The use of Göttingen Minipig in pharmacokinetic studies for safety and efficacy
Nicole H.P. Cnubben, PhD, ERT
Symposium The Göttingen Minipig in Pharmaceutical Research
TNO Triskelion BV
Pharmacokinetics in minipigs to support safety and efficacy studies

- Pharmacokinetics and immunogenicity of biopharmaceuticals
  - Anakinra (Kineret®)
  - Adalimumab (Humira®) and Infliximab (Remicade®)
- Oral bioavailability
- Microdosing
  - $^{14}$C-LPS in minipigs (AMS)
Pharmacokinetics and immunogenicity of biopharmaceuticals

The Göttingen minipig® as an alternative non-rodent species for immunogenicity testing: A demonstrator study using the IL-1 receptor antagonist anakinra

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The minipig as an alternative non-rodent model for immunogenicity testing using the TNFα blockers adalimumab and infliximab

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Anakinra (generic name for Kineret®)

- Drug used to treat rheumatoid arthritis
- Recombinant, non-glycosylated form of the human IL-1 receptor antagonist (IL-1Ra)
- Differs from native human IL-1Ra: single methionine residue at its amino terminus
- 153 AA, MW 17.3 kDa
- Anakinra interacts with pig IL-1α using the D10 bioassay
Outline PK- immunogenicity study with anakinra/Kineret® in Göttingen minipigs

- **Groups**
  - Vehicle – water for injection
  - Anakinra (Kineret®) - 0.5 mg/kg BW/day
  - Anakinra (Kineret®) – 5 mg/kg BW/day

- **Number of animals**
  - 3 ♂ and 3 ♀ per group

- **Treatment**
  - Daily for 28 days
  - Subcutaneous injection in the neck region

![Diagram showing treatment schedule and test procedures](chart.png)
Minipigs treated with anakinra/Kineret® develop anti-drug antibodies (ADA)
Pharmacokinetics of anakinra/Kineret® in minipigs

Figure 3. Anakinra plasma concentration vs time curves. Data shown are from Göttingen minipigs following a subcutaneous low (-○-) and high (-●-) dose (females) and low (-△-) and high (-▲-) dose (males) at Day 0 and Day 28. Mean values for each group are depicted.

- PK similar D0-28, Dose, Gender
- D28 Higher inter-individual variation - relation with formation of ADA
Minipig data compared to NHP and humans

<table>
<thead>
<tr>
<th></th>
<th>Anti Drug Antibodies</th>
<th>ADA neutralizing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minipig</td>
<td>Yes</td>
<td>Probably not</td>
</tr>
<tr>
<td>NHP</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Human</td>
<td>4-57%</td>
<td>2%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>$T_{\text{max}}$</th>
<th>$T_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minipig</td>
<td>2 - 4 h</td>
<td>1.5 - 2.4 h</td>
</tr>
<tr>
<td>NHP</td>
<td>2 h</td>
<td>1.9 - 2.7 h</td>
</tr>
<tr>
<td>Human</td>
<td>3 - 7 h</td>
<td>4 - 6 h</td>
</tr>
</tbody>
</table>

Comparable results are obtained with respect to the PK & immunogenicity testing of anakinra/Kineret® in minipigs and non human primates
Adalimumab (Humira®) and Infliximab (Remicade®)

- TNFα blockers used in chronic inflammation disorders such as rheumatoid arthritis

<table>
<thead>
<tr>
<th></th>
<th>Adalimumab</th>
<th>Infliximab</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Origin</strong></td>
<td>100% human</td>
<td>Chimeric mouse/human</td>
</tr>
<tr>
<td><strong>Dose</strong></td>
<td>40 mg / 2 wks</td>
<td>3-5 mg/kg / 6 wks</td>
</tr>
<tr>
<td><strong>Route</strong></td>
<td>Subcutaneous</td>
<td>Intravenous</td>
</tr>
<tr>
<td><strong>Biological active</strong></td>
<td>A.o. minipig, NHP, human</td>
<td>Chimpanzee and human</td>
</tr>
</tbody>
</table>
Outline PK- immunogenicity study with Adalimumab (Humira®) and Infliximab (Remicade®) in Göttingen minipigs

- **Groups**
  - Adalimumab – 0.1 mg/kg BW
  - Adalimumab – 1 mg/kg BW
  - Adalimumab – 5 mg/kg BW
  - Infliximab – 5 mg/kg BW

- **Number of animals**
  - 4 ♀ per group

- **Treatment**
  - days 0, 14, 28, 42, and 56
  - Subcutaneous injection in the neck region
  - 4 wk recovery period, necropsy day 84/85
Adalimumab levels decrease with ADA induction

0.1 mg/kg

![Graphs showing adalimumab and anti-adalimumab levels over study days.](image)
Adalimumab levels decrease with ADA induction

1 mg/kg

Adalimumab
anti-adalimumab
Adalimumab levels decrease with ADA induction

5 mg/kg
Serum adalimumab concentrations in RA patients decrease in presence of ADA

## Anti-adalimumab formation in cynomolgus monkeys

<table>
<thead>
<tr>
<th>Dose levels (mg/kg)</th>
<th>Administration route and frequency</th>
<th>N (per group)</th>
<th>% ADA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>s.c.</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>1, 3 and 10</td>
<td>i.v. single dose, s.c. single dose</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>i.v./s.c. 4x weekly</td>
<td>2/sex</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>i.v. single dose</td>
<td>3/sex</td>
<td>100</td>
</tr>
<tr>
<td>15.5</td>
<td>i.v. single dose, s.c. single dose</td>
<td>4</td>
<td>50, 75</td>
</tr>
<tr>
<td>15.5</td>
<td>i.v. 6x monthly, s.c. 6x monthly</td>
<td>4</td>
<td>50, 75</td>
</tr>
<tr>
<td>32</td>
<td>i.v. single dose</td>
<td>6/sex</td>
<td>17</td>
</tr>
<tr>
<td>32</td>
<td>i.v./s.c. 4x weekly</td>
<td>2/sex</td>
<td>12.5</td>
</tr>
<tr>
<td>32, 70.9 and 157.2</td>
<td>i.v. 4x weekly</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Sources: FDA / EMEA / J. of Imm. Methods vol 345, 2009, p17

- Human: anti-Fab
- Minipig: anti-Fab
- Cynomolgus: anti-Fab & anti-Fc
High plasma concentrations of Infliximab – no ADA

Induction of ADA against Infliximab in RA patients

Source: Sanquin Research, Amsterdam
Table 2. Pharmacokinetic parameters for adalimumab and infliximab following SC dosing to female Göttingen minipigs every other week for 8 weeks (n = 4 per group).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Adalimumab</th>
<th>Infliximab</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
<td>Day 56</td>
</tr>
<tr>
<td></td>
<td>0.1 mg/kg</td>
<td>1 mg/kg</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg.ml)</td>
<td>1.2 ± 0.1</td>
<td>7.9 ± 1.6</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (days)</td>
<td>3.0 ± 1.0</td>
<td>7.5 ± 1.0</td>
</tr>
<tr>
<td>Half-life (days)</td>
<td>0.7 ± 0.1</td>
<td>2.9 ± 3.6</td>
</tr>
<tr>
<td>$\text{AUC}_{(0-\infty)}$ (day*µg/ml)</td>
<td>8.5 ± 1.3</td>
<td>66.2 ± 25.9</td>
</tr>
<tr>
<td>Total clearance (L/kg*day)</td>
<td>0.012 ± 0.001</td>
<td>0.016 ± 0.007</td>
</tr>
</tbody>
</table>

| $T_{\frac{1}{2}}$ Minipig | 1 - 3 days | 13 - 20 days |
| $T_{\frac{1}{2}}$ Human    | 10 - 20 days | 15 - 17 days |
| $T_{\frac{1}{2}}$ NHP      | 14 - 21 days | 8 - 10 days |
Kineret:
From day 14 onwards ADA’s in all Minipigs
- Also in NHP (rhesus/cyno’s) ADA’s from day 14 onwards
- Like in NHP (rhesus/cyno’s) ADA’s are not neutralizing in minipigs
- In humans 3.8-57.2% of patients are ADA+, antibodies are limited (2%) neutralizing

Adalimumab:
From day 14 onwards ADA’s in 11/12 Minipigs
- Also in NHP (rhesus/cyno’s) ADA’s from day 14 onwards
- Like in NHP (rhesus/cyno’s) ADA’s are neutralizing in minipigs
- In humans approximately 70-80% of patients are ADA+

Infliximab (not biologically active in NHP and minipigs, only active in chimps and human):
No ADA’s detected against Infliximab in minipigs
- Also in NHP (cyno’s) no ADA’s detected
- In chimpanzee only low levels of ADA’s
- Is absence in ADA’s related to absence of biological activity of Infliximab in MP and cyno’s?

- Kineret T1/2 human ~ T1/2 NHP = T1/2 minipig
- Adalimumab T1/2 human = T1/2 NHP > T1/2 minipig
- Infliximab T1/2 human = T1/2 minipig ~ T1/2 NHP

Comparable results are obtained in respect to the immunogenicity testing of Kineret, Adalimumab and Infliximab in Göttingen Minipigs

Determine immunogenicity and PK to understand efficacy and safety
Current approach to predict oral bioavailability

Solubility data

HTS Permeability studies
PAMPA, Caco-2 cells, MDCKII-MDR1

In vitro human / animal metabolism studies

Animal pharmacokinetic and animal toxicity studies
However, correlation between animal data and human oral bioavailability is very poor...

![Graph showing animal versus human oral bioavailability](image)

*Oral bioavailability of 184 compounds in humans compared to mouse, rat, dog or non-human primates (NHP).*

From Münsther et al, 2013
Intestinal permeability: multiple transport routes and possible metabolism

Passive diffusion
- Transcellular
- Paracellular

Active Transport
- Uptake
- Metabolism
  - CYP3A
  - UGTs
  - MDR1
  - BCRP
  - MRP2
- Efflux

Large molecules and/or particles: endocytosis; uptake via M cells, Goblet cells
## Different intestinal epithelial barrier models

<table>
<thead>
<tr>
<th>Model</th>
<th>Similarity to human intestine</th>
<th>Complexity in vitro model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TNO</strong>: Porcine healthy intestinal tissue</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>an alternative for human tissue</td>
<td></td>
</tr>
<tr>
<td>PAMPA Artificial membrane</td>
<td>+ High-throughput, low costs</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>- No structural and biological properties</td>
<td></td>
</tr>
<tr>
<td>Caco-2 cells Transwells</td>
<td>+ Most well-established screenings cell model</td>
<td>Low</td>
</tr>
<tr>
<td></td>
<td>- Low paracellular transport</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Low metabolic CYP3A4 activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Variable expression of transporters</td>
<td></td>
</tr>
<tr>
<td>Minipig intestinal tissue</td>
<td>+ GI Physiology highly similar to human</td>
<td>High</td>
</tr>
<tr>
<td>“InTESTine”</td>
<td>+ More tissue form different regions available</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Some differences in metabolism/transport</td>
<td></td>
</tr>
<tr>
<td>Human intestinal tissue</td>
<td>+ Ultimate human model</td>
<td></td>
</tr>
<tr>
<td>“Ussing chamber”</td>
<td>- Low throughput</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Disease and drug history</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- <strong>Very limited availability of healthy tissue</strong></td>
<td></td>
</tr>
</tbody>
</table>
InTESTine™: Some features

- In house developed easy mounting system of ex vivo intestinal tissue from pigs
- Intestinal tissue from ‘left-over’ animals
- Disposable system, no contamination
- Horizontal exposure on a rocker platform
- In a high O₂/5% CO₂ humidified incubator
- Healthy GI tract available: regional differences
- Feasible with bio-relevant matrices
- Up to 96 incubations/experiment possible

According to 3R-mission of TNO:
No animals are sacrificed for this purpose only (‘left-over’ animals from minipig studies)
Measured apparent permeability data (Papp) of selected compounds

- Caco-2 cells Transwell
- Porcine jejunum InTESTine system
- Human jejunum Ussing chamber (1)

Data are average of at least three animals / humans

Especially for compounds with low, moderate permeability, the Papp values in porcine intestinal tissue are better comparable to Papp values in human intestinal tissue


Metabolism: CYP expression
Quantitative mRNA Expression relative to villin

21 days differentiated Caco-2 cells
N=5 Pig jejunum
N=4 Human jejunum

Figure simplified due to homologous forms of CYPs:
eg. CYP3A4 vs CYP3A46
CYP2D6 vs CYP2D25

Porcine intestinal tissue high CYP3A4 = comparable to human
Quantitative differences in CYP expression
The effect of these differences will be evaluated
Metabolism: UGT expression
Quantitative mRNA Expression relative to villin

21-days differentiated Caco-2 cells
N = 5 Pig jejunum
N = 4 Human jejunum

Porcine intestinal tissue high UGT1A1 en UGT1A10
= comparable to human
Quantitative differences in UGT expression
The effect of these differences will be evaluated

vdSteeg et al, 2014, in prep
Transporter protein abundance
Quantitative Protein abundance relative to villin using UPLC-MS/MS (outer plasma membrane$)

Abundance in Human intestinal tissue: ongoing
Differences in transporter abundance
The effect of these differences will be evaluated

Method adapted from Kamiie, et al. 2008
Minipig for oral bioavailability studies

- Additional model for oral bioavailability
- Similar physiologic characteristics involving the digestive system
- Porcine metabolic pathways (liver) have been found to be relatively similar to humans, with significant overlap in substrate specificity.
- GI tract - Differences & Similarities in drug metabolizing enzymes, drug transporters, permeability human – minipig tissue, relevance needs to be evaluated.
- Oral dosing via gavage (capsules) including 14C labelled test substance & metabolic cages for ADME – Mass balance studies.
\(^{14}\text{C}-\text{labelled LPS: a new marker to assess intestinal permeability in vivo}\)

- For maintenance of local and systemic homeostasis in optimal health, gut epithelial integrity is crucial;

- Barrier function of the gut can become disturbed due to unbalanced dieting, disease, pathogenic activity and other stressors;

- Compromised barrier function leads to leakage of the bacterial endotoxin LPS from the gut lumen into the systemic circulation, leading to systemic inflammation;

- Plasma levels of LPS is a physiologically-relevant read-out of intestinal barrier function.
Bacterial LPS:

- Endotoxin with diverse structure
- Pro-inflammatory
- Marker for gut permeability
- Difficult to measure
Reasons for using $^{14}$C-labelled LPS

- Currently, LPS assays are not very quantitative and not sensitive enough to detect very small changes in plasma levels.

- Plasma samples are easily contaminated with LPS from the environment;

- In healthy people, LPS plasma levels are estimated to be 0.06 pg/mL (approximately 0.00042 EU/mL);
Advantages of using a microdose of $^{14}$C-LPS via enterocoated capsules

- LPS is a physiologically relevant intestinal barrier marker;
- LPS is absorbed via different routes
- A microdose is defined as not therapeutic and/or bioactive.
- Kinetic profiling of plasma levels is possible
Accelerator Mass Spectrometer @TNO

- Small doses, huge machines,
- Zeptomole sensitivity: counting atoms
- Detector requires 100-1000 atoms for quantification
- Sensitivity down to ~10 atto (10^{-18}) gram/ml of plasma
Strategy to develop and evaluate $^{14}\text{C}$-LPS as intestinal permeability marker

- Biosynthesis, purification, quality check
- Set up analysis using Accelerator Mass Spectrometry (AMS)
- Permeability tests in \textit{in vitro} models (Caco-2, InTESTine)
- Permeability in \textit{in vivo} models (mini pig)
- Permeability test in humans (future)
**In vitro intestinal translocation of $^{14}$C-LPS**

- *In vitro* epithelial cell line: Caco-2 cells
  - Transport of $^{14}$C-LPS linear with time and concentration (up to 0.25 ng/ml LPS)
  - Permeability value ($P_{app}$) $0.30 \pm 0.11$ cm.$10^{-6}$/sec

- *Ex vivo* intestinal tissue segments: InTESTine™
  - Permeability value ($P_{app}$) $3.02 \pm 0.99$ cm.$10^{-6}$/sec

- Higher translocation of LPS in intestinal tissue than in Caco-2 cells because:
  - In intestinal tissue:
    - Larger paracellular pore size
    - Higher chylomicron synthesis facilitating LPS translocation
    - Presence of lipid rafts facilitating LPS translocation
Feasibility study:
In vivo intestinal translocation of orally dosed $^{14}$C-LPS in minipigs

- Subjects: 2 female minipigs
- Dose: 1.5 mg $^{14}$C-LPS (20 kBq)
- Formulation:
  - Pig 1 & 2: Enterocoated capsule
- Regular blood samples via vena jugularis (canulation)
- Plasma samples analyzed with AMS
  - total $^{14}$C in plasma
  - $^{14}$C-LPS in plasma
Results

- Rapid appearance of $^{14}$C–LPS in plasma
- Peak value at about 7 hours
- $^{14}$C specific label slowly disappears
Dermal studies
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Thank you very much for your attention

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