

MINIPIG TOXICOLOGY OVERVIEW

Peter McAnulty

Independent Consultant

Roskilde, Denmark

E-mail: pmca@bakkehuset.info

Minipig Toxicology Overview

INTRODUCTION

- The early days of the minipig
- The reasons why minipigs came to be used for non-rodent studies
- Examples of toxicity studies and compounds
- Examples of safety pharmacology studies
- Regulatory acceptability

The Early Days of Minipigs

- For many years the standard approach has been to use a rodent and a non-rodent for safety assessments.
- The normal species used have been:
 - MICE: single & repeat dose studies, carcinogenicity.
 - RATS: single & repeat dose studies, reproductive studies, carcinogenicity.
 - RABBITS: dermal studies, local tolerance, reproductive studies.
 - DOGS: single & repeat dose studies.
 - MONKEYS: used if dogs were not suitable.

The Early Days of Minipigs

- The RAT/DOG approach was seldom questioned.
- Occasionally other species would be used for various reasons, and this included both pigs and minipigs.
- More recently the value of minipigs as non-rodents for toxicology studies has been realised.
- Choice of species is now a regulatory issue.

The Early Days of Minipigs

- Miniature swine (Minipig later) were first developed in the latter part of the 1950s.
- The use of minipigs for biomedical research began in the 1960s.
- Following the early success of using minipigs for a wide range of biomedical studies, the use of minipigs erupted.
- From 1962 until the present time (08/03/11), PubMed has listed 5420 articles involving minipigs.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Dog, monkey or rabbit not the best choice?
 - Skin and dermal toxicity studies.
 - Drugs, chemicals, pesticides and food and additives with oversensitivity for dogs
 - Teratology studies if rabbits are not suitable.
 - Juvenile non-rodent studies if metabolism is the most suitable.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Skin and dermal toxicity studies.
 - Dermal or transdermal dosing.
 - Similarities of skin between minipigs and humans, due to the thickness of the dermis.
 - Sparse coat of hair in both species.
 - Göttingen minipig skin lightly pigmented.
 - Useful for local tolerance skin studies.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Compounds that cause oversensitivity in dogs.
 - Non-steroidal anti-inflammatory (NSAID) drugs can cause gastrointestinal lesions in dogs.
 - In one example using minipigs, there were no GI lesions at the lowest dosages, and only transient ulcers at the high dosage.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Compounds that cause oversensitivity in dogs.
 - Sympathomimetics & anti-hypertensives can cause cardiotoxicity in dogs, as can benzodiazepines.
 - In one benzodiazepine case, both dogs and minipigs developed tachycardia & increased heart rates, but the dogs developed myocardial necrosis whilst the minipigs had no histopathological heart changes.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Compounds that cause oversensitivity in dogs.
 - Endothelins can cause arteriopathy in coronary arteries in the heart of dogs, but not in minipigs.
 - Endothelins are also mediators of renal and endocrine functions, non-rodents toxicity studies species should be checked.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Compounds that cause oversensitivity in dogs.
 - Many compounds can have emetic effects on dogs, which can result in insufficient or erratic dosing.
 - Female dogs are very sensitive to oestrogens and anti-gestagens and can develop a severe type of anaemia.

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Compounds that cause oversensitivity in dogs.
 - Cytotoxic & cytostatic anticancer drugs can also cause emetic and GIT effects in dogs.
 - If the dog is not suitable, ICH S9 recommends to consider an alternative, but does not suggest a non-rodent species. Minipig?

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Decision to use minipigs for **teratology** studies.
 - Test articles or their metabolites may produce irrelevant results from rat, mouse or rabbit.
 - Poor predictors for humans.
 - First minipig studies in 1999.
 - Three CROs offering minipig studies in Europe.
 - A recent case had no possibility to use rats, mice or rabbits because of metabolites. The FDA recommended the minipig!

Reasons for Toxicologists Using Minipigs for Non-Rodent Studies

- Decision to use minipigs for **juvenile** toxicity studies.
 - The majority of juvenile tox studies are performed with rats, but if not suitable for metabolism reasons, then a non-rodent is used.
 - The FDA Document mentions minipigs as well as other non-rodents.
 - Many minipig studies run mainly at CROs.
 - Advantages: 5-6 offspring/litter, 400g at birth, rapid growth, rapidity to sexual maturity (3-5 months). Amenable to cross-fostering.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- If minipigs are used for non-rodent studies, the extrapolation of minipig tox data should be “human-like” with these criteria:
 - Comparable pharmacodynamic activity of the compound in humans & the minipig.
 - Comparable pharmacokinetic & metabolic parameters.
 - Comparable sensitivity & profile of reactions following toxic insults.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Most study results involving minipigs are not available in the public domain.
- In PubMed, there are only 35 references that mention both minipigs & toxicology!
- However, FDA Approval Packages and EMA Approval Documents can be accessed, e.g. PharmaPendium can help in this respect.
- The majority of minipig approval documents involve dermal toxicity studies.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- General minipig studies & compounds
 - The OECD Guideline for testing chemicals involving Repeated Dose 90-day oral toxicity studies in non-rodents recommends dogs, but also suggests minipigs as an alternative.
 - A general safety assessment of Castor Seed Oil (*Ricinus communis*) included minipigs and other species. Minipigs showed no skin irritation (no minipig oral studies).

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- General minipig studies & compounds
 - A comparison between dogs & minipigs was made by giving acrylamide in their diet. In both species there was distribution into muscle tissue, and no neurotoxic signs. Urine excretion was similar in both species, but minipigs had a higher recovery of acrylamide in the faeces than in dogs.
 - It was once proposed that the minipig might be a model for methanol-induced neuro-ocular toxicosis, but the minipigs did not appear to be overtly sensitive.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Central Nervous System
 - Organophosphorus compound-based (OP) chemicals and pesticides etc. are often tested with minipigs to determine the level of toxicity.
 - OPs act primarily by inhibiting acetylcholinesterase (AChE).
 - The treatment for exposure to OPs is AChE reactivators (oximes) such as obidoxime & pralidoxime.
 - Studies with minipigs have been performed with oximes to determine the similarities between minipigs & humans.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Central Nervous System
 - Minipigs have been used to investigate MPTP induced parkinsonism which can prolong during many months.
 - Minipigs have also been good models of diffuse brain trauma which may induce acute formation of amyloid plaques (Alzheimer's disease).
 - Juvenile toxicity studies have been run using minipigs, and they have been found to be able to perform in behaviour studies and learning tests.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Cardiovascular System
 - Minipigs have been used in cardiovascular safety studies to determine the effects propranolol, isoproterenol, nifedipine & clonidine, & to investigate QT-interval after treatment with terfenadine.
 - Studies with minipigs compared with dogs have been performed with minoxidil. In both species there was decreased arterial pressure & increased heart rate, followed by myocardial haemorrhages.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Respiratory System
 - Minipigs have been found to be useful for nasal toxicity studies.
 - One example was intranasal midazolam which showed rapid & reliable sedation.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Respiratory System
 - The safety of a novel adjuvant for intranasal immunisation of an influenza virus has been investigated with minipigs, showing no clinical signs or haematological effects.
 - Validation of minipig inhalation administration has been performed with verapamil, dofetilide, and lactose. The system works, but needs further validation.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Gastrointestinal System
 - Another example is HMG-CoA reductase inhibitors like cerivastatin which cause erosions & haemorrhages in the GIT of dogs, but not seen in minipigs.
 - The buccal epithelium of the minipig is unkeratinised as in humans, and this can be useful in administration of peptides in the buccal cavity.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Renal System
 - Ifosfamide (IFO) is used in the treatment of cancer, and can cause nephrotoxicity in both minipigs and humans.
 - Minipigs are used for toxicity testing of ciclosporin (CsA) because of nephrotoxicity similar to that seen in humans.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Renal System
 - Proquazone is a non-steroidal anti-inflammatory drug (NSAID), which usually cause GIT effects. Minipigs used in a toxicology study had GIT effects, but also had inflammatory renal changes.
 - In another case, Pramipexole tested in minipigs had no renal effects, and was chosen because dogs had extreme emesis.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Reproductive System
 - The synthetic progesterone, altrenogest, can inhibit the release of GNRH (gonadotrophin-releasing hormone) & maintain the fertility of minipigs.
 - Teratology studies with minipigs has already been mentioned. Minipigs have been shown to be susceptible to various teratogens, thalidomide, hydroxyurea & aminopterin, including known teratogens, pyrimethamine & tretinoin.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Musculoskeletal System
 - Bisphosphonates for treatment of post-menopausal women, especially osteoporosis.
 - In the past, aged dogs and monkeys were used.
 - Minipigs have not been used in non-clinical studies, but they have been used as models to determine the effects of administration of bisphosphonates (e.g. alendronate, alfacalcidol etc) on human patients.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Musculoskeletal System
 - Proliferol is a product that contains dextrose, glycerine, phenol, & lidocaine hydrochloride for treatment of chronic low back pain.
 - A toxicity study was performed with Yucatan miniature swine over 14 days.
 - There was no evidence of toxicity, and repair was underway with fibrosis & skeletal muscle regeneration at the injection sites.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Food Additives & Contaminants
 - The WHO “Guidelines for the preparation of toxicological working papers” recommends the use of both pigs and minipigs.
 - US FDA “Redbook 2000” specifies the dog as the principal non-rodent in toxicity studies – but minipigs are also referred to as suitable if there is scientific justification.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Food Additives & Contaminants
 - Thickening agents such as gelatine, pectin etc have been toxicity tested with minipigs. This demonstrated that these agents could cause atherosclerosis & hypercholesterolaemia.
 - Stabilisers have also been toxicity tested using minipigs with products such as brominated vegetable oil, which caused cardiotoxicity & accumulation of lactic dehydrogenase activity.

Results of Using Minipigs in Various Toxicology Studies and Various Compounds

- Food Additives & Contaminants
 - Food colours (e.g. carmoisine & sunset yellow FCF) have been tested in short term toxicity studies using minipigs.
 - Food colours have also been tested using minipigs to evaluate the immunotoxic potential of food additives.

Examples of Safety Pharmacology Studies with Minipigs

Examples of Safety Pharmacology Studies with Minipigs

- Central nervous function
 - Normally performed with mice & rats
 - However, now there have been several studies where minipigs & pigs have been used in neurobehavioural assessments.

Examples of Safety Pharmacology Studies with Minipigs

- *In vivo* cardiovascular function
 - Non-rodents have been recommended for cardiovascular safety pharmacology, including dogs, monkeys and swine.
 - Minipigs specifically are suitable for *in vivo* cardiovascular studies, despite the fact that they have a long QT interval compared with other non-rodents. This has been shown not to be a constraint by recent telemetric studies.

Examples of Safety Pharmacology Studies with Minipigs

- Respiratory, renal, & gastrointestinal functions.
 - Respiratory & renal functions can be combined with systemic toxicity testing.
 - GIT function tests maybe suited to minipigs because they are omnivorous, but they are not well established.

Regulatory Acceptability

- Use of minipigs in evaluation of toxicity of human products has increased over the past 10 years.
- Regulatory authorities have gained more experience in reviewing such studies.
- One of the ICH Guidelines, M3, does not specify which non-rodent species should be used for the evaluation of human drug products. To a certain degree, this is beginning to start with other products as well, although in some areas the use of dogs still continues automatically.

Regulatory Acceptability

- Acceptability of minipigs and other non-rodents to the authorities is not clearly spelled-out in regulatory guidance documents.
- The regulatory world still indicates that two species should be used, one a rodent and one a non-rodent.

Regulatory Acceptability

- Despite not being mentioned in guidelines, pharmaceutical EMA/CHMP Approval Documents & FDA Approval Packages can be accessed to determine if minipigs have been used.
- There are indeed many examples of minipigs supporting the safety of pharmaceutical products, & clearly regulators have accepted minipigs in this rôle.