Juvenile (immune) development in Göttingen Minipigs®

Geertje van Mierlo

*Experimental Immunology*

TNO Triskelion BV

*Contract Research Organisation*
Experimental Immunology Group

**IMMUNOTOXICOLOGY (including immunogenicity and food allergy)**
- unintended effects of drugs, food ingredients/supplements and industrial chemicals on the immune system

**IMMUNOPHARMACOLOGY/IMMUNOMODULATION**
- intended effects of drugs on the immune system

**IMMUNONUTRITION/IMMUNOMODULATION**
- intended effects of food ingredients/supplements/probiotics on the immune system

**PRECLINICAL SAFETY AND EFFICACY TESTING OF VACCINES AND (BIO)PHARMACEUTICALS**
- vaccine safety/efficacy studies (i.m./i.n./s.c.) for prophylactic and therapeutic vaccines
- challenge studies with mouse adapted influenza strains (prophylactic and therapeutic)
Minipigs: an alternative non rodent model for safety assessment?

- **Immunotoxicity of (bio)pharmaceuticals**
  - *Cyclosporin A and Dexamethasone*

- **Immunogenicity of (bio)pharmaceuticals**
  - *Kineret, Adalimumab and Infliximab*

- **Juvenile development in the minipig**
  - *Development of 26 organs/tissues from day 1 to 6 months*
Minipig is considered to be a useful alternative species to traditional non-rodent species for non-clinical toxicity studies, including immunotoxicity testing, of (bio)pharmaceuticals and food ingredients*. 

Why minipigs?

• (Mini)-pigs closely resemble man in many features of its anatomy, physiology, biochemistry and lifestyle.

• 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 non-rodent laboratory animals.
Why juvenile developmental studies?

› Better protection of children’s health
  ✓ > 90 % of medicines are off label used

› Development of better medicines for children
  ✓ Safer and more efficient
  ✓ more child-friendly applications

› Children are not small adults
  ✓ absorption, distribution, metabolism, excretion etc
The rat is the generally applicable species for juvenile toxicity studies

but

For logistic and/or scientific reasons the rat may not always be the appropriate species….

Our aim is to evaluate if for those cases the minipig might be a good alternative for rat?

Focused on development of immune system
Thymus effects on adult minipigs

Vehicle control  Dexamethasone treated

Females thymus weight

Females relative thymus weight

Thymus weight (g)

Vehicle  Cyclosporin A  Dexamethasone

Vehicle  Cyclosporin A  Dexamethasone

*  **
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
Juvenile development Göttingen Minipigs®

**Animals**
5 pregnant sows + 12 ♂ and 12 ♀ Göttingen Minipigs® of 2, 3 and 6 month were obtained from Ellegaard.

**Necropsy**
4 ♂ and 4 ♀ Göttingen Minipigs® per necropsy day.

Aged:
1-4 days, 7 days, 14 days, 4 wks, 2, 3 and 6 months.
Juvenile development Göttingen Minipigs®

› The mean gestation length was 113.4 days

› The mean number of piglets delivered by the 5 sows was 7 (range: 5-8)

› Two stillborn piglets were born in two litters

› Sex ratio was 58%: 19 male and 14 female piglets

› None of the piglets died other than scheduled between day 1 and 28
Body weight development

Piglet body weight

Mean BW (g) vs. days PN

Males
Females
males+females

Body weight juvenile and young pigs

Body weight (KG) vs. age (days)

males
females
Developmental landmarks

At birth the minipig is relatively mature regarding general physical and sensory parameters.
Quantitative and qualitative immunology endpoints

› quantitative endpoints

- WBC differentiation and clinical chemistry
- Lymphocyte subsets in PBMC’s
- Lymphoid organ weights + kidney and liver weights
- Lymphoid organ pathology

› qualitative (functional) endpoints

- Natural Killer (NK) cell activity (data not shown)
Absolute WBC-, Lymphocyte-, and RBL counts
Lymphocyte subset analysis in blood
Relative organ weights

Relative thymus weight

relative spleen weight

Relative cervical lymph node weight

relative mesenterial lymph node weight

(Charts showing data for males and females across different time intervals from 2 days to 6 months.)
Microscopy lymphoid organs
<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>Male genital system</td>
</tr>
<tr>
<td>Bone marrow (sternum)</td>
<td>Testes</td>
</tr>
<tr>
<td>Spleen</td>
<td>Epididymides</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Prostate</td>
</tr>
<tr>
<td>Peyer’s patches</td>
<td>Seminal vesicles/coagulation glands</td>
</tr>
<tr>
<td>Bronchus associated lymphoid tissues</td>
<td><strong>Female genital tract</strong></td>
</tr>
<tr>
<td></td>
<td>- Uterus</td>
</tr>
<tr>
<td></td>
<td>- Vagina</td>
</tr>
<tr>
<td></td>
<td>- Ovaries and oviduct</td>
</tr>
<tr>
<td><strong>Respiratory tract and lungs</strong></td>
<td><strong>Nervous system</strong></td>
</tr>
<tr>
<td>Lungs</td>
<td>- Sciatic (peripheral) nerve</td>
</tr>
<tr>
<td><strong>Pancreas, Liver and gallbladder</strong></td>
<td>- Brain</td>
</tr>
<tr>
<td>Pancreas</td>
<td>- Soft tissue</td>
</tr>
<tr>
<td>Liver</td>
<td>- Mamma</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>- Skin</td>
</tr>
<tr>
<td><strong>Endocrine system</strong></td>
<td></td>
</tr>
<tr>
<td>Adrenals</td>
<td></td>
</tr>
</tbody>
</table>

Ellegaard Göttingen Minipigs A/S and TNO Quality of Life; 2010
Thymus at day 10,
cortex and medulla, and epithelial-free area-100x

Thymus at day 24; C:M ratio; 25x

Thymus at day 182; large C:M ratio; 25x
Spleen

Male, day 8; 100x. Extramedullary hematopoiesis and distinct ellipsoids in the marginal zone and the red pulp.

Male day 14; 100x
Red pulp is filled with erythrocytes, widely dispersed ellipsoids

Female 182 days; 50x.
Germinal centre, capsule and trabeculae rich in elastic fibres.
Mesenteric LN

Male day 3  Male day 8  Male day 91
Peyer’s patches

Male, 10 days

Male, 14 days; 50x

Male 182 days; 50x
BALT
(day 28)
Bone Marrow

Bone marrow male day 60

Bone marrow male day 92
Conclusions

- Limited developmental landmarks are available in the minipig due to relative maturity (regarding general physical and sensory development) at birth.

- Lymphoid organ development was comparable to that in other species, except for the inverted morphology of the lymph nodes.

- As for other species, more basic information is still needed on juvenile (immune) development of the minipig.

- The minipig might be a suitable model especially to evaluate the potential effects of pharmaceuticals on the development of the nervous-, reproductive- and immune system.
Acknowledgements

Colleagues at TNO/TNO Triskelion:

André Wolterbeek - reproduction toxicology
André Penninks– experimental immunology
Ine Waalkens-Berendsen - reproduction toxicology
Frieke Kuper - pathology
Joost Bruijntjes - technician (immuno)histology
Cor Snel - technician
and many others
Dept Toxicology and Risk Assessment
TNO Triskelion BV
Zeist
Thank you for your attention

Geertje van Mierlo
Geertje.vanMierlo@tno.triskelion.nl
Minipig publications: book chapters


Minipig publications


Minipig publications


- Wolterbeek et al. Developmental issues of main organs/tissues (20) in the minipig from birth to six month of age. In preparation
Guidelines related to study the potential effect of pharmaceuticals on juvenile development

**EMA**
- Guideline on the need for non-clinical testing in juvenile animals of pharmaceuticals for paediatric indications (EMEA/CHMP/SWP/169215/2005), into effect 2008
- Guideline on the investigation of medicinal products in the term and preterm neonate (EMEA/536810/2008)

**FDA**
- Nonclinical Safety Evaluation of Pediatric Drug Products, February 2006

**ICH**
- Nonclinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals [M3(R2)]
- Nonclinical evaluation of anticancer pharmaceuticals (S9)
# DTH response in adult minipigs (diameter in mm)

## Males

<table>
<thead>
<tr>
<th>Group</th>
<th>PBS</th>
<th>1 mg KLH</th>
<th>2 mg KLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>7.2</td>
<td>11.4</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>0</td>
<td>6.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Dex</td>
<td>0</td>
<td>8.2</td>
<td>9.2</td>
</tr>
</tbody>
</table>

## Females

<table>
<thead>
<tr>
<th>Group</th>
<th>PBS</th>
<th>1 mg KLH</th>
<th>2 mg KLH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0</td>
<td>9.3</td>
<td>9.1</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>1.1</td>
<td>4.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Dex</td>
<td>0</td>
<td>11.8</td>
<td>9.0</td>
</tr>
</tbody>
</table>