Endogenous Biomarkers for Inhibition of Hepatic Transporters

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Outline

• Endogenous biomarkers to study transporter-related DDIs

• Evaluation of cynomolgus monkey as a translational model to
  – Identify endogenous biomarkers for hepatic uptake transporters OATP1B
  – Predict OATP1B-related DDIs with statins

• Summary
Why Biomarkers for Drug Transporters?

- **Low confidence to quantitatively predict transporter-related DDIs**
  - IVIVE-PBPK model is premature
  - Preclinical animal models are often limited by potential species difference on transporters/ADME

- **It is desirable to predict transporter-mediated DDIs early in drug development when**
  - The clinically efficacious dose is not known yet
  - The design of relevant DDI studies is difficult

- **Biomarkers predictive for transporter inhibition may allow an early read on DDI potential of drug candidates in phase I studies**
Development of Biomarkers to Predict Transporter-Related DDIs Is Rapidly Growing
Can Endogenous Biomarkers for Liver Transporters Be Identified?

- Bilirubin (conjugated/unconjugated)
- Bile acids (CA, CDCA, GUDCA, etc)
- Thyroid hormones (T3, T4, FT3, FT4)

Cynomolgus Monkey: a Translational Model to Identify the Biomarkers for OATP1B?

- OATPs are evolutionarily conserved between human and cynomolgus monkeys

<table>
<thead>
<tr>
<th>Cynomolgus Monkey (%)</th>
<th>Rhesus Monkey (%)</th>
<th>Human (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cOATP1B1</td>
<td>100</td>
<td>99.9</td>
</tr>
<tr>
<td>cOATP1B3</td>
<td>76.7</td>
<td>99.3</td>
</tr>
<tr>
<td>cOATP2B1</td>
<td>27.2</td>
<td>99.9</td>
</tr>
</tbody>
</table>

- Cynomolgus OATP1B1/1B3/2B1 demonstrate a similar transporter activity and inhibition profile as human orthologs

<table>
<thead>
<tr>
<th>Compound</th>
<th>cOATP1B1</th>
<th>hOATP1B1</th>
<th>cOATP1B3</th>
<th>hOATP1B3</th>
<th>cOATP2B1</th>
<th>hOATP2B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF</td>
<td>0.20 ± 0.08</td>
<td>0.55 ± 0.07</td>
<td>1.4 ± 0.5</td>
<td>0.46 ± 0.13</td>
<td>69.1 ± 7.5</td>
<td>40.1 ± 5.1</td>
</tr>
<tr>
<td>CsA</td>
<td>1.0 ± 0.3</td>
<td>0.87 ± 0.29</td>
<td>0.50 ± 0.11</td>
<td>0.80 ± 0.22</td>
<td>&gt;50</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>0.49 ± 0.14</td>
<td>1.3 ± 0.3</td>
<td>1.3 ± 0.6</td>
<td>4.0 ± 1.1</td>
<td>4.5 ± 1.8</td>
<td>10.7 ± 2.3</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>20.2 ± 5.8</td>
<td>41.4 ± 9.0</td>
<td>22.4 ± 6.6</td>
<td>50.1 ± 27.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Verapamil</td>
<td>13.5 ± 2.8</td>
<td>14.8 ± 3.1</td>
<td>52.7 ± 15.9</td>
<td>87.3 ± 35.8</td>
<td>&gt;200</td>
<td>&gt;200</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>2.9 ± 0.9</td>
<td>1.6 ± 0.4</td>
<td>3.6 ± 1.5</td>
<td>5.5 ± 3.0</td>
<td>12.3 ± 4.8</td>
<td>9.4 ± 3.2</td>
</tr>
</tbody>
</table>

- DDIs between rosuvastatin-rifampin and pitavastatin-rifampin have been observed in cynomolgus monkeys

Objectives/Experimental Approaches

• Identify endogenous biomarkers suitable to assess DDIs involving OATP1B in cynomolgus monkeys
  – Evaluate the effect of single dose rifampin on endogenous biomarkers
    • Unconjugated and conjugated bilirubin
    • Bile salts
    • Thyroid hormones
  – Establish IVIVC and mechanistic understanding
    • cOATP1B1, cOATP1B3, cNTCP, and cMRP2

• Evaluate if cynomolgus monkeys are a suitable translational model to predict OATP1B-related DDIs in humans
  – Evaluate the effect of single dose rifampin on PK of OATP1B probes
    • Rosuvastatin
    • Atorvastatin
Effect of Rifampin on Various Endogenous Biomarkers in Cynomolgus Monkey Plasma

- Significant elevation of conjugated and unconjugated bilirubin and GCA was observed, which correlated with rifampin exposure.
In VivoDisposition of Unconjugated and Conjugated Bilirubin

In Vitro Transport of Probe Substrates for cOATP1B1, cOATP1B3, cNTCP, and cMRP2

- Rifampin is a potent inhibitor for cOATP1B1, cOATP1B3, but not for cNTCP and cMRP2
Can Cynomolgus Monkey Be Used As a Translational Model to Study OATP-Mediated DDIs?
Rosuvastatin and Atorvastatin

**Rosuvastatin (RSV)**
- OATP1B1/1B3/2B1/NTCP
- Liver
- Bile
- Blood

**Atorvastatin (ATV)**
- OATP1B1/1B3/2B1/NTCP
- Liver
- CYP3A4
- 2-OH ATV, 4-OH ATV
- Bile
- Blood

Liver and bile are involved in the metabolism and excretion of both Rosuvastatin and Atorvastatin. BCRP (Breast Cancer Resistance Protein) is also present in the gut and lumen, potentially affecting the absorption and distribution of these drugs.
RSV and ATV Are Substrates for cOATP1B1, cOATP1B3, cOATP2B1, and cNTCP

Rosuvastatin (RSV)

- **cOATP1B1**
  - HEK293-cOATP1B1
  - HEK293

- **cOATP1B3**
  - HEK293-cOATP1B3
  - HEK293

- **cOATP2B1**
  - HEK293-cOATP2B1
  - HEK293

- **cNTCP**
  - HEK293-cNTCP
  - HEK293

Atorvastatin (ATV)

- **cOATP1B1**
  - HEK293-cOATP1B1
  - HEK293

- **cOATP1B3**
  - HEK293-cOATP1B3
  - HEK293

- **cOATP2B1**
  - HEK293-cOATP2B1
  - HEK293

- **cNTCP**
  - HEK293-cNTCP
  - HEK293

Manuscript to be submitted
Effect of Oral Rifampin on Rosuvastatin (RSV) PK in Cynomolgus Monkeys

• Rifampin showed a significant impact on RSV pharmacokinetics by inhibition of hepatic clearance and gut absorption
• In humans, RIF (600 mg PO) increased plasma AUC of RSV by 5.2-fold and \( C_{\text{max}} \) by 9.9-fold

\[ \text{RSV (3 mg/kg PO)} \]

\[ \text{RSV (1 mg/kg IV)} \]

Prueksaritanont et al., 2014

Manuscript to be submitted
Effect of Rifampin on Atorvastatin (ATV) PK in Cynomolgus Monkeys

- Rifampin caused <2-fold change of plasma AUC, $C_{\text{max}}$, and CL of ATV
- In humans, Rifampin 600 mg SD PO and IV increased plasma AUC of ATV by 8.5-12 fold\textsuperscript{1-2} and 7.3-fold\textsuperscript{3}
- **Why lack of translation: more extensive metabolism in cynomolgus monkey?**
  Is OATP1B-mediated uptake by ATV not rate-limiting in cynomolgus monkeys?

Summary

- Conjugated and unconjugated bilirubin could be sensitive endogenous biomarkers for inhibition of hepatic OATP1B in cynomolgus monkeys
  - Inhibition of other transporters, such as MRP2, cannot be excluded

- Utility of cynomolgus monkeys as a translational model to predict OATP-mediated DDIs with statins is compound dependent
  - Need to consider the species differences on ADME of drugs between cynomolgus monkeys and humans
Key Considerations on Transporters Biomarker Approaches

• Endogenous biomarkers could become a valuable tool to assess transporter-related DDI liability early in drug development

• Limitations/Caveats
  – Complex formation and disposition for endogenous biomarkers
  – Lack of selectivity: the drugs can have an impact on multiple pathways of endogenous biomarkers disposition and/or formation
  – Translation of drug-endogenous probe interactions to DDIs?
  – Sensitivity and how to extrapolate to patient population?

• Measurement of endogenous probes cannot replace in vitro and clinical DDIs studies
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