Mechanisms and Clinical Significance of Drug Interactions Involving Hepatic Efflux Transporters

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Conflict of Interest Disclosure

• Consultant for Merck Research Laboratories

• 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, recently acquired by BioIVT

• The Brouwer lab receives research funding from the National Institute of General Medical Sciences of the National Institutes of Health (R35 GM122576), Intercept Pharmaceuticals, Otsuka Pharmaceutical Development & Commercialization, Inc., and Gilead Sciences, Inc.
Session I: Pioneer Lectures
Outline

• Overview of Hepatic Efflux Transporters
• Drug Interactions with the Bile Salt Export Pump (BSEP) and Clinical Liability for Liver Injury
• Basolateral Multidrug Resistance-Associated Protein 3 (MRP3): Impact of Drug Interactions
• Organic Solute Transporter (OSTα/β) and Drug Interactions: An Overlooked Transporter
• Functional Impact of Hepatic Basolateral Efflux Transporter Induction and Clinical Implications
• Summary
Hepatic Efflux Transporters

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)

BSEP (Bile Salt Export Pump); NTCP (Sodium-Taurocholate Cotransporting Polypeptide); MRP (Multidrug Resistance–Associated Protein); OST (Organic Solute Transporter)
Impact of Drug Interactions with BSEP (ABCB11) on Hepatic Bile Acid Disposition

BSEP (Bile Salt Export Pump); NTCP (Sodium-Taurocholate Cotransporting Polypeptide); MRP (Multidrug Resistance–Associated Protein); OST (Organic Solute Transporter)

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
BSEP inhibition alone cannot accurately predict hepatotoxic potential of drugs.
Potency and Mechanisms of BSEP Inhibition by Tolvaptan and DM-4103, a Tolvaptan Metabolite

The inhibitory effect of tolvaptan and DM-4103 on 2 µM $^3$H-taurocholic acid (TCA) uptake was evaluated using inside-out membrane vesicles prepared from Sf9 cells over-expressing human bile salt export pump (BSEP).

Adapted from Slizgi...Brouwer, *Toxicol Sci*, 149:237, 2015

$K_i = 34.2 \mu M$  
Noncompetitive Inhibitor

$K_i = 3.77 \mu M$  
Competitive Inhibitor
Beyond BSEP: Compensatory Basolateral Efflux Transporters

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
MRP3 (ABCC3) 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
Hepatobiliary Disposition in Sandwich-Cultured Hepatocytes (SCH): B-CLEAR®

**Day 0**
- Overlay ~20 h after plating

**Day 1**
- change medium daily

**Day 4 (Rat)**

**Day 7 (Hu)**

**Standard Buffer**
- (cells + bile)

**Ca²⁺-free Buffer**
- (cells)

**Substrate in Bile Canaliculi**

<table>
<thead>
<tr>
<th>Biliary Excretion Index (BEI) (%)</th>
<th>=</th>
<th>Accumulation_{cells + bile} - Accumulation_{cells}</th>
<th>Accumulation_{cells + bile}</th>
<th>x 100</th>
</tr>
</thead>
</table>

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, now BioIVT.
Scheme Depicting the Uptake and Efflux Protocol for Estradiol-17β-Glucuronide (E₂17G) in Human SCH

Mechanistic Model Depicting the Disposition of Estradiol-17β-Glucuronide (E$_2$17G) in Human SCH

$X = E_217G$ mass
$C = E_217G$ compartmental concentration
$CL_{BL} = $ Basolateral Efflux Clearance
$C_{Bile} = $ Biliary Clearance

Impact of MRP3 Induction in Patients with Nonalcoholic Steatohepatitis (NASH)


Canet et al., *Drug Metab Dispos*, 43:829, 2015
Beyond BSEP: Compensatory Basolateral Efflux Transporters

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)

BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)
Organic Solute Transporter (OST) α/β

(OST) α/β is a Heteromeric Transporter

Both OSTα & OSTβ are Required for Membrane Localization and Transport

- chromosome 15
- 128 amino acids
- ~19 kDa
- 1 transmembrane domain

- chromosome 3
- 340 amino acids
- ~37 kDa
- 7 transmembrane domains

extracellular

OSTα

SLC51A

intracellular

OSTβ

SLC51B

HEK293 cells transfected with OSTα, OSTβ, or OSTα-OSTβ

Wang et al., PNAS 98:9431, 2001

Dawson et al., J Biol Chem 280:6960, 2005
Li et al., Biochem 407:363, 2007
OSTα/β (SLC51A/B) is a Bidirectional Heteromeric Transporter

OSTα/β Substrates
- Taurocholate; other bile acids
- Dehydroepiandrosterone sulfate
- Estrone sulfate
- Pregnenolone sulfate
- Prostaglandin E2
- Digoxin

OSTα/β-Mediated Substrate Transport in Novel *In Vitro* Model was Rapid and Time-Dependent

Taurocholate (TCA)

Estrone Sulfate (E₁S)

Dehydroepiandrosterone Sulfate (DHEAS)

OSTα/β Flp-In™ 293 cells; 20 µM; Mean ± SEM (n=3)

Malinen…Brouwer, *Am J Physiol Gastrointest Liver Physiol* **314**:G597, 2018
OSTα/β-mediated Initial Velocity in Novel In Vitro Model was Linear as a Function of Concentration

**Taurocholate (TCA)**

**Estrone Sulfate (E₁S)**

**Dehydroepiandrosterone Sulfate (DHEAS)**

OSTα/β Flp-In™ 293 cells; 30-sec uptake; Mean ± SD (n=3)

Malinen…Brouwer, *Am J Physiol Gastrointest Liver Physiol* 314:G597, 2018
OSTα/β-Mediated DHEAS Transport was Inhibited by Compounds Associated with Cholestatic DILI

OSTα/β Flp-In™ 293 cells; 30-sec uptake; Mean±SD (n=3); 10-min preincubation with 100 μM inhibitors

Malinen...Brouwer, *Mol Pharm*, in press, 2018
Organic Solute Transporter (OSTα/β) SLC51A/B is Upregulated in Liver Disease

Patients with Obstructive Cholestasis

Chai et al, *Plos One*, 2015

Is OSTα/β an Overlooked “Safety Valve” for Hepatocellular Efflux of Bile Acids?

Patients with Nonalcoholic Steatohepatitis (NASH) and Primary Biliary Cirrhosis (PBC)

Malinen…Brouwer, *Am J Physiol Gastrointest Liver Physiol* 314:G597, 2018
Functional Impact of Hepatic Efflux Transporter Induction in Human Sandwich-Cultured Hepatocytes (SCHH)

Farnesoid X Receptor Agonists Obeticholic Acid and Chenodeoxycholic Acid Increase Bile Acid Efflux in Sandwich-Cultured Human Hepatocytes: Functional Evidence and Mechanisms

Cen Guo, Carl LaCerte, Jeffrey E. Edwards, Kenneth R. Brouwer, and Kim L. R. Brouwer

Farnesoid X Receptor (FXR) Agonists:

- CDCA (chenodeoxycholic acid)
- OCA (6E-CDCA) (obeticholic acid)

FXR EC$_{50}$ = 8.6 μM (~100x increased potency) FXR EC$_{50}$ = 90 nM

https://www.sec.gov/Archives/edgar/data/1270073/000114420415068713/v425958_ex99-1.htm
Effect of FXR Agonists on Taurocholate (TCA) Uptake, Basolateral Efflux and Biliary Clearance in SCHH

OCA (1 µM), CDCA (100 µM) or Vehicle Control Treatment for 72 hr (n=3 SCHH)

Uptake and Efflux of TCA in SCHH
+ Ca²⁺
- Ca²⁺

Mechanistic PK Modeling

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

Immunoblot analysis

Time-course of TCA concentration in cell lysate and medium

FXR: Farnesoid X Receptor
TCA: Taurocholate
SCH: Transporter Certified™ Sandwich-Cultured Human Hepatocytes

Guo...Brouwer, *J Pharmacol Exp Ther*, 365:413, 2018
PK Model Described TCA Disposition in OCA-, CDCA- and Control-treated SCHH

Treatment:
- Control
- Obeticholic Acid
- Chenodeoxycholic Acid

Mean ± S.D.
(n=1 representative hepatocyte lot, triplicate measurement)

Guo…Brouwer, *J Pharmacol Exp Ther*, **365**:413, 2018
FXR Agonists Increased TCA Intrinsic Basolateral Efflux Clearance and Biliary Clearance in SCHH

Mean ± S.D. (n=3 hepatocyte donors)

**p<0.01; ***p<0.001 (treated vs. control)

OCA: Obeticholic Acid
CDCA: Chenodeoxycholic Acid

Guo...Brouwer, J Pharmacol Exp Ther, 365:413, 2018
FXR Agonist Treatment Upregulated OSTα/β and BSEP mRNA and Protein Expression in SCHH

<table>
<thead>
<tr>
<th>Transporter</th>
<th>mRNA (fold increase)</th>
<th>Protein (fold increase)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OSTα</td>
<td>3-7</td>
<td>&gt;3</td>
</tr>
<tr>
<td>OSTβ</td>
<td>21-187</td>
<td>13</td>
</tr>
<tr>
<td>BSEP</td>
<td>2-8</td>
<td>2</td>
</tr>
</tbody>
</table>

Data expressed as relative fold change

OCA: Obeticholic Acid
CDCA: Chenodeoxycholic Acid

Mean ± S.D. expressed as % of control (n = 3 hepatocyte donors) **p<0.01; ***p<0.001 (treated vs. control)

Jackson et al., Appl In Vitro Toxicol, 2:207, 2016; Guo...Brouwer, J Pharmacol Exp Ther, 365:413, 2018
CDCA Decreased TCA Uptake Clearance and Increased TCA Efflux Clearance in SCHH

![Diagram showing the effect of CDCA on bile secretion and transporter activity](image)

<table>
<thead>
<tr>
<th>Parameter (µL/min/mg protein)</th>
<th>Control Mean (CV%)</th>
<th>CDCA-Treated Mean (CV%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL\textsubscript{Uptake}</td>
<td>1.3 (21%)</td>
<td>0.78 (23%)</td>
</tr>
<tr>
<td>CL\textsubscript{Bile}</td>
<td>0.77 (10%)</td>
<td>1.3 (28%)</td>
</tr>
<tr>
<td>CL\textsubscript{BL}</td>
<td>0.24 (12%)</td>
<td>1.5 (10%)</td>
</tr>
</tbody>
</table>

Guo...Brouwer, *J Pharmacol Exp Ther*, 365:413, 2018
Summary

• Hepatic efflux transporters (canalicular and basolateral) play a key role in excretion of some compounds.

• Inhibition of hepatic efflux transporters may increase hepatocyte exposure and cause toxicity (e.g., drug-induced liver injury).

• 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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