Complexities of Hepatic Drug Transport: How Do We Sort It All Out?

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The Challenge

Intestinal uptake
OATP2B1, OATP1A2, PEPT-1, MRP3

Intestinal efflux
Pgp, MRP2, BCRP

CNS efflux
Pgp, OAT3, MRP1, MRP2, BCRP

Biliary secretion
Pgp, BSEP, MRP2, BCRP

Hepatic uptake
OATP1B1, OATP2B1, OAT2, OATP1B3, NTCP

Hepatic efflux
MRP3, MRP4, MRP6

Renal uptake
OAT1, OAT3

Renal re-uptake
OATP2B1, PEPT-2

Renal secretion
Pgp, MRP2, OAT4

in red ATP dependent efflux transporters
in yellow exchange/co-transport transporters

Courtesy J. Polli, GSK
Specific Challenge

To understand the role of multiple transport mechanisms in hepatobiliary drug disposition and the pharmacokinetic consequences of modulating those processes

- Characterize of a novel *in vitro* system
- Utilize an ideal probe substrate and elucidate its mechanisms of transport
- Examine the modulation of transport mechanisms and determine potential implications
The Liver

Modified from Albert, 1994
The Liver: Basolateral Transport Proteins
Rodent

blood flow

basolateral membrane

hepatocyte
tight junction

bile

Mrp3
Mrp4
Mrp5

ATP

GSH?

-70 mV

Oatp1a1
Oatp1a4
Oatp1b2

blood flow

basolateral membrane

Ntcp
Oat2
Oat3
Oct1

Na^+
α-ketoglutarate?
The Liver: Basolateral Transport Proteins
Human

- blood flow
- hepatocyte
- tight junction
- bile
- basolateral membrane
- OATP1B1
- OATP1B3
- OATP2B1
- GSH?
- -70 mV
- Na⁺
- α-ketoglutarate?
The Liver: Canalicular Transport Proteins Rodent

tight junction

hepatocyte

bile

Bsep

Mdr2

Mdr1a/b

Mrp2

Abcg5

Abcg8

Bcrp

ATP
The Liver: Canalicular Transport Proteins

Human

- BSEP
- MRP2
- BCRP
- MDR3
- MDR1
- ABCG5
- ABCG8

ATP

tight junction

hepatocyte

bile
## Multiplicity of Hepatic Transport Proteins

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Basolateral Membrane</th>
<th>Canalicular Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>E217B-glucuronide</td>
<td>Oatp1a1, Oatp1a4, Oatp1b2</td>
<td>P-gp, Mrp2</td>
</tr>
<tr>
<td>digoxin</td>
<td>Oatp1a4</td>
<td>P-gp</td>
</tr>
<tr>
<td>rhodamine 123</td>
<td>unknown</td>
<td>P-gp, Bcrp</td>
</tr>
<tr>
<td>DPDPE</td>
<td>Oatp1a1, Oatp1a4, Oatp1b2</td>
<td>(P-gp?), (Mrp2?)</td>
</tr>
<tr>
<td>20-S-camptothecin</td>
<td>unknown</td>
<td>MRP2, P-GP</td>
</tr>
<tr>
<td>irinotecan</td>
<td>unknown</td>
<td>MRP2, P-GP</td>
</tr>
<tr>
<td>fexofenadine</td>
<td>Oatp1a1, Oatp1a4, Oatp1b2</td>
<td>P-gp</td>
</tr>
<tr>
<td>pravastatin</td>
<td>Oatp1a4, Oatp1b2</td>
<td>Mrp2</td>
</tr>
<tr>
<td>bosentan</td>
<td>Oatp1a1, Oatp1a4, Oatp1b2</td>
<td>(Mrp2?)</td>
</tr>
<tr>
<td>enalapril</td>
<td>Oatp1</td>
<td>Mrp2</td>
</tr>
<tr>
<td>atorvastatin</td>
<td>OATP1B1</td>
<td>P-GP</td>
</tr>
<tr>
<td>doxorubicin</td>
<td>unknown</td>
<td>Mrp2, P-gp</td>
</tr>
<tr>
<td>BQ-123</td>
<td>Oatp1a1, Oatp1a4, Oatp1b2, Mrp6</td>
<td>Mrp2</td>
</tr>
<tr>
<td>rifampicin</td>
<td>OATP1B1, OATP1B3</td>
<td>P-GP</td>
</tr>
<tr>
<td>etoposide</td>
<td>MRP1, MRP3, MRP6</td>
<td>MRP2, BCRP, P-GP</td>
</tr>
<tr>
<td>saquinavir</td>
<td>(OATP1B1?), MRP1</td>
<td>MRP2, P-GP</td>
</tr>
<tr>
<td>azithromycin</td>
<td>unknown</td>
<td>Mrp2</td>
</tr>
<tr>
<td>cerivastatin</td>
<td>Oatp1a4</td>
<td>(MRP2?)</td>
</tr>
<tr>
<td>rosuvastatin</td>
<td>OATP1B1</td>
<td></td>
</tr>
</tbody>
</table>
Optimal Probe Substrate: [D-Penicillamine$^{2,5}$]enkephalin (DPDPE)

- Opioid peptide
- Enzymatically stable
- Extensively and rapidly excreted in bile
- Short t$_{1/2}$ ~ 12 min in rats
- Transport is somewhat characterized
- In vivo disposition characterized
In Vitro Systems to Study Hepatobiliary Drug Transport

- Expression systems
  - Only one or two transport proteins
- Isolated Membranes
  - Purity?
- Immortalized Cell Lines
  - Representative of normal physiology?
- Primary Hepatocytes
  - Immediately lose polarization
- Sandwich-Cultured (SC) Hepatocytes
  - Localization of canalicular proteins?
- Isolated Perfused Liver
  - Limited to preclinical species
Liver-Based *In Vitro* Systems to Study Hepatic Transport

Isolated Perfused Liver

Liver Perfusion:
10 min Ca$^{2+}$-free with chelator
10 min collagenase digestion

Hepatocyte Isolation
Liver capsule gently torn

Percoll Gradient
85 – 95% Viability

Suspended Hepatocytes

Sandwich-Cultured Hepatocytes

Day 1

Day 4

Pre-isolation

0 hours

24 hours

48 – 96 hours
Reestablishing Polarity in SC Hepatocytes

Hoffmaster KA, et al. Pharm Res, 2004
Representative Transport Protein Expression in SC Rat and Human Hepatocytes

Rat
- Oatp1a1
- Oatp1a4
- Actin

Day in Culture: 1 2 3 4

Canalicular Membrane
- P-gp
- Mrp2
- Actin

Day in Culture: 1 2 3 4

Human
- OATP1B1
- OATP1B3
- ACTIN

Day in Culture: 1 3 6

Canalicular Membrane
- P-GP
- MRP2
- ACTIN

Day in Culture: 1 3 4 6

Hoffmaster KA, et al. Pharm Res, 2004
Co-localization of P-gp and DPPIV in SC Rat and Human Hepatocytes

P-gp | DPPIV | rlgG | Merge
---|---|---|---
Rat Day 4

A

B

C

D

Human Day 6

E

F

G

H

Hoffmaster KA, et.al. Pharm Res, 2004
Localization of P-gp and DPPIV in SC Rat Hepatocytes Over Time in Culture
Localization of P-gp and DPPIV in SC Human Hepatocytes Over Time in Culture

Day 1

Day 3

Day 4

Day 6

Day 7

Day 10

Hoffmaster KA, et.al. Pharm Res, 2004
Localization of Mrp2 in SC Rat and Human Hepatocytes

Hoffmaster KA, et.al. Pharm Res, 2004
Quantification of Biliary Excretion in Sandwich-Cultured Hepatocytes

$$\text{BEI} \, (\%) = \frac{\text{Accumulation}_{\text{Cells+BC}} - \text{Accumulation}_{\text{Cells}}}{\text{Accumulation}_{\text{Cells+BC}}} \times 100$$

$$\text{Cl}_{\text{b, in vitro}} = \frac{\text{Accumulation}_{\text{Cells+BC}} - \text{Accumulation}_{\text{Cells}}}{\text{AUC}_{0-T}}$$
In Vitro Biliary Excretion of $^3$H-DPDPE SC Rat Hepatocytes at 10 min

DPDPE Accumulation (pmol/mg protein)

Days in Culture | BEI (%) |
--- | --- |
1 | < 0 |
2 | 22 ± 11 |
3 | 38 ± 9 |
4 | 44 ± 12 |
5 | 35 ± 10 |

*p<0.05

Hoffmaster KA, et.al. Pharm Res, 2004

Oatp1a4
**In Vitro** Biliary Excretion of $^3$H-DPDPE in Day 6 SC Human Hepatocytes

BEI (10 min) = 21%

Hoffmaster KA, et.al. Pharm Res, 2004
In Vitro Biliary Excretion of CDF in SC Rat and Human Hepatocytes

Rat

Day 1
Day 4

Human

Day 1
Day 6

Hoffmaster KA, et.al. Pharm Res, 2004
Summary: Characterization of SC Rat and Human Hepatocytes

- Transport protein expression maintained
- P-gp and Mrp2 trafficked to the canalicular membrane
- Biliary excretion of probe substrates correlated with appropriate protein localization
- Slight decrease in $^{3}$H-DPDPE uptake reflective of decrease in Oatp1a4 protein expression
Uptake of 1µM $^3$H-DPDPE in Suspended Rat Hepatocytes

Condition | Transport Protein
---|---
Choline | Ntcp
BSP | Oatp1a1
BSP | Oatp1b2
DIG | Oatp1a4
DELT II | Oatp1a1
TEA | Oct1
PAH | Oat1
PAH | Oat2
GF120918 | ???
TR-hepatocytes | ???

Hoffmaster, KA, et.al. DMD, 2005
Accumulation of $^{3}$H-DPDPE in Control and TR$^{-}$ Sandwich-Cultured Rat Hepatocytes

Control

BEI %  37 ± 10

Cl$_{b, in vitro}$  1.37 ± 0.4

($\mu$L min$^{-1}$ mg protein$^{-1}$)

Hoffmaster, KA, et.al. DMD, 2005
Accumulation of $^3$H-DPDPE in Control and TR$^-$ Sandwich-Cultured Rat Hepatocytes

Control

<table>
<thead>
<tr>
<th>BEI %</th>
<th>37 ± 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{b, in vitro}$</td>
<td>1.37 ± 0.4</td>
</tr>
</tbody>
</table>

$Cl_{b, in vitro}$ (µL•min$^{-1}$•mg protein$^{-1}$)

TR$^-$

<table>
<thead>
<tr>
<th>BEI %</th>
<th>6.2 ± 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{b, in vitro}$</td>
<td>0.23 ± 0.2</td>
</tr>
</tbody>
</table>

$Cl_{b, in vitro}$ (µL•min$^{-1}$•mg protein$^{-1}$)

Hoffmaster, KA, et.al. DMD, 2005
Canalicular Transport Protein Expression on Day 4 in Control and TR- Sandwich-Cultured Rat Hepatocytes

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>TR-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mrp2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-gp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bcrp</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Actin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Influence of P-gp on DPDPE *In Vivo* Disposition

**Brain Disposition**

Mdr1a(-/-) Mice vs. Control

P-gp Competent Mice + GF120918 vs. Control

**Systemic Pharmacokinetics**

Chen and Pollack, 1998, 1999
Uptake of $^3$H-DPDPE (0.5-250 µM) in Suspended Rat Hepatocytes

![Graph showing uptake rate vs. DPDPE concentration](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cl_{uptake}$ ($\mu l \cdot min^{-1} \cdot mg$ protein)</td>
<td>1.93</td>
<td>2</td>
</tr>
<tr>
<td>$Vm_{uptake}$ ($pmol \cdot min^{-1} \cdot mg$ protein)</td>
<td>385</td>
<td>3</td>
</tr>
<tr>
<td>$Km_{uptake}$ ($\mu M$)</td>
<td>28.9</td>
<td>7</td>
</tr>
</tbody>
</table>

Hoffmaster KA, et.al. *DMD*, 2005
Pharmacokinetic Modeling of $^3$H-DPDPE (0.5-250 µM) Accumulation in SC Rat Hepatocytes

**Parameter** | **Estimate** | **CV%**
--- | --- | ---
$k_{\text{uptake}}$ (min$^{-1}$) | 0.00331 | 6
$V_{\text{medium}}$ (mL) | 3000 | n/a
$K_{m,\text{uptake}}$ (µM) | 28.9 | n/a
$V_{m,\text{uptake}}$ (pmol•min$^{-1}$) | 320 | 19
$V_{m,\text{blefflux}}$ (pmol•min$^{-1}$) | 4.8 | 65
$V_{\text{hepatocyte}}$ (mg protein) | 1.85 | n/a
$k_{\text{bile}}$ (min$^{-1}$) | 0.245 | 8
$k_{\text{leakage}}$ (min$^{-1}$) | 1.6 | 34

Hoffmaster KA, et.al. DMD, 2005
Accumulation of $^3$H-DPDPE (0.5-250 µM) in SC Rat Hepatocytes

Cells + BC

Cells

Hoffmaster KA, et.al. DMD, 2005
Summary: Hepatic Uptake and Biliary Excretion Mechanisms of DPDPE

<table>
<thead>
<tr>
<th>Basolateral Membrane</th>
<th>Canalicular Membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oatp1a1</td>
<td>Mrp2</td>
</tr>
<tr>
<td>Oatp1a4</td>
<td>P-gp?</td>
</tr>
<tr>
<td>Oatp1b2</td>
<td></td>
</tr>
</tbody>
</table>

- DPDPE not transported by Ntcp, Oat2, Oat3, Oct1
- Transport protein expression maintained and DPDPE uptake mechanism intact in TR^- hepatocytes
- Uptake of DPDPE involves a linear and a saturable component
- Modeling supports a basolateral excretion process
- Multi-experimental approach assisted with model development
Modulation of DPDPE Hepatobiliary Transport: Study Design

- Determine the role of Mrp2 and P-gp?
- Can we specifically modulate each process?

→ Recirculating isolated perfused liver
→ $^3$H-DPDPE infusion
→ Control and TR$^-$ rat livers
→ Presence and absence of GF120918

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TR$^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-GF120918</td>
<td>-GF120918</td>
</tr>
<tr>
<td>P-gp</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Mrp2</td>
<td>++</td>
<td>XX</td>
</tr>
<tr>
<td></td>
<td>XX</td>
<td>XX</td>
</tr>
</tbody>
</table>
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

$^{3}$H-DPDPE Biliary Excretion

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

\[ k_o \]

Perfusate \[ \rightarrow \]
Liver

Mrp2, P-gp

Bile

\[ \text{Biliary Excretion Rate (nmol/min)} \]

\[ \text{Time (min)} \]

\[ \text{Control} \]
\[ \text{TR}^+ \]

\[ \text{3H-DPDPE Biliary Excretion} \]

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

$^{3}\text{H}$-DPDPE Biliary Excretion

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

$^{3}$H-DPDPE Biliary Excretion

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

$\text{Perfusate} \xrightarrow{k_0} \text{Liver} \xleftarrow{\text{Mrp2}} \xrightarrow{\text{P-gp}} \text{Bile}$

$^{3}\text{H}-\text{DPDPE Perfusate Concentration}$

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

{

$^{3}$H-DPDPE Perfusate Concentration

Hoffmaster KA, et.al. JPET, 2004

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

Hoffmaster KA, et.al. JPET, 2004
Modulation of DPDPE Hepatobiliary Transport: Isolated Perfused Liver Studies

$^3$H-DPDPE Perfusate Concentration

Hoffmaster KA, et.al. JPET, 2004
Average $^3$H-DPDPE [Liver]/[Perfusate] Post Infusion: Isolated Perfused Liver Studies

Hoffmaster KA, et al. JPET, 2004
Data Analysis: The Utility of the Multi-experimental Approach

- Is \(^3\text{H}\)-DPDPE uptake different in TR\(^-\) livers?
  - No- Suspended/SC hepatocyte data

- Does GF120918 affect \(^3\text{H}\)-DPDPE uptake?
  - No- Suspended hepatocyte data
  - 15.2 ± 2.5 vs. 14.9 ± 2.1 pmol•min\(^{-1}\)•mg protein

- Are perfusate concentrations above the \(K_m\) for the uptake process?
  - \(K_m = 28.9 \ \mu\text{M}, \ C_{\text{max}} \approx 7 \ \mu\text{M}\)

- Model structure…initial parameter estimates?
  - Linear uptake, basolateral excretion

\[ k_o \]

Hoffmaster KA, et.al. JPET, 2004
Pharmacokinetic Modeling: Isolated Perfused Liver

All Conditions

$k_{\text{uptake}}$ fixed at 0.12 min\(^{-1}\)

$V_{\text{perfusate}}$ fixed at 88 mL

<table>
<thead>
<tr>
<th>Condition</th>
<th>$V_{m,\text{blefflux}}$ (nmol•min(^{-1}))</th>
<th>CV%</th>
<th>$k_{\text{bile}}$ (min(^{-1}))</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>16</td>
<td>18</td>
<td>0.12</td>
<td>12</td>
</tr>
<tr>
<td>Control + GF120918</td>
<td>0</td>
<td>n/a</td>
<td>0.14</td>
<td>21</td>
</tr>
<tr>
<td>TR(^-)</td>
<td>37</td>
<td>4</td>
<td>0.037</td>
<td>5</td>
</tr>
<tr>
<td>TR(^-) + GF120918</td>
<td>19</td>
<td>21</td>
<td>0.0024</td>
<td>18</td>
</tr>
</tbody>
</table>

Hoffmaster KA, et.al. JPET, 2004
Pharmacokinetic Modeling: Isolated Perfused Liver

Hoffmaster KA, et.al. JPET, 2004
Summary of Modulation Studies

- DPDPE is excreted into bile primarily by Mrp2
- The impact of P-gp on biliary excretion is only observed in the absence of functional Mrp2
- $^3$H-DPDPE appears to be a substrate for a basolateral efflux mechanism that is sensitive to GF120918
Implications

• P-gp may serve as a back-up excretion mechanism in the absence of functional Mrp2
• A multi-experimental approach is helpful in understanding hepatic disposition
• Concentration at the site of elimination is what drives the clearance process
• Interpretation of drug interactions may be confounded by modulation of transport processes
• Simultaneous inhibition of apical and basolateral transport mechanisms may impact organ accumulation
Implications of Multiple Sites of Transport Inhibition: Clearance Calculations

\[
\text{Cl}_{\text{biliary}} = \frac{X_{\text{bile}}}{\text{AUC}_{\text{plasma}}}
\]

\[
\text{Cl}_{\text{biliary}} = \frac{X_{\text{bile}}}{\text{AUC}_{\text{liver}}}
\]

<table>
<thead>
<tr>
<th></th>
<th>Control Livers</th>
<th>TR Livers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-GF120918</td>
<td>+GF120918</td>
</tr>
<tr>
<td>Biliary Clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{perfusate}\rightarrow\text{bile}) (ml/min)</td>
<td>5.22 ± 0.3</td>
<td>17.0 ± 0.3(^*)</td>
</tr>
<tr>
<td>Biliary Clearance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\text{liver}\rightarrow\text{bile}) (ml/min)</td>
<td>1.89 ± 1.3</td>
<td>1.58 ± 0.6</td>
</tr>
</tbody>
</table>

\(^*\) p < 0.05 compared to Control -GF120918

\(^†\) p < 0.05 compared to Control -GF120918
Implications of Multiple Sites of Transport Inhibition: Interpreting Drug Interactions

Wu and Benet, 2003

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Troleandomycin</th>
<th>GG918</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC (ng/ml - min)</td>
<td>2,260 ± 430</td>
<td>5,200 ± 2,470*</td>
<td>1,730 ± 270*</td>
</tr>
<tr>
<td>AUC\textsubscript{m} (ng/ml - min)\textsuperscript{a}</td>
<td>22,900 ± 10,900</td>
<td>72,100 ± 7,900*</td>
<td>12,100 ± 4,500</td>
</tr>
<tr>
<td>AUC\textsubscript{m}/AUC\textsubscript{b}</td>
<td>9.35 ± 1.94</td>
<td>10.3 ± 2.4</td>
<td>6.34 ± 1.74</td>
</tr>
</tbody>
</table>

Concluded that decreases in AUC were due to GF120918 based inhibition of P-gp mediated biliary excretion of tacrolimus and primary metabolite coupled with subsequent enhanced metabolism of both parent compound and primary metabolites…

Tacrolimus

Perfusate

Liver

\textbf{P450}

Bile

\textbf{P450}
Implications of Multiple Sites of Transport Inhibition: Liver Accumulation

- Perfusate
- Liver
- Bile

- Mrp2
- P-gp

- $k_o$

Graph showing the amount in liver (nmol) over time (min) with a peak at 30 minutes.
Implications of Multiple Sites of Transport Inhibition: Liver Accumulation

- Perfusate
- Liver
- Bile

$k_0$

Perfusate $\rightarrow$ Liver $\rightarrow$ Bile

Mrp2

Pgp

Time (min)

0 10 20 30 40 50 60 70 80 90

Amount in Liver (nmol)

0 200 400 600 800 1000 1200

Control

Control + GF120918

Amount in Liver (nmol) vs Time (min)
Implications of Multiple Sites of Transport Inhibition: Liver Accumulation

- Perfusate
- Liver
- Bile

$\lambda_o$

Mrp2, P-gp

Control, Control + GF120918, TR$^-$

Amount in Liver (nmol)

Time (min)
Implications of Multiple Sites of Transport Inhibition: Liver Accumulation

Toxicity?  Efficacy?  Target?
An Attempt to Sort it All Out…

- Continue to develop novel systems to investigate hepatobiliary disposition
- Apply pharmacokinetic modeling to provide useful insights into hepatobiliary disposition
- Utilize a multi-experimental approach to study hepatic transport and/or hepatic disposition
Acknowledgements

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