The minipig in toxicity testing
Geertje van Mierlo
Species selection in toxicology studies

- Generally, rats and dogs have been the rodent and non-rodent species of choice in safety evaluation.
- For biologicaals non-human primates are often used for safety evaluation.
Criteria for the selection of the non-rodent toxicology model

- Pharmacological activity comparable to human
- Pharmacokinetic and metabolic parameters comparable to human
- Comparable sensitivity and profile of reactions in test species
- Comparative physiology to select for certain end point/target organ(s)
- Practical issues

- Case-by-case decision, justification needed
Minipig as a model for safety testing*

- The pig closely resembles man in many features of its anatomy, physiology, biochemistry and life style. In particular, the cardio-vascular system, skin and gastro-intestinal tract resemble the human situation better than that in other species.
- Because of these similarities the toxic effects of chemicals and drugs in pigs may resemble the effects in man more closely than do some other commonly used laboratory animals.

Minipig: a non rodent model for safety testing*

- Easy to house
- Less zoonotic diseases than nonhuman primates
- Easy to breed (large litter sizes and quick development time)
- Defined health status
- Ample genetic and genomic background data available
- Growing body of literature and background data
- Will be more acceptable for the community as animal test species than dog/primate

Safety and toxicity studies

- Safety pharmacology
- Toxicokinetic and pharmacokinetic studies
- General toxicity (acute, repeat dose, chronic toxicity)
  - Local tolerance
  - Immunotoxicity
- Phototoxicity testing
- Genotoxicity
- Reproduction toxicity including juvenile toxicity

ICH guideline M3(R2) on non-clinical safety studies for the conduct of human clinical trials and marketing authorization for pharmaceuticals, Step 5
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Safety pharmacology in minipigs

› Cardiovascular function by telemetry
  › Heart rate and blood pressure comparable to humans
  › QT interval slightly longer in minipig but does not preclude its use
› Respiratory function
  › Tidal volume, respiratory rate, minute volume
› Central nervous system function
  › FOB
  › Cognitive testing

See: Authier et. al. 2011 and Gieling et. al. 2013
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General toxicity studies, administration routes

- Oral
- Dermal
- Intravenous
- Inhalation
- Intranasal
- Subcutaneous
- Intramuscular
- Intradermal
General toxicity studies, observations

- All standard observations possible
- A.o. Clinical signs, body weight, physiological parameters, ophthalmoscopy, hematology, clinical chemistry, histology
- High blood sampling volume (no satellite groups necessary)
- Local tolerance can be included
- Immunological parameters can be included
Quantitative immunotoxicity testing: Factors to consider in Standard Toxicity Studies*

- Hematological changes (leukocytosis, lymphopenia)
- Alterations in immune system organ weights and/or histology
- Changes in serum globulins might be an indication for changes in immunoglobulins
- Increased incidence of infections
- Increased occurrence of tumors (sign of immunosuppression?)

ICH S8 “Immunotoxicity studies for Human pharmaceuticals” (ICH step 4, in operation from April 2006)
Qualitative (functional) immunotoxicity testing

- T cell dependent antibody response
- Lymphocyte subset analysis
- NK activity
- Lymphocyte proliferation
- Delayed type hypersensitivity
- Cytokine production
- Host resistance studies
Quantitative and functional immunotoxicity in minipigs: a demonstrator study

Start treatment

KLH immunization (i.m.)

KLH immunization (i.m.)

DTH immunization (i.d.)

- d0
- d13
- d27
- d39/40

PBMC

PBMC

PBMC

- Serum primary IgM/IgG
- Serum secondary IgM/IgG

- Proliferation assay
- Lymphocyte subset analysis
- NK activity assay

✓ Vehicle
✓ Cyclosporin A (20 mg/kg/day)
✓ Dexamethasone (0.4 mg/kg/day)

4 ♂ and 4 ♀ per group

- No treatment related clinical observations

Immunotoxicity study in minipigs

- Quantitative endpoints
  - Hematology
  - Immune system organ weights and histology

and

- Qualitative (functional) endpoints
  - Ex vivo lymphocyte proliferation
  - NK cell activity
  - Delayed type hypersensitivity reaction (DTH)
  - Lymphocyte subset analysis (still needs attention)
  - T cell dependent antibody response (TDAR)

Were overall successfully implemented in minipigs.

The effects observed with CsA and DEX were generally in line with those described in other species.
Decreased thymus weight by Dexamethasone and Cyclosporin A

** P<0.01
**In vitro proliferation assay**

**ConA**

- **0**
- **50000**
- **100000**
- **150000**
- **200000**

**Daynumber**

- **-1**
- **13**
- **27**

**Vehicle**

**Cyclosporin A**

**Dexamethasone**

**CPM**

**Males and females combined**

**p<0.01**
DTH response 72 hours
Mean skin reaction (diameter (mm))

**Males**

<table>
<thead>
<tr>
<th>Group</th>
<th>PBS</th>
<th>2 mg KLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>11.4</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>Dex</td>
<td>0</td>
<td>9.2</td>
</tr>
</tbody>
</table>

**Females**

<table>
<thead>
<tr>
<th>Group</th>
<th>PBS</th>
<th>2 mg KLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>9.1</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Dex</td>
<td>0</td>
<td>9.0</td>
</tr>
</tbody>
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Minipig in phototoxicity testing

(a) (b) (c)
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Minipigs in reproduction toxicity

› Polyoestrus with short cycle 19-21 days (dog mono-estrous with long interval), oestrus can be synchronised
› Gestation period 114 days (dog 59-67 days, NHP ca. 150 days)
› Fetal exposure of small molecules resembles that in humans
› No placental transfer of antibodies, so less useful for biologicals (mAbs)
› Ca. 5 offspring/litter (dog ca. 6, NHP 1-2)
› Cross-fostering and bottle-feeding possible
› Size of piglets permits all dosing routes from an early age
› Size of piglets permits several investigations (blood sampling, ECG, ophthalmoscopy etc.)
› Rapid development to sexual maturity, 3-5 months (entire juvenile period can be covered).
Juvenile development Göttingen Minipigs

Aim
To get more knowledge about the following parameters during development of minipigs to 6 months of age:

- physical and sensory development (developmental landmarks)
- hematology and clinical chemistry
- immunological parameters
- the weight and histopathology of 26 main organs

Necropsy
4 ♀ and 4 ♂ minipigs per necropsy day aged 1-4 days, 7 days, 14 days, 4 wks, 2, 3 and 6 months
Juvenile development of the minipig

- The minipig pig is born relatively mature regarding general physical and sensory development. So, limited developmental landmarks available.

- Comparable development of the lymphoid organs in minipigs and other species (inverted morphology of lymph nodes).

- The minipig is especially suitable for evaluation of toxicity on development of the nervous-, reproductive- and immune system.
### Age-related postnatal-developmental histopathology in Göttingen minipigs® (1 day – 6 months)

<table>
<thead>
<tr>
<th>Cardiovascular system</th>
<th>Urogenital tract system</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Heart</td>
<td>- Kidneys</td>
</tr>
<tr>
<td><strong>Hemopoietic system</strong></td>
<td>- Urinary bladder</td>
</tr>
<tr>
<td>- Thymus</td>
<td></td>
</tr>
<tr>
<td>- Bone marrow (sternum)</td>
<td>- Male genital system</td>
</tr>
<tr>
<td>- Spleen</td>
<td>- Testes</td>
</tr>
<tr>
<td>- Lymph nodes</td>
<td>- Epididymides</td>
</tr>
<tr>
<td>- Peyer’s patches</td>
<td>- Prostate</td>
</tr>
<tr>
<td>- Bronchus associated lymphoid tissues (BALT)</td>
<td>- Seminal vesicles/coagulation glands</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory tract and lungs</th>
<th>Male genital system</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Lungs</td>
<td>- Testes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pancreas, Liver and gallbladder</th>
<th>Female genital tract</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Pancreas</td>
<td>- Uterus</td>
</tr>
<tr>
<td>- Liver</td>
<td>- Vagina</td>
</tr>
<tr>
<td>- Gall bladder</td>
<td>- Ovaries and oviduct</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Endocrine system</th>
<th>Nervous system</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Adrenals</td>
<td>- Sciatic (peripheral) nerve</td>
</tr>
</tbody>
</table>

|                                | Soft tissue                                           |
|                                | - Mamma                                               |

|                                | Skin                                                  |
|                                |                                                       |

Ellegaard Göttingen Minipigs A/S and TNO Quality of Life; 2010
Development Mesenteric Lymph node

Day 3

Day 91
Development spleen in minipig

Day 10

Day 91
Minipigs, what can we offer?

- Standard toxicity studies (STS); oral, i.m., s.c., i.d., dermal by patch testing or semi-occlusive treatment
- Kinetics and Metabolism

We generated special experience in the following areas
- Immunotoxicity testing
- Immunogenicity
- Juvenile (immune)development
Our minipig publications

**Book chapters:**


**Scientific journals:**


