TOXICOLOGY SERVICES
• General toxicology in all species
• Special toxicology
  - infusion
  - Inhalation
  - Dermal
  - Ocular
• Immunotoxicology
• Regenerative medicine
• Reproductive toxicology including minipigs and NHPs
• Carcinogenicity studies also in rassH2 and p53+/- mice
• Genetic toxicology: ICH compliant package
• In vitro toxicology: BCOA, h-CLAT, KeratinoSens™, DPRA, Photo 3T3-NU, EpiSkin™, chicken eye test
• Agrochemical / chemical / REACH
• QSAR
• Physico-chemical testing
• Ecotoxicology: wide range of test species

SAFETY PHARMACOLOGY
• Integrated safety pharmacology in toxicity studies
  - CV (JET), BP
  - Respiratory (JET), plethysmography
  - CNS (FOB) and JET-EEG
• Safety pharmacology core battery
• In vitro assays
  - GLP compliant ion channel testing panel (hERG +5)
  - CNS ex vivo models for seizure liability screening
  - Screening and follow-up models
    - Rodent and non-rodent LVP telemetry
    - Anesthetized models
    - Polysomnography
    - Gastrointestinal motility

DMPK, BIOANALYSIS, BIOMARKERS
• 1C and 1ADME studies in all species
• In vitro metabolic clearance, metabolite ID and profiling, DDI package (metabolism and transporters)
• Bioanalysis: LC-MS/MS, GC-MS/MS, LC-ICP/MS, LC-Radiodetection, ELISA, RIA
• Toxicogenomics, miRNA: Affymetrix™ accredited service provider, next generation sequencing (illumina™)
• Immunology:
  - 10-color flow cytometer, Luminex, Meso Scale

MEDICAL DEVICE
• Biocompatibility testing
• Cardiovascular stents, electrophysiology and structural heart studies
• Long-bone defects and cranio maxillofacial/dental models
• Spinal fusion models
• Joint and cartilage repair models
• Regenerative medicine (growth factors, biomaterials, cell and gene therapy)

SPECIALIZED EXPERTISE
• Juvenile studies including minipigs
• Otoxicity in rats
• Fertility studies in rodents and NHPs
• Radiation safety and efficacy studies
• Drug transporter studies and Drug-Drug interactions
• Tissue Cross Reactivity (TCR): human and animal tissue banks
• Gene therapy vector biodistribution via pQCR
• ES cell testing: devTOX™ and cardioTOX™ (with Stemina)
• Lead optimization and predictive toxicology services: LeadScreen™

Effects of phenobarbital (PB)
acute exposure on minipig liver
gene expression

Jeremy Silvano, Philippe Ancian, Carine Bansard, Lysiane Richert, Jonas Nielsen, Pramila Singh and Roy Forster

GLP CERTIFIED

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Effects of phenobarbital (PB) acute exposure on minipig liver gene expression

Jeremy Silvano1, Philippe Ancian1, Carine Bansard1, Lysiane Richert², Jonas Nielsen1, Pramila Singh1 and Roy Forster1


INTRODUCTION
Phenobarbital (PB) is a widely used anti-seizure medication. Oral administration of PB in laboratory animals results in hepatomegaly and induction of CYP450 xenobiotic metabolizing enzymes in the liver. Long-term treatment of rats and mice with PB results in hepatic tumors. While PB has been extensively studied in rodents, little is known about the impact of PB treatment on the gene expression profile in minipig liver. The present study was aimed at analyzing the effect of repeated oral administration of PB on minipig liver gene expression using Affymetrix arrays.

MATERIAL AND METHODS
Three male Göttingen minipigs aged 4-5 months were treated orally with placebo (empty capsules) or 15 mg/kg/day PB for 6 days. On day 7, animals were euthanized and an 80 mg section of flushed liver was snap frozen and stored at -80°C pending RNA extraction. Total RNA was extracted from liver samples using a combined Trizol/RNaseasy method and cRNA product was then hybridized to Affymetrix GeneChips Porcine Arrays scanned using an Affymetrix Scanner 3000. Differentially expressed genes were filtered using an absolute fold-change threshold at 1.5 with a corrected p-value lower than 0.05. PCA (from all probes) and hierarchical clustering of (differentially expressed genes) were performed using the JMP Genomics software. Filtered genes were classified into controlled vocabularies (ontologies), associated with biological processes, cellular components and molecular functions. The classification was performed using David software v6.8 (NIADDK, NIH) to determine the gene ontology terms significantly enriched.

RESULTS
Treatment with phenobarbital PB was generally well tolerated. The PB treated minipigs were subdued and at necropsy hepatomegaly was observed (+46% increase in absolute liver weight compared with controls). Taken together, these results show that the selected dose was in the pharmacological range of PB. PCA revealed a clear separation of samples in the first principal component associated with the treatment (Figure 1). A total of 161 and 130 probesets were found to be significantly down and upregulated in minipig liver by PB treatment, respectively. Hierarchical clustering of samples was performed (using average linkage clustering with a Pearson correlation measure of similarity) and revealed a clear separation between the treated and the control groups (Figure 2).

Table 1: Selection of modulated genes by PB treatment in minipig liver

<table>
<thead>
<tr>
<th>Gene Title</th>
<th>Gene Symbol</th>
<th>Affymetrix</th>
<th>Change vs control</th>
<th>Ontology / Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytochrome P450, family 1, subfamily A, polypeptide 2</td>
<td>cyP1A2</td>
<td>1.58</td>
<td>1.62</td>
<td>1.71</td>
</tr>
<tr>
<td>cytochrome P450, family 3, subfamily IIIA, polypeptide 22</td>
<td>cyP3A22</td>
<td>ND</td>
<td>ND</td>
<td>1.27</td>
</tr>
<tr>
<td>cytochrome P450, family 4, subfamily A, polypeptide 24</td>
<td>cyP4A24</td>
<td>1.55</td>
<td>1.63</td>
<td>0.27</td>
</tr>
<tr>
<td>cytochrome P450, subfamily B, polypeptide 1</td>
<td>cyP7B1</td>
<td>-1.20</td>
<td>-1.38</td>
<td>-1.29</td>
</tr>
<tr>
<td>cytochrome P450, family 3A, subfamily A, polypeptide 29</td>
<td>cyP3A29</td>
<td>-1.20</td>
<td>-1.35</td>
<td>-1.07</td>
</tr>
<tr>
<td>cytochrome P450, subfamily C, polypeptide 42</td>
<td>cyP2c42</td>
<td>3.73</td>
<td>5.28</td>
<td>2.74</td>
</tr>
<tr>
<td>fatty acid desaturase 1</td>
<td>fADs1</td>
<td>11.31</td>
<td>17.35</td>
<td>16.39</td>
</tr>
<tr>
<td>glutathione s-transferase alpha 2</td>
<td>gS TA2</td>
<td>1.25</td>
<td>1.33</td>
<td>1.35</td>
</tr>
<tr>
<td>glutathione s-transferase A4-like</td>
<td>gS TA4</td>
<td>1.63</td>
<td>1.58</td>
<td>1.73</td>
</tr>
<tr>
<td>hypothetical protein-like</td>
<td>loc100521030</td>
<td>-2.07</td>
<td>-3.02</td>
<td>-2.25</td>
</tr>
<tr>
<td>loc100514063</td>
<td>UDP-glucuronosyltransferase 2B31-like</td>
<td>1.27</td>
<td>1.26</td>
<td>1.43</td>
</tr>
<tr>
<td>multidrug resistance protein 3-like</td>
<td>loc100522630</td>
<td>-1.31</td>
<td>-1.43</td>
<td>-1.41</td>
</tr>
<tr>
<td>sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1</td>
<td>sUlT1A1</td>
<td>11.62</td>
<td>7.43</td>
<td>11.08</td>
</tr>
<tr>
<td>tsukushin-like</td>
<td>loc100522628</td>
<td>16.62</td>
<td>18.05</td>
<td>16.42</td>
</tr>
<tr>
<td>UDP glucuronosyltransferase 1 family, polypeptide A1</td>
<td>UGT-A1</td>
<td>1.31</td>
<td>1.39</td>
<td>1.27</td>
</tr>
</tbody>
</table>

Transcripts of phase II metabolizing enzymes such as UGTs, SULT and GSTs were also found to be significantly up-regulated by PB treatment in minipig liver and among the top 20 modulated transcripts, 4 are involved in glucosonogenesis (SDS) and fatty acid metabolism (acetoacetyl-CoA synthetase-like and FADS).

PB is known to activate the transcriptional activity of CAR by translocation to the nucleus where the protein can bind to specific DNA response elements. In our data, there was no modulation of CAR expression levels, but CAR and PXR regulated genes showed increased expression (Tables 1 and 2).

Table 3: Significantly enriched pathways and ontology classifications in liver samples from treated animals

<table>
<thead>
<tr>
<th>Annotation cluster</th>
<th>Enrichment score</th>
<th>Gene sets</th>
<th>Data sets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathway 1</td>
<td>4.08</td>
<td>Gene set 1</td>
<td>Data set 1</td>
</tr>
<tr>
<td>Pathway 2</td>
<td>3.97</td>
<td>Gene set 2</td>
<td>Data set 2</td>
</tr>
</tbody>
</table>

Pathways and ontology classification using the DAVID software revealed that the oxireductase, retinol metabolism, cellular cancerogenesis and steroid hormone biosynthesis pathways were significantly enriched, with modulated genes such as CYP1A1 and SULT1A1 (Sulfotransferase Family 1A Member 1), as well as fatty acid desaturation FADS1 and FADS2 (Table 3).

CONCLUSION
This study improves our understanding of gene regulation and activation responses in the liver following acute oral PB administration in minipigs. Further investigation will help determine whether or not the transcriptional and post-transcriptional mode of action of PB demonstrated in rodents is reproduce in this non rodent model.

REFERENCE
Percutaneous administration of phenobarbital in rats and in guinea pigs of the Citoxlab strain induces the expression of cytochrome P450 isozyme (minor metabolism of parahydroxyphenobarbital). CYP2C24 can be considered as a porcine equivalent of hu-CYP2C19; this CYP isoform was strongly induced by PB treatment.

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