Probe-drug cocktails to assess transporter activity in humans: Concept, probe selection and in vitro evaluation

Thomas Ebner, Naoki Ishiguro, Mitchell Taub
Background

- Increasing role of transporters recognized for PK, safety and efficacy in the clinic
- Regulatory agencies require assessment of potential drug-drug interactions (DDI)

however...

- Difficult extrapolation of in vitro results to clinical effect due to lack of specific substrates and inhibitors, complex experimental systems
- Recognized high variability of results between experimental systems, different labs

Highly conservative approach taken by regulators to ensure patient safety
- Expensive and time consuming
- Complex for transporters
- Inconsistent between FDA and EMA
If a drug interacts with one transporter, it will most likely interact with many transporters

- A single clinical DDI study may not be sufficient to explore the entire „interaction space“ between a drug and a single transporter.
- Most transporter DDI studies result in slight exposure changes compared to CYP450 DDI studies and are often not critical for safety (except for NTR drugs).
- Result: Lengthy labels due to excessive transporter DDI studies

Example: For telaprevir, effects on/by 21 comedications (+ 2 post approval) were investigated
In vivo transporter cocktail studies - Alternative approach

Cocktail DDI studies as a possible problem solver?

Mentioned / recommended by FDA (draft) and EMA guidelines

”Negative results from a well-conducted cocktail study may eliminate the need for further evaluation of particular CYP enzymes and transporters.”

EMA: Half-page (section 5.4.2) dedicated to cocktail studies

“In vivo cocktail studies may also be used to replace studies of the in vitro inhibition and induction potential of parent drug (and metabolites) on enzymes (and transporters).”
In vitro data → in vivo transporter cocktail studies: Pros and cons

Pros & Potential Benefits:
- Recommended in current FDA (draft) and EMA DDI guidances
- Addresses potential hepatic and extrahepatic DDIs
- Covers interactions caused by parent drug and/or its metabolites
- Can be designed to assess time-dependent inhibition, induction, complex DDIs
- Avoids uncertainties of IVIVE predictions and simulations

Cons & Challenges:
- No previously published / validated cocktails
- No specific test drugs for transporter in vivo DDI studies
- No specific test inhibitors for in vivo cocktail validation
- Overlapping substrate specificity for transporters
- Potential co-interaction of test drugs with transporters and DMEs
Conceptual questions & requirements for suitable probe drugs

- Cassette size – how many probes, how many target transporters?
- Only transporters or combined assessment of DMEs and transporters?
- Conventional dosing or microdose?
- Which probe drugs?

- Probe drugs must be safe, and should be selective and sensitive
- No mutual interaction of probe drugs
- Address key processes of transporter-mediated drug disposition

- hepatic uptake by OATPs
- renal elimination by OATs, OCTS, MATEs
- intestinal secretion by P-gp, BCRP
## Selectivity

<table>
<thead>
<tr>
<th>Drug</th>
<th>P-gp</th>
<th>BCRP</th>
<th>MRP2</th>
<th>OATP1B1</th>
<th>OATP1B3</th>
<th>OATP 2B1</th>
<th>OAT1</th>
<th>OAT3</th>
<th>OCT2</th>
<th>MATE1</th>
<th>S/I other transporters</th>
<th>Metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cimetidine</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
<td>S / I</td>
<td>S / I</td>
<td>S / I</td>
<td>S / I</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Cyclosporine A</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Fexofenadine</td>
<td>S</td>
<td></td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
<td>S</td>
<td>S</td>
<td></td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
<td>S</td>
<td></td>
<td></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Pravastatin</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
<td></td>
<td>yes</td>
<td>minor</td>
<td>minor</td>
</tr>
<tr>
<td>Probenecid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>S / I</td>
<td>S</td>
<td></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Rifampin</td>
<td>I</td>
<td>I</td>
<td>S / I</td>
<td>S / I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>S</td>
<td></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
<td>S / I</td>
<td></td>
<td></td>
<td>yes</td>
<td>minor</td>
<td>minor</td>
</tr>
</tbody>
</table>

- S: substrate
- I: inhibitor

- There are most likely no clinically usable drugs available that interact with only a single defined transporter and are not metabolized.
- Compromises must be made with respect to selectivity of cocktail probe drugs.
Safety & applicability in an industrial setting

• Single dosing
• Low dose size of marketed drug
• Only drugs with acceptable safety profile
  – potential OAT probe drug tenofovir is considered unsuitable
• Marketed globally – easily accessible and usable in the clinic
  – potential OAT probe drug cephradine is marketed only in a few countries
• Avoid manufacturing steps to prepare cocktail test medication
## Proposed four-component transporter cocktail – PK properties

<table>
<thead>
<tr>
<th></th>
<th>Digoxin</th>
<th>Rosuvastatin</th>
<th>Furosemide</th>
<th>Metformin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target transporters</strong></td>
<td>(P-gp)</td>
<td>(OATP, BCRP)</td>
<td>(OAT1, OAT3)</td>
<td>(OCT2, MATEs)</td>
</tr>
<tr>
<td><strong>Dose in cocktail (mg)</strong></td>
<td>0.25</td>
<td>10</td>
<td>5</td>
<td>500</td>
</tr>
<tr>
<td><strong>Half-life (h)</strong></td>
<td>18 - 36</td>
<td>21</td>
<td>2</td>
<td>4 - 6</td>
</tr>
<tr>
<td><strong>Bioavailability (%)</strong></td>
<td>60 - 80 (tablet)</td>
<td>20</td>
<td>65 (tablet)</td>
<td>50 - 60</td>
</tr>
<tr>
<td><strong>PPB (fu, %)</strong></td>
<td>75</td>
<td>12</td>
<td>1.3 - 4.1</td>
<td>100</td>
</tr>
<tr>
<td><strong>Route of elimination (i.v. dosing)</strong></td>
<td>51% urine</td>
<td>28% urine</td>
<td>83% urine</td>
<td>100% urine</td>
</tr>
<tr>
<td></td>
<td>15% feces</td>
<td></td>
<td>7.5% feces</td>
<td></td>
</tr>
<tr>
<td><strong>Metabolism</strong></td>
<td>minor</td>
<td>minor, ~20%</td>
<td>minor</td>
<td>negligible</td>
</tr>
<tr>
<td><strong>In vitro transporter (recommended by guidelines)</strong></td>
<td>P-gp, OATP1B3, OCT2</td>
<td>P-gp, BCRP, OAT3, OATP1B1, OATP1B3</td>
<td>BCRP, OAT1, OAT3</td>
<td>MATE1, MATE2-K, OCT2, P-gp, BCRP</td>
</tr>
</tbody>
</table>

# In vitro transport inhibition of cocktail components

<table>
<thead>
<tr>
<th></th>
<th>In vitro inhibitory effect, IC\textsubscript{50} (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P-gp</td>
</tr>
<tr>
<td><strong>Digoxin</strong></td>
<td>125</td>
</tr>
<tr>
<td><strong>Rosuvastatin</strong></td>
<td>&gt;400</td>
</tr>
<tr>
<td><strong>Furosemide</strong></td>
<td>&gt;2,000</td>
</tr>
<tr>
<td><strong>Metformin</strong></td>
<td>&gt;30,000</td>
</tr>
</tbody>
</table>

- IC\textsubscript{50} values generally high
- Static DDI model indicates only remote risk for mutual interactions

Transporter profiling of furosemide

Passive permeability and efflux ratio of furosemide in CaCo-2 cells and effects of inhibitors of P-gp and BCRP

Uptake of furosemide in SLC-transporter expressing HEK293 cells

Transporter profiling of furosemide

- Furosemide is a substrate of BCRP, OAT1, OAT3, OATP1B1, OATP1B3 and probably of MRP2
- Furosemide is no substrate of P-gp, OCT2, MATE1 and MATE2-K

Uptake of furosemide in OATP expressing HEK293 cells and effect of OATP inhibitor rifampicin

Conclusion

- Mutual DDI upon oral administration of the four selected drugs is unlikely to occur.
- Based on literature and in vitro data, a 4-component probe drug cocktail is proposed for clinical validation trials:
  - digoxin
  - rosuvastatin
  - furosemide
  - metformin
- Proposed low doses of probe drugs are expected to be clinically safe.
- Suitable dose strengths are readily available (globally).
## Transporter probe drug cocktails – current status

<table>
<thead>
<tr>
<th>Originator</th>
<th>Cocktail 1</th>
<th>Cocktail 2</th>
<th>Cocktail 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Boehringer Ingelheim</td>
<td>Merck</td>
<td>Gilead</td>
</tr>
<tr>
<td>First published</td>
<td>2015</td>
<td>2016</td>
<td>2017 (Poster)</td>
</tr>
<tr>
<td>Dosing /formulation</td>
<td>Conventional</td>
<td>Microdose</td>
<td>Conventional</td>
</tr>
<tr>
<td>Pathway / Target</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A</td>
<td>None</td>
<td>Midazolam (10 µg)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atorvastatin (50 µg)</td>
<td></td>
</tr>
<tr>
<td>P-gp</td>
<td>Digoxin (0.25 mg)</td>
<td>Dabigatran etexilate (0.375 mg)</td>
<td>Dabigatran etexilate (75 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atorvastatin (50 µg)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosuvastatin (25 µg)</td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td>Rosuvastatin (10 mg)</td>
<td>Rosuvastatin (25 µg)</td>
<td>Rosuvastatin (10 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atorvastatin (50 µg)</td>
<td></td>
</tr>
<tr>
<td>OATP1B1, OATP1B3</td>
<td>Rosuvastatin (10 mg)</td>
<td>Pitavastatin (10 µg)</td>
<td>Rosuvastatin (10 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosuvastatin (25 µg)</td>
<td>Pravastatin (20 mg)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Atorvastatin (50 µg)</td>
<td></td>
</tr>
<tr>
<td>OAT1 and OAT3</td>
<td>Furosemide (5 mg)</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>OCT2, MATE1, MATE2-K</td>
<td>Metformin (500 mg)</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Summary and Acknowledgements

- A 4-component cocktail is proposed for clinical validation
- Transporter probe drug cocktails are recognized as valuable tool for DDI assessment
- Further evolution of concepts and new clinical data of transporter cocktail trials will provide purpose-driven cocktail approaches to various aspects of transporter DDIs
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