Use of Humanized Mice to Study the Function of Human Glucuronyltransferases

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Senior Director
Pfizer
Presentation Outline

- Introduction and hypothesis
  - Why would a humanized UGT mouse be useful?
  - Success and failure in predicting human pharmacokinetics
- Identifying a leading collaboration to advance the science
  - Dr Robert Tukey, UCSD (taking advantage of Pfizer and UCSD strengths)
- UGT Humanized mouse model
  - (UGT1A1*28/ugt1-/-) characterization
- In vitro results
  - Enzyme kinetics using UGT probe substrates with liver microsomes
- In vivo results
- Summary and future directions
The art of human pharmacokinetic predictions

- Multiple methods are used for human PK predictions
  - No consistency / limited rationale in method choice
- How to assess the confidence of these predictions
Thus, a retrospective analysis was conducted...

- **The data**
  - Collected clinical data on 50 drug candidates
  - Subdivided the results into major clearance mechanisms (P450, nonP450, passive, transport).

- **Re-predicted** (retrospectively), using multiple in vitro & in vivo methodologies

- **Assessed the method quality** of oral half-life predictions based on the accuracy relative to clinical findings

- **Identified the most predictive methods** considering all clearance mechanisms

Hosea et. al, J Clinical Pharmacology, May 2009
Thus, a retrospective analysis was conducted…

We learned that…

- There is increased confidence in predicting low (or moderate) clearance.
- Increased confidence is a function of agreement between experimental approaches.

- For P450-dependent clearance, agreement between preclinical species and human results in high confidence.
- For transport and non-P450-dependent clearance, confidence is medium or low.

Hosea et al, J Clinical Pharmacology, May 2009
One major gap in clearance predictions: UDP-Glucuronyltransferases

- A major pathway for drug elimination
  - UGT’s catalyze the conjugation of glucuronic acid to a nucleophilic substrate
  - Primary function is to inactivate by increasing the solubility of the conjugate to facilitate excretion
  - Primarily found in liver, but also the GI tract and kidney

- However, there are limited means for studying UGTs
  - In Vitro
    - Expressed UGT enzyme systems
    - Correlation analysis with HLM against an activity known to be specific for an UGT
  - Preclinical species-such as mouse, rat, dog, monkey
  - Species specific UGT enzymes with many unknowns
Human UGTs: 18 enzymes

- UGT1A5
- UGT1A3
- UGT1A4
- UGT1A1
- UGT1A6
- UGT1A7
- UGT1A8
- UGT1A10
- UGT1A9
- UGT2B10
- UGT2B28
- UGT2B11
- UGT2B7
- UGT2B4
- UGT2B15
- UGT2B17
- UGT2A1
- UGT2A2

- Bilirubin
  - Tertiary Amines
  - Heterocyclic Aromatics
- Drugs, Pollutants, $C_{18}$ Steroids
- Bioactive lipids
- Bile acids
- Androgens
- Odorant molecules
Role of UGT1A1 in Homeostasis

- Three forms of hyperbilirubinemia
- Crigler-Najjar Syndrome I
- Crigler-Najjar Syndrome II
  - Functional homozygous mutations in exons 1-5
- Gilbert’s Syndrome (UGT1A1*28)
  - Promoter mutation (with decreased Vmax)

Neonatal Jaundice

Phenobarbital treatment

↓ UGT1A1 ⇒ ↑ bilirubin

↑ UGT1A1 ⇒ ↓ bilirubin

Genetically Modified Mouse: Definitions

- Homozygous (ugt1-/- or ugt1+/+)
- Heterozygous (ugt1+/−)
- Transgenic (UGT1A1/ugt1+/+)
- Humanized (UGT1A1/ugt1-/-)

- These mice express all the human UGT1A enzymes except UGT1A1 being the genetic variant *28
The humanized mice do express UGT1A1, and the enzyme is inducible by phenobarbital.

Right panel shows individual human liver microsomes genotyped for the *1 and *28 alleles.
Characterization of the drug metabolism capacity of UGT humanized mice

- First, *keep it simple* (start in vitro)
  - Using 3 probe substrates, determine the enzyme kinetic parameters using hUGT1A1*28 and wt (C57BL6) mouse liver microsomes
  - Conduct a parallel assessment for human liver microsomes genotyped as UGT1A1*28 (Gilberts) and UGT1A1*1 (normal)
- Step 2, determine the pharmacokinetic profiles for the same compound in hUGT1A1*28 and wt mice
- Finally, use phenobarbital to alter enzyme levels and potentially, pharmacokinetic profiles
Tool Compounds

1. SN-38 (UGT1A1)
   - SN-38 glucuronide

2. Ezetimibe (UGT1A1, UGT1A3, 2B15)
   - Ezetimibe glucuronide

3. Naloxone (UGT2B7)
   - Naloxone glucuronide

EB 2010
SN-38 with human liver microsomes (wt or *28)

- $V_{max}$ decreased up to 8-fold between wt and *28 samples
- Consistent with $V_{max}$ and literature data (DMD, 2007)
SN-38 with MLM (wt, Hu*28, Hu*28 w/pb)

### Table

<table>
<thead>
<tr>
<th>MLM genotype</th>
<th>Km</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>3.6</td>
<td>201</td>
</tr>
<tr>
<td>Hu*28</td>
<td>2.4</td>
<td>44</td>
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<tr>
<td>Hu*28 w/pb</td>
<td>1.5</td>
<td>497</td>
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> Vmax 5-fold from Hu*28 to wt and 10-fold to Hu*28 w/pb

> Good in vitro mouse to human translation
Ezetimibe with HLM (wt or *28)

\[ v \text{ (pmol/min/mg)} \]

<table>
<thead>
<tr>
<th>HLM genotype</th>
<th>Km</th>
<th>Vmax</th>
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</thead>
<tbody>
<tr>
<td>HH112 (wt)</td>
<td>15</td>
<td>1515</td>
</tr>
<tr>
<td>HH9 (*28)</td>
<td>17</td>
<td>1619</td>
</tr>
<tr>
<td>HH81 (*28)</td>
<td>12</td>
<td>1330</td>
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</tbody>
</table>

\( \hat{\gamma} \) Vmax ↔, Minor role of UGT1A1 in Ezetimibe clearance
Ezetimibe with MLM (wt, Hu*28, Hu*28 w/pb)

\[ v \text{ (pmol/min/mg)} \]

**Concentration [\text{uM}]**

<table>
<thead>
<tr>
<th>MLM genotype</th>
<th>Km</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>15</td>
<td>859</td>
</tr>
<tr>
<td>Hu*28</td>
<td>36</td>
<td>1093</td>
</tr>
<tr>
<td>Hu*28 w/pb</td>
<td>20</td>
<td>1025</td>
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</table>

\[ V_{\text{max}} \leftrightarrow, \text{Consistent with HLM} \]
Naloxone with HLM (wt or *28)

<table>
<thead>
<tr>
<th>HLM genotype</th>
<th>Km</th>
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<tbody>
<tr>
<td>HH83 (wt)</td>
<td>58.5</td>
<td>1593</td>
</tr>
<tr>
<td>HH112 (wt)</td>
<td>54.6</td>
<td>2059</td>
</tr>
<tr>
<td>HH9 (*28)</td>
<td>34.5</td>
<td>1882</td>
</tr>
<tr>
<td>HH81 (*28)</td>
<td>63.1</td>
<td>1986</td>
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</table>

\[ \text{Vmax} \leftarrow, \text{consistent with no UGT1A1 involvement (UGT2B7 substrate)} \]
Naloxone with MLM (wt, Hu*28, Hu*28 w/pb)

<table>
<thead>
<tr>
<th>MLM genotype</th>
<th>Km</th>
<th>Vmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>278</td>
<td>1635</td>
</tr>
<tr>
<td>Hu*28</td>
<td>363</td>
<td>1787</td>
</tr>
<tr>
<td>Hu*28 w/pb</td>
<td>432</td>
<td>1723</td>
</tr>
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</table>

$\hat{V}_\text{max}$, consistent with HLM
Enzyme kinetics summary

- For SN-38 and HLM, the decrease in $V_{\text{max}}$ for HLM expressing the *28 genetic variant vs ‘wt’ was consistent with the literature.

- For SN-38 in MLM, $V_{\text{max}}$ shifts (between Hu*28 and wt and Hu*28 treated with pb) were consistent with what was seen w/ HLM (in vitro mouse to human translation).

- For Ezetimibe and Naloxone, there was consistent $V_{\text{max}}$ data between HLM and MLM.
Moving from In Vitro to In Vivo: Mouse PK Study Design

**Study Design**

- IV only (mainly liver UGT enzymes)
- All males for consistency
- Tail vein injection
- 3 time points per mouse
  - Retro-orbital bleed
  - One timepoint per eye and terminal bleed
- N = 2 (for most per time points) or 1

**PK parameters**

- AUC = area under curve
- Cl = IV dose/AUC
SN-38 PK at 1 mpk (wt, Hu*28, Hu*28 w/pb)

<table>
<thead>
<tr>
<th></th>
<th>mouse</th>
<th>wt</th>
<th>Hu*28</th>
<th>Hu*28 w/pb</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AUC (µM·h)</strong></td>
<td>0.14</td>
<td>0.535</td>
<td>0.149</td>
<td></td>
</tr>
<tr>
<td><strong>Cl (mL/min/kg)</strong></td>
<td>320</td>
<td>79.9</td>
<td>285</td>
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</tbody>
</table>

~4 fold differences in AUC and CL
Consistent with Vmax shifts in MLMs ⇒ **qualitative IVIVC** in mouse
SN-38 PK at 2 mpk (wt, Hu*28)

<table>
<thead>
<tr>
<th></th>
<th>mouse</th>
<th>wt</th>
<th>Hu*28</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (μM·h)</td>
<td>0.369</td>
<td>0.886</td>
<td></td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>230</td>
<td>95.9</td>
<td></td>
</tr>
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</table>

> 2 fold differences in AUC and CL
Ezetimibe PK at 1 mpk (wt, Hu*28)

<table>
<thead>
<tr>
<th></th>
<th>mouse</th>
<th>wt</th>
<th>Hu*28</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µM·h)</td>
<td>0.30</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>136</td>
<td>132</td>
<td></td>
</tr>
</tbody>
</table>

No difference in AUC or CL
Ezetimibe PK at 5 mpk (wt, Hu*28)

2-fold differences in AUC and CL
Partial role of UGT1A1 in Ezetimibe CL

<table>
<thead>
<tr>
<th></th>
<th>mouse wt</th>
<th>Hu*28</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µM·h)</td>
<td>1.71</td>
<td>3.25</td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>120</td>
<td>62.6</td>
</tr>
</tbody>
</table>
Naloxone PK at 5 mpk (wt, Hu*28)

<table>
<thead>
<tr>
<th>mouse</th>
<th>wt</th>
<th>Hu*28</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (µM·h)</td>
<td>1.31</td>
<td>1.21</td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>194</td>
<td>210</td>
</tr>
</tbody>
</table>

No difference in AUC or CL as expected
Summary

For SN-38 significant changes in pharmacokinetics were seen between Hu*28 and wt & Hu*28 with phenobarbital-treated mice, which is consistent with Vmax shifts in MLM (from hu*28 and wt and Hu*28 treated with phenobarbital) (qualitative IVIVC in mouse).

Ezetimibe exhibited small differences in pharmacokinetics at a high dose (5 mpk).

Overall, these mice are valuable in identifying the (major) role of UGT1A1 in overall clearance of a UGT substrate.

Can be used to understand and has the potential to predict UGT1A1-mediated clearance.
Acknowledgements

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