Transport vs. metabolism: what determines the PK and PD of drugs?
Insights from the extended clearance model

Gabby Patilea-Vrana
Tuesday, 6/20/17

Dr. Jashvant Unadkat Lab
Department of Pharmaceutics
University of Washington
How does the extended clearance model help elucidate the implication of transporters and enzymes in PK and PD of drugs?

- Rate-determining step(s) to clearance – 4 scenarios
  - Site(s) of action/toxicity
  - Implications for DDI predictions
  - Applicability to IVIVE
- Quantitative methods for assessing the rate-determining step
Simple liver model

Mechanistic liver model

Legend:
- Drug
- Metabolite
- Passive diffusion
- Sinusoidal active transport
- Canalicular active transport
- Metabolism
**Hepatic extended clearance model (ECM)**

**Advantages:**
- Permeability limitations acceptable
- Active transport present (sinusoidal and canalicular membranes)
- More comprehensive

**Assumptions:**
- Sinusoidal transport \( CL = \) active + passive
- Canalicular transport \( CL \) is active only
- Metabolism and canalicular efflux are parallel (thus additive) processes
- Linear kinetics

\[
CL_{\text{hepatic}} = \frac{Q_h \times fuCL^s_{\text{in}} \left(CL_{\text{met}} + CL^c_{\text{ef}} \right)}{Q_h \left(CL^c_{\text{ef}} + CL_{\text{met}} + CL^c_{\text{ef}} \right) + fuCL^s_{\text{in}} \left(CL_{\text{met}} + CL^c_{\text{ef}} \right)}
\]
Scenario 1: When does sinusoidal uptake clearance determine hepatic clearance of drugs?

When \( CL_{ef}^s \ll CL_{met} + CL_{ef}^c \)

then

\[
CL_h = \frac{Q_h fuCL_{in}^s}{Q_h + fuCL_{in}^s}
\]

When sinusoidal efflux is significantly smaller than metabolism and canalicular (biliary) efflux, sinusoidal uptake becomes the rate-determining step to hepatic clearance despite presence of metabolism and biliary excretion.
Scenario 1: Sinusoidal uptake is the rate-determining step in hepatic clearance

Condition: $CL_{ef}^S \ll (CL_{met} + CL_{ef}^C)$

Assumptions:
- Liver is the only eliminating organ
- 90% inhibition

Liver System

No inhibition

Inhibition of sinusoidal uptake $CL$  

Inhibition of metabolism and canalicular efflux $CL$

$CL_{in}^S \uparrow \quad CL_{ef}^S$

$CL_{in} \downarrow \quad CL_{ef}^C$

$CL_{ef} \downarrow \quad CL_{met}$

$CL_{ef} \downarrow \quad CL_{met}$

Patilea-Vrana & Unadkat. CPT. 2016
Atorvastatin – Systemic PK ≠ PD

> Inhibition of OATPs and not CYP3A4 ↑ atorvastatin plasma AUC

> ↓ OATP activity, ↔ hepatic AUC, ↔ PD

> ↓ CYP3A activity, ↑ hepatic AUC, ↑ PD

Shitara et al., Biopharm Drug Dispos. 2013 34:45-78
Maeda. Biol Pharm Bull. 2015 38:155-68
CAUTION: atorvastatin AUC increases when CYP inhibitors are given orally

↑ 4.5 fold
ATORVA + clarithromycin

F_g ↑
F_h ↔
PET imaging as a tool to elucidate tissue drug disposition

**Coronal 2 min SUV images of \(^{11}\text{C}\)-Rosuvastatin**

- **A** - RIF OATP inh
- **B** + RIF OATP inh

**Rosuvastatin:**
- \(\log \text{D} = -0.33\)
- Substrate of OATPs
- Substrate of BCRP and MRP2
- Metabolized in the rat, minimal metabolism in humans
- <30% renally excreted

He et al., Mol Pharm. 2014
PET imaging elucidates liver exposure of rosuvastatin in the rat

When the liver is the primary elimination organ, inhibition of rosuvastatin uptake does not change the hepatic AUC in the rat but it impacts the hepatic concentration-time curve.

He et al., Mol Pharm. 2014
Summary – Scenario 1

When $\text{CL}_{\text{ef}}^S << (\text{CL}_{\text{met}} + \text{CL}_{\text{ef}}^C)$, $\text{CL}_{\text{in}}^S = \text{rate-determining step}$

- Inhibition of $\text{CL}_{\text{in}}^S$ will increase systemic but not hepatic AUC when the liver is the main elimination organ. The hepatic conc.-time curve will change.
- Inhibition of $\text{CL}_{\text{met}} + \text{CL}_{\text{ef}}^C$ will increase hepatic but not systemic AUC

Circumstances arise when systemic AUC $\neq$ PD response

Special consideration for oral dosing if significant Fg
Scenario 2/3: When does metabolic and/or canalicular efflux clearance determine hepatic clearance of drugs?

When $CL_{in}^s = CL_{ef}^s \gg (CL_{met} + CL_{ef}^c)$

then $CL_h = \frac{Q_h fu(CL_{met} + CL_{ef}^c)}{Q_h + fu(CL_{met} + CL_{ef}^c)}$

Metabolism and canalicular efflux become the rate-determining step(s) when the sinusoidal membrane is essentially transparent and no longer a barrier to distribution.
Docetaxel – hepatic CYP3A/P-gp interplay

Knock-out mice | Systematic AUC fold ↑
--- | ---
Mdr1a/1b−/− | 2
Cyp3a−/− | 4.9
Cyp3a−/−/Mdr1a/1b−/− | 17

Toxicity observed only in the dual KO mice

When $CL_{in}^S = CL_{ef}^S >> (CL_{met} + CL_{ef}^C)$ then $CL_{met} + CL_{ef}^C = RDS$

- When sinusoidal membrane is not a barrier to distribution, metabolism and canalicular efflux determine hepatic CL

- Because $CL_{met}$ and $CL_{ef}^C$ are parallel elimination pathways, not accounting for canalicular transport can cause
  > Underprediction of hepatic CL during IVIVE
  > Overestimation of the impact of metabolism on drug disposition for inhibitors that are dual transporter/enzyme inhibitors
Scenario 4: When do all hepatobiliary clearances determine hepatic clearance of drugs?

Many drugs will have characteristics (moderate or low passive diffusion or relatively low metabolic/canalicular efflux clearance) where their systemic clearance will be determined by both transport (sinusoidal/canalicular) and metabolism. The full ECM is needed.
Scenario 4: All hepatobiliary clearance pathways determine hepatic clearance

Condition: $CL_{ef}^s \geq \text{or} \leq (CL_{met} + CL_{ef}^c)$ and $CL_{in}^s \neq CL_{ef}^s$

No inhibition

Inhibition of sinusoidal uptake $CL$

Inhibition of sinusoidal efflux $CL$

Inhibition of metabolism and canalicul efflux $CL$

Assumptions:
- Liver is the only eliminating organ
- 90% inhibition

Patleia-Vrana & Unadkat. CPT. 2016
Repaglinide transporter/enzyme interplay

<table>
<thead>
<tr>
<th>Inhibition</th>
<th>OATPs</th>
<th>CYP3A fm=0.3</th>
<th>CYP2C8 fm=0.7</th>
<th>Systemic AUC fold ↑</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1 polymorphism</td>
<td>⊗</td>
<td></td>
<td></td>
<td>2</td>
<td>←</td>
</tr>
<tr>
<td>Itraconazole</td>
<td></td>
<td>⊗</td>
<td></td>
<td>1.4</td>
<td>←</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>⊗</td>
<td>⊗</td>
<td></td>
<td>1.6</td>
<td>←</td>
</tr>
<tr>
<td>Cyclosporine</td>
<td>⊗</td>
<td>⊗</td>
<td></td>
<td>2.4</td>
<td>↑</td>
</tr>
<tr>
<td>Gemfibrozil</td>
<td>⊗</td>
<td>⊗</td>
<td></td>
<td>8</td>
<td>↑↑</td>
</tr>
<tr>
<td>Itraconazole + Gemfibrozil</td>
<td>⊗</td>
<td>⊗</td>
<td>⊗</td>
<td>20</td>
<td>↑↑↑</td>
</tr>
</tbody>
</table>

> When none of the extreme conditions apply, both metabolism and transport play a role-determining role in hepatic clearance

## How to identify the rate-determining step(s)?

<table>
<thead>
<tr>
<th>Method</th>
<th>Pros</th>
<th>Cons</th>
<th>( CL_{\text{met}} )</th>
<th>( CL_{\text{in}} )</th>
<th>( CL_{\text{ef}} )</th>
<th>( CL_{\text{c_ef}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLM, cytosol, S9</td>
<td>Good IVIVE for CYPs</td>
<td>Need proper co-factors and test S9/cytosol for non CYP/UGT metabolism</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicles</td>
<td>Easy, fast, available</td>
<td>Mixture of inside-out/right-side out vesicles</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Transfected cell lines</td>
<td>Assess impact of individual transporters</td>
<td>Need expression based scaling factors to reflect in vivo</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Might miss transporters</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Suspended hepatocytes</td>
<td>Easy, fast</td>
<td>Need selective inhibitors</td>
<td>✓</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Capture all uptake transporters</td>
<td>Proteomics for transporters expression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandwich-cultured human hepatocytes</td>
<td>Quantify all pathways</td>
<td>Multi step inhibition</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Most representative in vitro system</td>
<td>Down-regulation of transporters &amp; enzymes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo – animal species</td>
<td>KO &amp; inhibition</td>
<td>Translations to humans</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Humanized proteins</td>
<td>Misrepresentation of microenvironment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vivo - PET</td>
<td>Most representative</td>
<td>Expensive, time consuming</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
Summary - Scenario 4

- All hepatobiliary clearance pathway determine hepatic CL when none of the extreme conditions are met and there is active transport and/or metabolism
  - Transport and metabolism interplay to determine CL

- Quantitative tools for determining rate-determining step(s)
  - *Examples shown for liver but applicable to other organs*
Impact of transporters in distribution organs: Verapamil and P-gp transport at the human BBB

11C-Verapamil brain:blood ↑ 88% in the presence on P-gp inhibition by cyclosporine indicating P-gp’s contribution to limiting brain drug exposure

Eyal et al., Clin Pharmacol Ther. 2010
Predicted maximum verapamil brain accumulation (complete P-gp inhibition) is ~4-5 fold

Eyal et al., Clin Pharmacol Ther. 2010
Ke et al., J Nucl. Med. 2013
Importance of transporters at the site of action: Metformin and OCT1

While metformin is primarily excreted unchanged in the urine, its site of action is in the liver, therefore, the activity of OCT1 in the liver will **profoundly impact PD but not PK**.

Shu et al. J of Clinical Investigation. 2007
Take-home message

Transport vs. Metabolism: What determines the PK and PD of drugs?

> Rate-determining step(s)
  - Sinusoidal uptake when $CL_{ef}^S \ll (CL_{met} + CL_{ef}^C)$
  - Metabolism and canalicular efflux when $CL_{in}^S = CL_{ef}^S \gg (CL_{met} + CL_{ef}^C)$
  - All hepatobiliary CL pathways when none of the above conditions apply

> Sole-eliminating organ
  - Changes to sinusoidal uptake/efflux CL do not impact hepatic AUC

> Eliminating vs. distribution organ

*Principles discussed here apply to other organs beyond the liver*

UNIVERSITY of WASHINGTON
Acknowledgements:

- Dr. Jashvant Unadkat
- Unadkat lab members
- Dr. Bhagwat Prasad & Prasad lab members

Other Collaborators:
- Dept. of Radiology: Jeanne Link, David Mankoff, Todd Richards, Janet Eary, Satoshi Minoshima, Ken Maravilla, Mark Muzi, Steve Shoner and the PET suite team
- Dept. of Medicine: Ann Collier and her team
- Dept. of Anesthesiology: Karen Domino
- Dept. of Pharmaceutics: Edward Kelly, Carol Collins

Funding and support:
NCATS TL1 TR000422, Elmer M. Plein Research Endowed Award, NIH P01DA032507
NIH MH63641, P50 HD44404, RR 00166, HD47892, AG031485, RC1NS068904, UWRAPT funded by
Genentech, Merck, Biogen, Gilead, BMS, Takeda; BioreclamationIVT