest that it may provide a suitable model of Parkinson’s disease of a progressive nature, with alterations in behavior and monoamine neurotransmission. Furthermore, from an animal welfare point of view, the proteasome inhibition model is not as debilitating as the MPTP model. This model is currently in use to investigate non-dopaminergic aspects of Parkinson’s disease.

Adeno-associated viral vector alpha-synuclein model
Point mutations, duplications and triplications in the alpha-synuclein gene can be causative in rare forms of familial Parkinson’s disease. Based on this, virus vectors encoding for the wild-type or mutant human alpha-synuclein have been used to transduce the nigral dopamine neurons in rat, mouse and non-human primate leading to neurodegeneration in the substantia nigra, reduced tissue dopamine content and abnormal accumulation of alpha synuclein protein and thus, replicating key components of the disease in humans. Alpha-synuclein overexpression in these animal studies relies on the use of recombinant adeno-associated viral vectors for in vivo transfer of transgenes. This model is currently in very early stages of development in minipig.
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