Hepatic Efflux Transporters: Relevance to Drug-Drug Interactions and Drug Toxicity

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Session I: Pioneer Symposium
Outline

• Overview of Hepatic Efflux Transporters
• Role of Hepatic Efflux Transporters in:
  – Drug-Induced Liver Injury (DILI)
  – Systemic and Hepatic Exposure to Drugs and Metabolites
    ➢ Rosuvastatin
    ➢ Morphine glucuronides
    ➢ $^{99m}$Tc-Mebrofenin
• Functional Impact of Hepatic Basolateral Efflux Transporter Induction
• Summary
Conflict of Interest Disclosure

- The Brouwer lab receives research funding from the National Institutes of Health, National Institute of General Medical Sciences [R01 GM041935-24, R35 GM122576], Intercept Pharmaceuticals, and Otsuka Pharmaceutical Development & Commercialization, Inc.

- Dr. Kim Brouwer is co-inventor of the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®) and related technologies, which have been licensed exclusively to Qualyst Transporter Solutions, LLC
Importance of Hepatic Efflux Transporters in Health... Hepatocyte Hopping of Bilirubin Glucuronide

Beyond BSEP: Other Hepatic Bile Acid Uptake and Efflux Transporters

(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)
BSEF inhibition alone cannot accurately predict hepatotoxic potential of drugs

Dawson et al., *Drug Metab Dispos*, **40**:130, 2012
MRP3 Inhibitors Exhibited an Increased Risk of Cholestatic Potential Among BSEP Non-Inhibitors

The inhibitory potency of non-cholestatic (n=40) and cholestatic (n=48) drugs on MRP3-mediated E$_2$17G transport was examined in membrane vesicles prepared from MRP3-overexpressing HEK293T cells.

Köck...Brouwer, *Drug Metab Dispos*, 42:665, 2014
MRP4 Inhibitors Exhibited a Significantly Increased Risk of Cholestatic Potential Among BSEP Non-Inhibitors

The inhibitory potency of non-cholestatic (n=40) and cholestatic (n=48) drugs on MRP4-mediated DHEAS transport was examined in membrane vesicles prepared from MRP4-overexpressing HEK293T cells.

Köck...Brouwer, *Drug Metab Dispos*, 42:665, 2014
DILI is Multifactorial: Inhibition of Multiple Hepatic Efflux Transporters Confers Additional Risk

Aleo et al., Chem Res Toxicol, 30:1219, 2017
OSTα/β is a Bidirectional Heteromeric Transporter that is Upregulated in Liver Disease

Patients with Primary Biliary Cirrhosis

Patients with Obstructive Cholestasis

Boyer et al., Am J Physiol Gastrointest Liver Physiol, 290:G1124, 2006

Chai et al., Plos One, 10: e0120055, 2015
Hepatobiliary Disposition in Sandwich-Cultured Hepatocytes (SCH; B-CLEAR®)

Biliary Excretion Index (BEI) (%) = \[
\frac{\text{Accumulation}_{\text{cells} + \text{bile}} - \text{Accumulation}_{\text{cells}}}{\text{Accumulation}_{\text{cells} + \text{bile}}} \times 100
\]

B-CLEAR® technology is covered by US Pat. No. 6,780,580 and other US and International patents, both issued and pending, and is exclusively licensed to Qualyst Transporter Solutions.
Basolateral Efflux in SCH

Standard Buffer (cells+bile)

\[
\text{Buffer} \quad C_{\text{Buffer}}^+ \quad V_{\text{Buffer}}
\]

\[
\text{Cell} \quad C_{\text{cell}}^+ \quad V_{\text{cell}}
\]

\[
\text{Bile} \quad X_{\text{bile}}
\]

\[
K_{\text{flux}} \quad \text{CL}_{\text{up}} \quad \text{CL}_{\text{BL}}
\]

\[
\text{loading phase} \quad \text{efflux phase}
\]

Ca\(^{2+}\)-free Buffer (cells)

\[
\text{Buffer} \quad C_{\text{buffer}}^- \quad V_{\text{Buffer}}
\]

\[
\text{Cell} \quad C_{\text{cell}}^- \quad V_{\text{cell}}
\]

\[
\text{Bile} \quad X_{\text{bile}}
\]

\[
\text{loading phase} \quad \text{efflux phase}
\]

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Hepatic Disposition of Rosuvastatin (RSV): Importance of Basolateral Efflux Transporters

RSV is a substrate of human MRP4

<table>
<thead>
<tr>
<th></th>
<th>Human SCH</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>CL_{Bile} (mL/min/g liver)</td>
<td>0.037 ± 0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CL_{Basolateral} (mL/min/g liver)</td>
<td>0.10 ± 0.02</td>
<td></td>
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</tbody>
</table>

Increased Expression of Hepatic Efflux Transporters in Patients with Nonalcoholic Steatohepatitis (NASH)

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Normal</th>
<th>Steatosis</th>
<th>NASH (fatty)</th>
<th>NASH (not fatty)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP3</td>
<td></td>
<td></td>
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<tr>
<td>MRP4</td>
<td></td>
<td></td>
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<tr>
<td>P-gp</td>
<td></td>
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<tr>
<td>BCRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Pan-Cadherin</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Human liver tissue

Hardwick et al., *Drug Metab Dispos*, 39:2395, 2011
Increased Expression of Hepatic Efflux Transporters in Patients with Nonalcoholic Steatohepatitis (NASH)

MRP3

~3-fold increase

Hardwick et al. Drug Metab Dispos, 39:2395, 2011
Mrp3 Mediates Morphine-3-Glucuronide Efflux from Hepatocyte to Plasma

van de Wetering K et al., Mol Pharmacol, 72:387, 2007
Increased Serum Concentrations of Morphine Glucuronide, an MRP3 Probe, in Patients with NASH

<table>
<thead>
<tr>
<th>MG Parameters</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nM)</td>
<td>225 (194-261)</td>
<td>343** (284-413)</td>
</tr>
<tr>
<td>$AUC_{0-\text{last}}$ ($\mu$M*min)</td>
<td>37 (32-44)</td>
<td>59 ** (42-83)</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>187 (153-229)</td>
<td>146 (104-205)</td>
</tr>
</tbody>
</table>

Geometric mean (95% CI); ** p<0.01 t-test on log transformed data

Ferslew...Brouwer, Clin Pharmacol Ther, 97:419, 2015
Altered MRP2 Localization and Expression in Liver Tissue of Patients with NASH

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
Farnesoid X Receptor (FXR): Key Regulator of Bile Acid Homeostasis

Acute Exposure:
- inhibitory effects on uptake and efflux
- transporter mobilization

Chronic Exposure:
- Increased FGF19 (hepatic and intestinal) and SHP
- Decreased CYP7a1 - leading to a decrease in the total endogenous bile acid pool
- No change or decreased NTCP-mediated bile acid uptake
- Increased bile acid efflux
  - BSEP (canalicular)
  - OSTα/β (basolateral)

Adapted from Camilleri et al., *Am J Physiol Gastrointest Liver Physiol*, 2014
Chenodeoxycholic Acid (CDCA), an Endogenous FXR Agonist, Upregulates BSEP in Human SCH

Jackson et al., *Appl In Vitro Toxicol* 2:1, 2016
Chenodeoxycholic Acid (CDCA) Upregulates OSTα/β

- CDCA exposure leads to a dose-dependent increase in expression of the basolateral efflux transporter OSTα/β.
- Induction of OSTα/β increased media concentrations of endogenously generated bile acids in human sandwich-cultured hepatocytes.

Increased function of basolateral efflux transporters can be an important “safety valve” if BSEP-mediated efflux is compromised.

Jackson et al., *Appl In Vitro Toxicol* 2:1, 2016
Farnesoid X Receptor (FXR) Agonists

- OCA is the first-in-class FXR agonist approved for primary biliary cirrhosis (PBC)
- OCA treatment for non-alcoholic steatohepatitis (NASH) is in Phase III clinical trials

FXR: nuclear receptor regulating bile acid

FXR EC$_{50}$ = 8.6 μM (~100x increased potency) FXR EC$_{50}$ = 90 nM
Estimation of Taurocholate (TCA) Clearance in Human SCH after OCA and CDCA Treatment

OCA (1 μM) and CDCA (100 μM) Treatment for 72 hr

Uptake and Efflux of TCA in SCH

+ Ca²⁺

- Ca²⁺

Time-course of TCA concentration in cell lysate and medium

Mechanistic PK Modeling

Clearance:
- Uptake CL
- Basolateral efflux CL
- Biliary CL

SCH: Sandwich-cultured Human Hepatocytes
TCA: taurocholate
OCA (1 µM) and CDCA (100 µM) Treatment for 72 hr

Uptake and Efflux of TCA in SCH
+ Ca^{2+}
  - Ca^{2+}

Donor 1

Control

OCA (1 µM)

CDCA (100 µM)

Donor 2

Donor 3

Clearance: Uptake Efflux Simulation

FXR Agonists Treatment with Human SCH
Uptake and Efflux Study of TCA in Human SCH

**Standard Buffer (Cell+Bile)**

Loading Phase

**Ca$^{2+}$-free Buffer (Cell)**

Loading Phase

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SCH: Sandwich-cultured Hepatocytes
TCA: taurocholate
HBSS: Hanks’ Balanced Salt Solution

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**Uptake and Efflux Study of TCA in Human SCH**

**Standard Buffer (Cell+Bile)**

**Ca\(^{2+}\) -free Buffer (Cell)**

SCH: Sandwich-cultured Hepatocytes  
TCA: taurocholate  
HBSS: Hanks’ Balanced Salt Solution

Mechanistic PK Modeling

Standard HBSS ($X_{Cell+Bile}, X_{Buffer}^+$):

Medium → Cell → Bile canaliculi

- $C_{Cell}^+$
- $C_{Buffer}^+$
- $V_{Cell}$
- $V_{Buffer}$
- $C_{Bile}$
- $CL_{Uptake}$
- $CL_{Bile}$
- $CL_{BL}$
- $K_{Flux}$

Ca$^{2+}$-free HBSS ($X_{Cell}, X_{Buffer}^-$):

Medium → Cell

- $C_{Buffer}^-$
- $C_{Cell}^-$
- $V_{Buffer}$
- $V_{Cell}$
- $CL_{Uptake}$
- $CL_{Bile}$
- $CL_{BL}$

$CL_{Uptake}$: uptake clearance
$CL_{Bile}$: biliary efflux clearance
$CL_{BL}$: basolateral efflux clearance
$K_{Flux}$: flux from bile networks into the medium

Summary

• Basolateral and canalicular efflux transporters play a critical role in hepatic and systemic exposure for some drugs, endogenous compounds, and metabolites.
• Inhibition of hepatic efflux transporters may increase hepatocyte exposure and cause toxicity.
• Induction of basolateral efflux transporters may decrease intracellular concentrations and increase systemic exposure.
• Sandwich-cultured hepatocytes and modeling/simulation are useful tools to evaluate how drug interactions influence the function of these proteins, and to predict the impact of transporter changes on the disposition of endogenous compounds, drugs and metabolites.
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