Coproporphyrin I and III as endogenous biomarkers for transporter-mediated Drug-Drug Interactions

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Jessica Riley, Shells
Artwork from Reflections Art in Health
Why all the efforts?

Liver hepatocytes

Kidney proximal tubule cells

Transporter Activity → Tissue & Systemic Exposure → Efficacy & Safety
Why all the efforts?

“The first step in solving a problem is admitting that there is a problem to be solved”

Transporter DDI prediction performance based on IVIVE EMA cut-off criteria

- **OATP1B**
  - 7% Missed (FN)
  - 31% False DDI (FP)
  - 62% Correctly predicted (TN+TP)
  - Based on 107 DDI studies

- **OAT**
  - 0% Missed (FN)
  - 34% False DDI (FP)
  - 66% Correctly predicted (TN+TP)
  - Based on 59 DDI studies

- **OCT2/MATE**
  - 0% Missed (FN)
  - 30% False DDI (FP)
  - 70% Correctly predicted (TN+TP)
  - Based on 33 DDI studies

- **P-GP**
  - 11% Missed (FN)
  - 23% False DDI (FP)
  - 66% Correctly predicted (TN+TP)
  - Based on 53 DDI studies

- Moderate in vivo DDI predictability based on in vitro IC_{50} data
- In contrast to PBPK DDI prediction for metabolism-based DDIs, predictions for transporter-based DDIs has less confidence
- Conservative guidance from health authorities result in increased number of unnecessary clinical DDI studies

#Lee et al (FDA). Advanced Drug Delivery Reviews. 116 (2017) 100-118
Biomarker validation

The challenge

Selectivity

Sensitivity

Robustness
The right timing...

Coproporphyrin (CP) I & III:
- Metabolic byproduct of the heme synthesis
- Light sensitive compounds: must be protected from light at all times
- Not metabolized but secreted in urine and bile as intact molecules
  - Secretion involves **OATP1B1, OATP1B3 & MRP2**

Rifampicin-Rosuvastatin DDI study:
- **in vitro** OATP1B inhibitor
- **in vivo** increased rosuvastatin AUC~5-fold; Cmax~13-fold

Part 1:
Method development and proof of concept
(retrospective analysis of DDI studies)
Qualified Analytical method

- Chromatography: 8.5 min run
  - MFA: 0.01 M ammonium formate + 0.1% FA
  - MFB: MeCN

- LLOQ and range in 2% BSA (Surrogate matrix):
  - Range 0.020 – 100 ng/mL (6500)
  - SIL of CPI and CP-III (\(^{15}\)N4)

- Sample prep: SPE (Oasis MAX)
  - Sample volume 200 µL
  - Injection solvent: 6M FA/ACN (3/1, v/v)

- Analytical column –
  - 150 mm x 3mm x 2,0 µm Ace Excel 2, C18 PFP

Strong perpetrator

<table>
<thead>
<tr>
<th>Perpetrator</th>
<th>Rifampicin 600 mg p.o. HV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>in vitro</strong></td>
<td>OATP1B1 (IC₅₀ &lt; 3 µM) OATP1B3 (IC₅₀ &lt; 3 µM)</td>
</tr>
<tr>
<td><strong>in vivo</strong></td>
<td>increases Cmax of Rosuvastatin by 13-fold and AUC by 5-fold</td>
</tr>
</tbody>
</table>

→ **Strong in vivo OATP1B perpetrator**

Part 2:

Scientific validation of an LC-MS/MS method to quantify CP-I and CP-III in plasma samples
Assessment involves a comparison of calculated concentrations of the unspiked matrix pool determined by two methods: 1) extrapolation of the spiked authentic matrix curve through the negative x-axis using the method of standard addition and 2) calculation by direct measurement using the surrogate matrix calibration curve (interpolation). Agreement between these values serves as a demonstration of parallelism.
Scientific Validation: Design

1. Parallelism and dilution integrity:
   - Surrogate matrix: 2% BSA in 50 mM PBS, pH 7.2
   - Acceptance criteria for calibration curve: $|\% \text{ RE}| < 20$ (25@LLOQ)
   - QC’s in plasma: Which levels to use in the validation?
     - Acceptance criteria for QC’s: $|\% \text{ RE}| < 20$ (25% @LLOQ level)

2. Selectivity and matrix effect:
   - Required or not?

   - Criteria for plasma: $|\% \text{ RE}| < 20\%$. Solvent: $|\% \text{ RE}| < 10\%$
Scientific Validation: Design

1. Parallelism (2%BSA as surrogate matrix):

   — Calibration curve in **surrogate matrix** and **plasma: to evaluate curves**
   — 1 A&P run
   — QC’s prepared in plasma at five levels (n=6)

<table>
<thead>
<tr>
<th></th>
<th>CP-I</th>
<th>CP-III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adapted Range</strong></td>
<td>0.05-10 ng/mL</td>
<td>0.025-5 ng/mL</td>
</tr>
<tr>
<td></td>
<td>0.08-15.3 nM</td>
<td>0.04-7.6 nM</td>
</tr>
<tr>
<td>QC-Blank</td>
<td>Blank</td>
<td>Blank</td>
</tr>
<tr>
<td>QC-LLOQ</td>
<td>Blank +0.05</td>
<td>Blank +0.02</td>
</tr>
<tr>
<td>QC-L</td>
<td>Blank + 0.2</td>
<td>Blank + 0.1</td>
</tr>
<tr>
<td>QC-M</td>
<td>Blank + 1</td>
<td>Blank + 0.5</td>
</tr>
<tr>
<td>QC-H</td>
<td>Blank + 5</td>
<td>Blank + 2</td>
</tr>
</tbody>
</table>
Scientific Validation: Design

1. Parallelism (2%BSA as surrogate matrix):

--- Concentration of QC blank determined by 2 approaches:
   a. From surrogate calibration curve
   b. Extrapolation from calibration curve established with QC’S

Criteria 1:

\[
\frac{(\text{Endo}_{\text{surrogate curve}}) - (\text{Endo}_{\text{plasma curve}})}{\text{mean}((\text{Endo}_{\text{surrogate curve}}) + (\text{Endo}_{\text{plasma curve}}))} < 25\%
\]
Scientific Validation: Results

1. Parallelism curve (surrogate matrix vs plasma)

- **CP-I**: High response with plasma curve
- **CP-III**: Nearly superimposable curves: Slightly high response with 2%BSA due to less matrix effect?
Scientific Validation: Results

Parallelism: CP-I

<table>
<thead>
<tr>
<th>Endo_{plasma} curve (ng/mL)</th>
<th>0.270</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Endo_{surrogate} curve (ng/mL)</td>
<td>0.28</td>
</tr>
<tr>
<td>Parallelism (%E)</td>
<td>5.37</td>
</tr>
</tbody>
</table>

Parallelism: CP-III

<table>
<thead>
<tr>
<th>Endo_{plasma} curve (ng/mL)</th>
<th>0.028</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Endo_{surrogate} curve (ng/mL)</td>
<td>0.025</td>
</tr>
<tr>
<td>Parallelism (%E)</td>
<td>-12.6</td>
</tr>
</tbody>
</table>
Scientific Validation: Results

1. Dilution integrity: CP-I and CP-III

- QC’s prepared in plasma were diluted 10 and 20-fold in surrogate matrix (2%BSA)
- Diluted QC calibrated with calibration line in surrogate matrix

<table>
<thead>
<tr>
<th>Conc. (ng/mL)</th>
<th>Dilution of 1 ng/mL</th>
<th>Accuracy (%)</th>
<th>Dilution of 0.5 ng/mL</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 (n=3)</td>
<td>20-fold</td>
<td>86</td>
<td>10-fold</td>
<td>91.5</td>
</tr>
<tr>
<td>0.1 (n=3)</td>
<td>10-fold</td>
<td>94</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>
## Scientific Validation: Results

### 2. Selectivity and matrix effect:
- **Required or not?**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>Suggestions?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrix effect</td>
<td>Not done because SIL was used.</td>
<td></td>
</tr>
<tr>
<td>Selectivity</td>
<td>Not done because of the endogenous presence of CP-I and CP-III</td>
<td>Other columns/solvents</td>
</tr>
</tbody>
</table>

### 3. Stability in solvent and LTS:
- Stability in DMSO: 7 months @ -20°C and 3 days at RT
- LTS: 141 days @ -20°C
Conclusion and perspective

- **Biomarker validation**
  - CP-I and CP-III are sensitive, selective and robust biomarkers: OATP1B1, 1B3

- **Scientific validation:**
  - Proven parallelism and dilution integrity
  - 2% BSA as surrogate matrix = appropriate matrix for calibration curve

- **First-in-man studies:**
  - Two studies were supported
  - IC$_{50}$ of investigational compounds were in the range to trigger DDI studies
  - DDI liability is being assessed for the investigational drugs

- **Future perspective:**
  - Continue to generate data to support CP-I and CP-III as endogenous markers to predict DDI liabilities
  - Discuss with regulators
Acknowledgements

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Thank you

Jessica Riley, *Shells*
Artwork from Reflections Art in Health
Summary: Retrospective Biomarker Evaluation

➢ **Transporter Selectivity:**
  - CPI: OATP1B-MRP pathway
  - CPIII: OATP-MRP pathway

➢ **Biomarker Sensitivity:**
  - CPI exposure allows predictions of mild, moderate & strong OATP1B-mediated DDIs
  - CPIII exposure allows predictions of moderate & strong OATP-mediated DDIs

➢ **Biomarker Robustness:**
  - CP baseline levels are constant over time
  - Consistent baseline levels obtained in different studies
  - Not all samples were collected in amber tubes. Back-up samples with less exposure to light were useful

Biomarker Validation – *In Vitro* Transporter Selectivity

**Uptake Transporters**

- **OATP1B1**
- **OATP1B3**
- **OATP2B1** (CPIII only)
- × OCT1
- × OCT2
- × OAT1
- × OAT2
- × OAT3
- × OAT4
- × NTCP

**Efflux Transporters**

- **MRP2**
- **MRP3**
- × MRP4
- × MATE1
- × MATE2-K
- × P-gp
- × BCRP
- × BSEP

Biomarker Validation – Clinical Sensitivity

Moderate perpetrator

<table>
<thead>
<tr>
<th>Perpetrator</th>
<th>Simeprevir: 150 mg q.d. HV (Cmax ~ 4 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>in vitro</td>
<td>OATP1B1 (IC$_{50}$ 0.7 μM)</td>
</tr>
<tr>
<td></td>
<td>OATP1B3 (IC$_{50}$ 0.6 μM)</td>
</tr>
<tr>
<td>in vivo</td>
<td>increases Cmax and AUC of Rosuvastatin by 3-fold</td>
</tr>
</tbody>
</table>

→ Moderate in vivo OATP1B perpetrator

Simeprevir: 150 mg q.d, HCV patients

- CPI
- CPIII

**Case Example: Internal Application**

FDA **new product** label information: Simeprevir: **150 mg qd**

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*Simeprevir Inhibits OATP1B1/3 and P-glucoprotein (P-gp) transporters. Co-administration of the **new product** with drugs that are substrates for OATP1B1/3 and P-gp transport may result in increased plasma concentration of such drugs.*

“**Would a dose-reduction to 100 mg or 75 mg Simeprevir still result in an OATP1B-mediated DDI liability? Could a label impact be avoided?**”

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*A. Kunze et al. 2018. Clinical Pharmacokinetics. 1-12*
Case Example: Internal Application

- **Simeprevir: 75 mg q.d., HCV patients**
  - CP plasma concentration (nM)
  - Simeprevir plasma concentration (µM)

- **Simeprevir: 100 mg q.d., HV**
  - CP plasma concentration (nM)
  - Simeprevir plasma concentration (µM)

- **Simeprevir: 150 mg q.d., HCV patients**
  - CP plasma concentration (nM)
  - Simeprevir plasma concentration (µM)

- Ø 75 mg Simeprevir in HCV patients is unlikely to cause OATP1B-mediated DDIs
- Ø Consistent results obtained for CP measurements in 3 independent clinical studies