Nonrodent Models: Minipig Specialty Capabilities

The Göttingen minipig has gained favor in pharmaceutical development programs due to its physiological similarity to humans (skin structure, digestive, cardiovascular, and urinary systems), ready availability, known disease status, and feasibility of genetic and phenotypic manipulation. Minipigs also experience fewer species-specific adverse reactions to compound classes than other large animal models, making them a viable alternative in those cases. As such, regulatory authorities accept the minipig as a suitable nonrodent species for use in safety evaluation assessment and efficacy studies of pharmaceutical products when scientifically appropriate.

Studies using minipigs as a nonrodent species for toxicity testing have been conducted and submitted to regulatory authorities worldwide by Charles River Laboratories for more than 20 years. These include pharmacokinetic, safety pharmacology, and toxicity studies, including DART and juvenile, using various dose routes including oral, dermal, subcutaneous, intravenous, target tissue, intranasal, ocular, wound healing, and intrathecal.

Intravenous Administration
Intravenous administration can be performed as bolus injections via ear vein or implanted catheter, or as intravenous infusions using cannulated methods for continuous or intermittent infusion regimens for up to six months. Cannulated methods typically utilize cannulation of the vena cava, performed with entry via the femoral vein (a procedure comparable to that used in rodent and other nonrodent species). While the cannula is in place, continuous saline infusion is used to maintain patency (no anticoagulant is used) and historical background has shown infection rates maintained at < 1%.

Intrathecal Administration
Intrathecal administration is an invasive procedure; however, we have maintained a very low rate of complications using two approaches in the minipig. With the first approach, we can surgically implant a catheter via a microlaminctomy followed by a durotomy at the lumbar level. Once the catheter is secured on the dura,
it is connected to an access port which is placed in the
subcutaneous space on the dorsal aspect of the animal.
This approach allows dosing on a conscious animal, and is
particularly well suited for repeat dose studies. The second
method is performed under anesthesia by direct injection in
the intrathecal space. A spinal needle is slowly inserted in
between two vertebrae at the lumbar level; once the dura is
performed, a flow of cerebrospinal fluid (CSF) in the hub of
the needle confirms correct placement. The syringe with the
formulation is then connected to the needle and the dose
is administered slowly. This method is less invasive than
the catheter-based approach, and is ideal for single dose
studies. We can also perform collection of CSF using direct
puncture under anesthesia or via the lumbar catheter.

Subcutaneous Administration
Subcutaneous administration can be performed as
injections or infusions using bolus injections or a temporary
indwelling cannula combined with rotation of sites. This
model may be of marked interest for evaluation of the
irritancy potential of formulations intended for subcutaneous
administration. In particular, it allows for evaluation of
reactions from the epidermis to the subcutis following
single administrations at various levels/sites, or repeat
administrations at given site(s) within the same animal
to mimic or surpass the clinical plan. A poster detailing a
subcutaneous infusion model which utilizes a rotation of
sites was presented at the annual meeting of the American
College of Toxicology in 2011.

Wound Healing
Wound healing assessment in the minipig closely resembles
that in humans, based on the similar characteristics between
minipig and human skin and wound healing process as
discussed above. Specific types of wounds can vary based
on specific need, and wounds ranging from partial thickness
(thermal, incisional, or excisional) to full thickness (incisional
or excisional) can be created on the dorsal surface. Dosing
of the material of interest follows the intended route of clinical
exposure (topical, subcutaneous, intravenous). Macroscopic
evaluation throughout the study interval and microscopic
evaluation at termination allow a full assessment throughout
the healing process. Specialized staining and tensile strength
/incisional wounds only) can be incorporated.

Intranasal Administration
A clinical device or commercially available nasal spray
pump device is used. Alternatively, when the dose needs
to be administered based on the animal’s body weight,
a calibrated micropipette or a 1 cc syringe could also be
used to deliver the dose in each nostril. The dose volumes
that can be administered are similar to those used in other
nonrodent models (0.1 to 1.0 mL/nostril) and dosing can be
performed several times per day.

Intraocular Administration
Of the species used for ocular safety evaluations, the
minipig’s eye most closely approximates the size of the
human eye. The minipig also has similar innervation and
vascular structure to humans, making it one of the most
suitable eyes for intraocular administration in preclinical
ocular toxicology studies. The larger eye size permits the
implantation of medical devices and conduct of clinically
relevant surgical procedures and facilitates translation of
preclinical results to the development and support of
clinical protocols.

Safety Pharmacology
As the use of the minipig in preclinical safety testing is
increasing, it follows that this species should also be used
in safety pharmacology testing. Minipig models are now
available for use in cardiovascular safety pharmacology
testing, and data demonstrate that the minipig constitutes a
suitable model for telemetry.
We use jacketed external telemetry (JET™, Data Sciences International, St. Paul, MN) for the collection of continuous electrocardiographic and hemodynamic data in large animal stand-alone and repeat-dose settings. JET employs surface electrodes for ECG collection, and implanted probes for blood pressure measurement. The JET transmitter has a direct connection to the ECG sensors, and radio frequency communication to the optional blood pressure probe. The jacketed device then sends both types of data wirelessly via Bluetooth® to a licensed acquisition system.

This system provides a unique signal for each animal and allows for continuous collection of electrocardiograms and real-time visualization of trends. Measurements are on alert, unrestrained animals, improving sensitivity to detect changes in the absence of the sympathetic stimulation of manually restrained approaches. Recordings can be limited to study days with minimal room entry to provide hours of undisturbed data. The technology offers a viable alternative to both snapshot techniques and fully implantable telemetry and is amenable to use in long-term repeat-dose (toxicity) studies.

**DART and Juvenile Toxicology**
When it comes to regulatory teratogenicity and juvenile toxicology, the minipig once again has several advantages over the more traditional species. They have a relatively large litter size, which reduces the number of pregnant females required in the overall study. Furthermore, they reach sexual maturity earlier, and have a shorter cycle length when compared with other large animal models.

The species also exhibits the same teratogenic sensitivities as humans, demonstrating susceptibility to similar fetal malformations induced by thalidomide, hydroxyurea, pyrimethamine, and ethanol, amongst others.

**Metabolism**
For certain enzyme systems, the use of the minipig is favored over more traditional species. Minipigs are the first non-primate choice when the drug candidate is metabolized by aldehyde oxidase, which is practically non-existent in dogs but present in pigs. This would also apply to substrates of N-acetyltransferases (NAT1 and NAT2), which are practically absent in the dog liver. Therefore, minipigs should be included in any in vitro or in vivo assessment of metabolism of a compound.

Safety guidelines include studies to investigate tissue distribution. Quantitative whole-body autoradiography (QWBA) is a powerful tool in determining whole-body tissue distribution as well as distribution to and localization within specific tissues. QWBA has, however, been traditionally restricted to the investigation of distribution in smaller species. In what appears to be the first application for such a type of investigation, Charles River used QWBA techniques to investigate the tissue distribution of radioactivity in the minipig. The work was presented at the national meeting of the International Society for the Study of Xenobiotics in 2012.

**Diabetes**
Although a variety of rodent models for type I diabetes exist, such models have genuine limitations and poor predictability due to the stark differences between rodents and humans. In many respects, diabetic minipig models more closely resemble the human condition; for example, human insulin and porcine insulin differ by only a single amino acid on the B-chain.
The type 1 diabetic minipig can be induced with streptozotocin. Monitoring and control of diabetes is facilitated with daily monitoring of blood glucose and appropriate insulin injection to reduce blood sugar fluctuations. This has allowed animals to be maintained for greater than 365 days in the laboratory setting\textsuperscript{20}. Standard preclinical testing can be conducted in the diabetic minipig, along with specialized protocols for specific endpoints (oral glucose tolerance test, etc.).

**An Alternative Approach**

The use of the minipig as a nonrodent species has been well documented, and in 2010 was the subject of the RETHINK European FP6 project. The RETHINK project evaluated the potential impact of toxicity testing in the minipig and established the use of this species as a valid alternative approach in regulatory toxicity testing that can contribute to the replacement, refinement and reduction of animal testing (3Rs)\textsuperscript{21}.

The species demonstrates numerous practical advantages over other large animal models in the preclinical toxicology space:

1. Minipigs tolerate NSAIDs, which can cause gastrointestinal lesions in other species.
2. Minipigs tolerate sympathicomimetic and antihypertensive drugs, which cause cardiotoxicity even at low doses in the traditional large animal model.
3. Minipigs do not develop arteriopathy with endothelin receptor antagonists.
4. Minipigs are not prone to vomiting, nor are they susceptible to anaphylactic reactions as a result of a histamine release, which can occur following administration of some vehicles/excipients.
5. Minipigs are not sensitive to sex hormones with estrogenic activity, which can result in aplastic anemia in other large animal models.

**Conclusion**

The minipig is recognized as a suitable alternate nonrodent species associated with efficacy and safety assessment of certain types of pharmaceutical products, and its prevalence and relevance in preclinical testing are growing. There is a good supply of high-quality animals of known disease status with sufficient background data now available to allow unequivocal data interpretation. Minipigs offer certain advantages over the traditional species in relation to specific responses to particular drug classes, and therefore can also offer advantages over the traditional species in relation to the ethical considerations and cost of animals in biomedical research.

**References**


19. Madden S, Patterson A, Stevenson K. The utilisation of quantitative whole body autoradiography (QWBA) methods to investigate whole body tissue distribution in the minipig (2012).
