Discovery of Endogenous Biomarkers for Organic Anion Transporter-mediated Drug-Drug Interactions

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Disclosure

• Co-Founder of Apricity Therapeutics, 2013
  • Transporters in Therapeutics
Endogenous Biomarkers

• A biological indicator of:
  – normal function
  – disease progression
  – drug target interaction
  – drug response
  – Drug itself or enzyme/transporters that govern its PK-ADME-DDI profile
Transporter Mediated Drug-Drug Interactions Can Cause Toxicities

• Cerivastatin was withdrawn from the market in 2001 because many cases of rhabdomyolysis

• Half of the cases where patients using cerivastatin + gemfibrozxil.


• Gemfibrozil ↑↑ cerivastatin plasma concentration 5.6 fold

OATP1B1 (SLCO1B1) and CYP2C8 Mediated
In Vitro Studies Trigger Clinical Drug-Drug Interaction Studies


OATP1B1/1B3, OCT2, OAT1/3, P-gp, BCRP, MATE1

Is the NME an inhibitor of OCT2, OAT1 or OAT3?
Criteria: determine the IC_{50} of NME against MPP^+, for OCT2; PAH for OAT1 or OS for OAT3 or other model substrates

Yes

Unbound C_{max}/IC_{50} of the NME ≥ 0.1
Clinical DDI study with a sensitive substrate (see footnote)

Unbound C_{max}/IC_{50} of the NME < 0.1
DDI study is not needed

No

Poor or not an inhibitor of OCT2, OAT1 or OAT3
In Vitro IC$_{50}$ Does Not Translate to In Vivo

The Effect of Nizatidine, a MATE2K Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin in Healthy Volunteers

Kari M. Morrissey$^{1,3}$ · Sophie L. Stocker$^{1,4}$ · Eugene C. Chen$^{1,5}$ · Richard A. Castro$^1$ · Claire M. Brett$^2$ · Kathleen M. Giacomini$^1$

DOI 10.1007/s40262-015-0332-9

The Effect of Famotidine, a MATE1-Selective Inhibitor, on the Pharmacokinetics and Pharmacodynamics of Metformin

Jennifer E. Hibma$^{1,2}$ · Arik A. Zur$^1$ · Richard A. Castro$^1$ · Matthias B. Wittwer$^1$ · Ron J. Keizer$^1$ · Sook Wah Yee$^1$ · Srijib Goswami$^1$ · Sophie L. Stocker$^1$ · Xuexiang Zhang$^3$ · Yong Huang$^3$ · Claire M. Brett$^4$ · Radojka M. Savic$^1$ · Kathleen M. Giacomini$^1$

DOI 10.1007/s40262-015-0346-3
**In Vitro IC\(_{50}\) Does Not Translate to *In Vivo***

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- Poor or not an inhibitor of OCT2, OAT1 or OAT3

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Can endogenous biomarkers serve as *in vivo* biomarkers?

**Clinical relevant drug interactions**
Approach/Tool to Identify Endogenous Biomarker of Transporters

<table>
<thead>
<tr>
<th>Methods</th>
<th>In Vitro</th>
<th>In Vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Transfected cell lines exposed to media and</td>
<td>Mouse: Knockout; cynomolgus or human:</td>
</tr>
<tr>
<td></td>
<td>serum</td>
<td>with and without inhibitor; human: with and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>without specific genotype</td>
</tr>
<tr>
<td>Examples</td>
<td>Thiamine and OCT1</td>
<td>Mice/Primate: Bilirubin and OATP1B1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human: N(^1)-Methylnicotinamide and MATE1;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6β-hydroxycortisol and OAT3</td>
</tr>
<tr>
<td>Limitations</td>
<td>Time point, bovine serum may not reflect</td>
<td>Primate: Candidate metabolites Mouse:</td>
</tr>
<tr>
<td></td>
<td>human</td>
<td>Differences to human</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Human: Not know about another inhibitor,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>different disease state</td>
</tr>
</tbody>
</table>
Endogenous Biomarkers on Drug Metabolizing Enzymes (CYP3A4)

**Goals:**
Utilize endogenous biomarker to:

(i) Avoid dosing of CYP3A probe (e.g. midazolam)

(ii) CYP3A activity
Endogenous Biomarkers on Drug Metabolizing Enzymes (CYP3A4)

Plasma (metabolite/parent): 4β-Hydroxycholesterol/cholesterol

Plasma and urine: 6β-Hydroxycortisol/cortisol (renal clearance)
Endogenous Biomarkers on Drug Metabolizing Enzymes (CYP3A4)

Plasma (metabolite/parent):
4β-Hydroxycholesterol/cholesterol

Plasma and urine: 6β-Hydroxycortisol/cortisol (renal clearance)
Rifampicin, CYP3A4 Inducer
Ketoconazole, CYP3A4 Inhibitor
Affect CYP3A4 Metabolite

Endogenous Biomarkers on Drug Metabolizing Enzymes (CYP3A4)

Plasma (metabolite/parent):
4β-Hydroxycholesterol/cholesterol

Plasma and urine: 6β-Hydroxycortisol/cortisol (renal clearance)
Endogenous Biomarkers on Drug Metabolizing Enzymes (CYP2D6)

Goal: Phenotyping using endogenous biomarkers, an alternative method of assessing CYP2D6 activity.

Examples: CYP2D6 substrates: 5-methoxy-N,N- dimethyltryptamine, pinoline, progesterone, anandamide
Endogenous Biomarker of Transporters

Human Clinical DDI Study
Genomewide Association Studies (GWAS) Approach to Identify Biomarkers of OATP1B1


- Disease/Physical Traits: 1974 (81.2%)
- Metabolites and Others In Serum/Plasma: 275 (11.3%)
- Pharmacogenomics: 182 (7.5%)

https://www.ebi.ac.uk/gwas/
Organic Anion Transporter, OATP1B1, Is Expressed in Abundance in the Liver

Representative Pharmacologic Substrates of OATP1B1

- Simvastatin
- Pravastatin
- Rosuvastatin
- Repaglinide
- Glyburide
- Docetaxel
- Saquinavir
Organic Anion Transporter, OATP1B1, Is Endogenous Biomarkers

Goal: To discover endogenous metabolites of OATP1B1
- Reveal its biological role
- Use as biomarkers of potential drug-drug interactions

Representative Endogenous Biomarkers of OATP1B1
- Bilirubin and conjugates
- Bile acids
- Coproporphyrin I

Hepatocyte
Discover Endogenous Metabolites of \textit{SLCO1B1}: Genomewide Association Studies

N > 7,000 Individuals- Blood Sample*

Metabolomic Approach

200 - 500 Metabolites

Genomewide Chip

GWAS of Each Metabolite

Which metabolites associate with \textit{SLCO1B1, 521T>C, Val174Ala})?

Examples of Metabolites Associated with SNPs in the SLCO1B1 Locus Are Discovered

- X-11529: SLCO1B1
  - p<10^-300
- Tetradecane-dioic Acid: SLCO1B1
  - p<10^-60
- Hexadecane-dioic Acid: SLCO1B1
  - p<10^-59
- CYP4A: SLCO1B1
  - p<10^-69
- X-11429: SLCO1B1
  - p<10^-50
- X-11905: SLCO1B1
  - p<10^-50
- X-14626: SLCO1B1
  - p<10^-50
20 Metabolites Associated with *SLCO1B1*, Val174Ala, rs4149056 (p<5x10^{-8})

<table>
<thead>
<tr>
<th>Group</th>
<th>Metabolites</th>
<th>Best p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile Acids Conjugates</td>
<td>Glycochenodeoxycholate glucuronide, glycodeoxycholate sulfate</td>
<td>6 x 10^{-315}</td>
</tr>
<tr>
<td>Fatty Acid Dicarboxylic acid</td>
<td>Tetradecanedioic acid, hexadecanedioic acid, octadecanedioic acids</td>
<td>3 x 10^{-59}</td>
</tr>
<tr>
<td>Lysolipid</td>
<td>1-arachidonoylglycerophosphoethanolamine, 1-arachidonoylglycerophosphoinositol</td>
<td>4 x 10^{-31}</td>
</tr>
<tr>
<td>Steroid Conjugates</td>
<td>4-androsten-3beta,17beta-diol-disulfate, 5alpha-androstan-3beta,17beta-diol-disulfate</td>
<td>1 x 10^{-18}</td>
</tr>
</tbody>
</table>
Validate Metabolites as Biomarkers of OATP1B1: Clinical Pharmacokinetic Studies

OATP1B1 (Val174Ala)
Ten TT
Seven TC
Three CC

Pravastatin

Time (hr)

Pravastatin (ng/mL)

Two doses of placebo/CSA:
7 pm night before +
30 min before pravastatin

Manuscript under reviewed
If a metabolite such as X-11529 is an endogenous substrate of OATP1B1, its levels will increase in patients treated with cyclosporine.
Validate Metabolites as Biomarkers of OATP1B1: Clinical Pharmacokinetic Studies

Manuscript under reviewed
Validate Metabolites as Biomarkers of OATP1B1: Cellular Studies

Uptake of Metabolites (Fold Over Empty Vector)

- TDA
- TDA+inh.
- HDA
- HDA+inh.

Manuscript under reviewed
TDA and HDA Are Not Substrates and Inhibitors of OATP1B3, OATP2B1 and OATP1A2
What about specificity? Renal Organic Anion Transporters?

**a**

<table>
<thead>
<tr>
<th>Metabolite Uptake</th>
<th>OAT1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDA</td>
<td>1</td>
</tr>
<tr>
<td>TDA+Inh.</td>
<td>2</td>
</tr>
<tr>
<td>HDA</td>
<td>3</td>
</tr>
<tr>
<td>HDA+Inh.</td>
<td>4</td>
</tr>
</tbody>
</table>

**b**

<table>
<thead>
<tr>
<th>Metabolite Uptake</th>
<th>OAT3</th>
</tr>
</thead>
<tbody>
<tr>
<td>TDA</td>
<td>5</td>
</tr>
<tr>
<td>TDA+Inh.</td>
<td>6</td>
</tr>
<tr>
<td>HDA</td>
<td>7</td>
</tr>
<tr>
<td>HDA+Inh.</td>
<td>8</td>
</tr>
</tbody>
</table>
Summary and Future Studies

1. Novel metabolic substrates of OATP1B1 have been identified through GWAS.

2. These metabolites were also increased after cyclosporine administration in human healthy volunteers.

3. In vitro studies confirmed that these metabolites are substrates.

4. Future studies:
   • Replicate and validate these in other clinical studies with OATP1B1, OAT1 and OAT3 inhibitors.
   • Determine the sensitivity, selectivity, other factors that could modulate these biomarkers.

Dicarboxylate Fatty Acids
How will Biomarker be Used?

IND Phase I Study

Measure Biomarker Before And After Drug Administration

e.g. CYP3A4, SLCO1B1

If Biomarker(s) Increases: Consider Clinical DDI Study

If No Increase: No Clinical DDI Study

X-11529

A metabolite or a panel

How will Biomarker be Used?

IND Phase I Study

Measure Biomarker Before And After Drug Administration

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X-11529

A metabolite or a panel
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