Application of Endogenous Probes for Early Assessing OATP Drug-drug interactions in Human

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Gilead Sciences
Acknowledgement


Drug Metabolism, Gilead Science
Outlines

- Current strategies to assess clinical DDIs for OATP inhibitor
- Endogenous probes for OATP inhibition
  - Preclinical evaluation
  - Clinical studies
  - Comparisons
- Summary
Current FDA DDI decision trees for OATP inhibition

Is total $C_{\text{max}}/IC_{50}$ of the investigational drug $\geq 0.1$ for OATP1B1 or OATP1B3?

Yes

Is the AUC of statin (e.g., rosuvastatin, pravastatin, pitavastatin) predicted to increase $\geq 1.25$-fold in the presence of the investigational drug using extrapolation (e.g., $R$-value$^{[a]} \geq 1.25^{[b]}$)?

Yes

In vivo DDI study with a sensitive substrate (e.g., rosuvastatin, pravastatin, pitavastatin)

No

In vivo study is not needed

No

In vivo study may not be needed

$R = 1 + (fu \ast I_{\text{in, max}}/ IC_{50})$

EMA

Is $K_i \leq$ than 25$^\circ \text{C} \text{max}_{\text{inlet}}$ (hepatic uptake transporters), 50$^\circ \text{C} \text{max}_{u}$ (hepatic efflux and renal transporters) or 0.1$^\circ \text{dose/250}$ (intestinal efflux transporters)?

Cut-offs: $R \geq 1.25$ or 1.04 (EMA)
Variability in Ki or IC50 of inhibitors

Relationships between observed AUCR and $\frac{I_{\text{max(free)}}}{K_i}$ or IC50.

The potential for a high rate of false-positive (and negative) prediction has been a particular concern.

(Prueksaritanont et al., 2013; Tweedie et al., 2013)

Dilemma also remains:

- Timing of human DDI studies
- Dose—how high?
- Selection of probe substrates
- Extrapolating DDIs to different population or ethnic group
- ___
## Endogenous OATP1B substrates

<table>
<thead>
<tr>
<th>Category</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bile acids</strong></td>
<td>Cholic acid</td>
</tr>
<tr>
<td></td>
<td>Glycocholic acid</td>
</tr>
<tr>
<td></td>
<td>Glycoursodeoxycholic acid</td>
</tr>
<tr>
<td></td>
<td>Tauroliothocholic acid 3-sulfate</td>
</tr>
<tr>
<td></td>
<td>Taurocholic acid</td>
</tr>
<tr>
<td></td>
<td>Tauroursodeoxycholic acid</td>
</tr>
<tr>
<td><strong>Thyroid hormones</strong></td>
<td>Thyroxine</td>
</tr>
<tr>
<td></td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td></td>
<td>Iodothyronine sulfates</td>
</tr>
<tr>
<td><strong>Eicosanoids</strong></td>
<td>Leukotriene C₄</td>
</tr>
<tr>
<td></td>
<td>Leukotriene E₄</td>
</tr>
<tr>
<td></td>
<td>Prostaglandin E₂</td>
</tr>
<tr>
<td></td>
<td>Thromboxane B₂</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td>Bilirubin</td>
</tr>
<tr>
<td></td>
<td>Bilirubin monoglucuronide</td>
</tr>
<tr>
<td></td>
<td>Bilirubin diglucuronide</td>
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<tr>
<td></td>
<td>CCK-8</td>
</tr>
<tr>
<td></td>
<td>DHEAS</td>
</tr>
<tr>
<td></td>
<td>E₂17βG</td>
</tr>
<tr>
<td></td>
<td>E₃S</td>
</tr>
</tbody>
</table>
Bilirubin and bile acids

Of 18 probes tested, Rifampin caused significant elevation of both conjugated and unconjugated bilirubin.

AUC and Cmax of rosvastatin (p.o) was significantly increased by 6 and 10.3 fold respectively, in the presence of rifampin

Chu et al DMD 2015
Dehydroepiandrosterone-3-sulfate (DHEAS)

Substrate: OATP1B1/1B3/2B1/1A2, OAT3/4

Watanabe, et al Drug Metab. Pharmacokinet. 2015, 30 (2), 198-204.

<table>
<thead>
<tr>
<th>DHEAS</th>
<th>(C_{\text{max}}) (ng/mL)</th>
<th>AUC(_{0.5-8h}) (ng h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>83.3 ± 38.3</td>
<td>530 ± 282</td>
</tr>
<tr>
<td>+2 mg/kg RIF</td>
<td>101 ± 38</td>
<td>609 ± 240*</td>
</tr>
<tr>
<td>+10 mg/kg RIF</td>
<td>165 ± 80*</td>
<td>1030 ± 550*</td>
</tr>
</tbody>
</table>

*p < 0.05, compared to the control values obtained without RIF administration.

Rifampin (10 mpk) caused significant elevation of plasma DHEAS (~2-fold \(C_{\text{max}}\) and AUC0.5~8h) in Monkey
Fatty acids and bile acid metabolites

- CsA caused elevation of plasma of fatty acids (TDA and HDA) and bile acid metabolites.

- TDA and HDA is a substrate of OATP1B1, OAT1 and OAT3

Note: X-11529: Glycochenodeoxycholate glucuronide; X-13429: glycodeoxycholate sulfate or isomer; TDA: Tetradecanedioate; HDA: Hexadecanedioate; X-1105: Unknown; X-14626: Unknown; 4-Andros.: 4-androsten-3-beta,17-beta-diol disulfate 2°; ODA: Octadecanedioate; TLS: Tauroliothicholate 3-sulfate; X-11440: Unknown; X-12850: Glycochenodeoxycholate sulfate or isomer; 1,5-AG: 1,5-anhydroglucitol.
Learning from “Rotor Syndrome”

Rotor Syndrome: complete deficiency of OATP1B1 & 1B3

**Coproporphyrin I**

**Coproporphyrin III**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Urinary Ratio of CP I (%)</th>
<th>Urinary Ratio of CP III (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference Subjects</td>
<td>~30</td>
<td>~70</td>
</tr>
<tr>
<td>Rotor Syndrome</td>
<td>~70</td>
<td>~30</td>
</tr>
</tbody>
</table>

Van de Steeg et al., *JCI* 2012, 122:519
Coproporphyrin I and III (CP I and III)

- CP I and CP III are byproducts of heme synthesis.
- They are not further metabolized by the liver and excreted in bile and urine. CP I predominates (70%) over CP III in bile whereas the reverse is found in urine.
Substrate specificity of CPs

- CP-I and CP-III are substrates of OATP1B1 and 1B3
- Both CP-I and III are not substrates for OAT1/2/3/4, OCT2, MATE1 and MATE2k
- CPs are metabolically stable in human, monkey and mouse hepatocytes

(Shen et al., JPET 2016)
Deletion of Oatp1a/1b genes increased plasma conc. and urinary excretion of CPs in mice

CP Plasma Levels at 3 h:
7.4- and 15.2-fold for CP-I and III, respectively

CP Urinary Excretion over 24 h:
12.4- and 18.4-fold for CP-I and III, respectively

- Oatp1a/1b cluster genes deleted: Slco1a1, 1a4, 1a5, 1a6 and 1b2.

Shen et al., JPET 2016, 357: 382
CsA (and RIF) increased plasma CPs and urinary excretion of CP-I in monkeys

<table>
<thead>
<tr>
<th>Fold Change</th>
<th>CP I</th>
<th>CP III</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$</td>
<td>3.3</td>
<td>6.9</td>
</tr>
<tr>
<td>$AUC_{(0-24\text{ h})}$</td>
<td>2.6</td>
<td>5.2</td>
</tr>
</tbody>
</table>

Shen et al., *JPET* 2016, 357: 382
Clinical study on CPs as probes for OATP inhibition


Phase 1 (n= 12)
600 mg RIF, PO
Day 1

Phase 2 (n= 11)
5 mg RSV, PO
Day 8

Phase 3 (n= 11)
600 mg RIF + 5 mg RSV, PO
Day 15

Sample Collection at Each Phase:
- Plasma: pre-dose + PK samples
- Urine: -7 to 0, 0 to 7, and 7 to 24 h
RIF significantly increased plasma conc. of CP-I and CP-III, and urinary excretion of CP-I

<table>
<thead>
<tr>
<th>Fold Change</th>
<th>CP-I (RIF, RIF+RSV)</th>
<th>CP-III (RIF, RIF+RSV)</th>
<th>RSV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$</td>
<td>5.7, 5.9</td>
<td>5.4, 6.5</td>
<td>13.2</td>
</tr>
<tr>
<td>$AUC_{(0-24\ h)}$</td>
<td>4.01, 3.8</td>
<td>3.4, 3.3</td>
<td>5.0</td>
</tr>
<tr>
<td>$X_e \ (0-24\ h)$</td>
<td>3.6, 3.4</td>
<td>2.1, 1.34*</td>
<td>1.6, 1.4</td>
</tr>
</tbody>
</table>

*NS
RIF increased (marginally) the levels of total- and indirect-, but not direct-bilirubin in plasma.
RIF did not increase the level of plasma DHEAS
RIF significantly increased HTD and TDA

(A) HDA
- Period 1 - RIF Alone
- Period 2 - RSV Alone
- Period 3 - RIF + RSV

(B) TDA
- Period 1 - RIF Alone
- Period 2 - RSV Alone
- Period 3 - RIF + RSV
RIF increased the level of bile acids in plasma (statistically significant)
Comparison of effect size of endogenous biomarkers detected in RIF treated subjects

(A) RIF

(B) RIF + RSV
Comparative Evaluation of Plasma Bile Acids, Dehydroepiandrosterone Sulfate, Hexadecanedioate, and Tetracanedioate with Coproporphyrins I and III as Markers of OATP Inhibition in Healthy Subjects

Hong Shen, Wei Qi Chen, Dieter M. Drexler, Sandhya Mandlekar, Vinay K. Holenarsipur, Eric E. Shields, Robert Langish, Kurex Sidik, Jinping Gan, W. Griffith Humphreys, Punit Marathe, and Yurong Lai

Pharmaceutical Candidate Optimization (H.S., W.C., R.L., J.G., W.G.H., P.M., Y.L.) and Global Biometrics Sciences (K.S.), Bristol-Myers Squibb Company, Princeton, New Jersey; Pharmaceutical Candidate Optimization, Bristol-Myers Squibb Company, Wallingford, Connecticut (D.M.D., E.F.S.); Bristol-Myers Squibb India Pvt. Ltd. (S.M.) and Syngene International Ltd. (V.K.H.), Biocon BMS R&D Center, Bangalore, India

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Consideration of Endogenous probes for DDI assessment

• **Specific**
  - A specific endogenous substrate of the transporter
    - **Questioning HAD/TDA, DHEAS**
  - Not a biomarker of a disease or a dietary constituent—
    - **Questioning bile acids, and bilirubin as probes**
  - No or minimum involvement of metabolic enzymes
    - **Questioning on bile acid and hormone metabolites**
  - Reflect instant response, v.s. delayed (compensatory) effects

• **Predictive and Translational**
  - Correlate with the extent of transporter inhibition, e.g fold increases of statins?
  - Reflect site of inhibition, e.g I_max/IC50

Modified from Dr. Giacomini AAPS transporter workshop 2015
Additional consideration of endogenous probes for transporter DDI assessment

- **Accessible**
  - Non-invasive sampling from either blood or urine
  - Can be monitored during early drug development phase, e.g. Phase I does finding trials

- **Reproducible**
  - Rapid, accurate and reproducible detection, e.g. LC/MS

- **Cost effective**
  - Manageable lists
Summary

- Plasma CP-I and CP-III are relatively consistent, with minimum fluctuation over the three study periods.
- CPs do not involved into metabolic enzymes, and seem to specific to OATP transporters.
- The determination of CPs is amendable to high throughput/GLP setting, and can be monitored during phase I dose finding trials.
- CPs in plasma are the most sensitive markers reflecting RIF inhibition, as compared to other reported markers.
- Changes of CPs with less potent OATP inhibitors, and the impacts of genetic polymorphisms, gender/age, disease state, and organ impairments etc need to be further investigated.
Integration of endogenous probes into OATP DDI decision trees

**Is the drug candidate a potent OATP inhibitor?**
- **Yes**: Phase I does escalation trials:
  - Is the concentrations of endogenous probe panel e.g CPs in plasma and/or urine significantly increased (e.g. ≥1.25-fold) in the presence of the investigational drug across all dose ranges?
  - **Yes**: In vivo human study to assess OATP inhibition DDIs
  - **No**: In vivo study may not be needed
- **No**: No OATP inhibition DDI liability

In vivo study may not be needed
Thank you