Clinical and Mechanistic Evidence for Associations between Drug-Transporter Interactions and DILI: BSEP and Beyond

Kim L. R. Brouwer, PharmD, PhD
W.R. Kenan, Jr., Distinguished Professor

UNC Eshelman School of Pharmacy and Curriculum in Toxicology
The University of North Carolina at Chapel Hill

Apical (Canalicular) Membranes
Basolateral Membranes
Tight Junction
Conflict of Interest Disclosure

• **Commercial Financial Interest:** 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.

• **Scientific Consulting:** Merck Research Laboratories, Nuvelution Pharma, Inc.

• **Grants/Research Support:** National Institute of General Medical Sciences of the National Institutes of Health (R35 GM122576); Intercept Pharmaceuticals; Otsuka Pharmaceutical Development & Commercialization, Inc.; Gilead Sciences, Inc.
Outline

• Bile Salt Export Pump (BSEP) Inhibition and Clinical Liability for Liver Injury

• Compensatory Basolateral Efflux Transporters – Clinical Evidence for Induction and DDI Risk
  - Multidrug Resistance-Associated Protein 3 (MRP3) and MRP4
  - Organic Solute Transporter (OSTα/β)

• Factors that May Predispose Patients to Drug Interaction-mediated Liver Injury
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

Mechanisms of Drug-Induced Liver Injury (DILI)
- Reactive Metabolites
- GSH Depletion
- Mitochondrial Dysfunction
- Adverse Immune Response
- Lipid Peroxidation
- DNA Damage
- Inhibition of Bile Acid Transport
  - Relevant Cellular Concentrations
  - Parent vs. Metabolite
  - Mechanism of Inhibition
  - BSEP variants

Pedersen et al., Toxicol Sci, 136:328, 2012
A Common Variant (rs2287622; p.V444A) in the Gene Encoding BSEP is Associated with an Increased Risk of Cholestatic DILI

The p.444V BSEP (reference) and p.444A BSEP (variant) don’t exhibit substantial differences in protein expression, stability, or taurocholate (TCA) transport.

1. Does the transport kinetics of other bile acids differ for variant BSEP?
2. Do some cholestatic medications inhibit variant BSEP selectively?

Ali…Brouwer, Mol Pharm, 16:1406, 2019
Glycocholate (GCA) Transport Kinetics for Reference and Variant BSEP

Mean ± SEM (n=3 in triplicate).

Ali...Brouwer, Mol Pharm, 16:1406, 2019
Inhibition of Reference and Variant BSEP by Cholestatic Medications was Similar

<table>
<thead>
<tr>
<th></th>
<th>TCA</th>
<th>GCA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reference</td>
<td>Variant</td>
</tr>
<tr>
<td>DMSO</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amox-Clav</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diclofenac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipyridamole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ibuprofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoniazid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketoconazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketorolac</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naproxen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nimesulide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranitidine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regorafenib</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rofecoxib</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Roxithromycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valacyclovir</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Inhibition by test compounds of TCA and GCA transport mediated by reference and variant BSEP. Mean values (% activity based on the grayscale bar on right; 100% indicates no inhibition; 0% indicates complete inhibition) were normalized to DMSO (mean values from 1-2 experiments in triplicate). Amoxicillin-clavulanate (Amox-Clav).

Data expressed as % activity normalized to DMSO, where 100% indicates no inhibition (mean ± SD; n=1 in triplicate).

Beyond BSEP: Compensatory Basolateral Efflux Transporters

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)
MRP4 mRNA and Protein are Significantly Increased in Liver Tissue from Patients with Progressive Familial Intrahepatic Cholestasis (PFIC)

Keitel et al., Hepatology, 41:1160, 2005
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
Noncompetitive Inhibition of MRP4-mediated \[^3\text{H}\text{]DHEAS\ Transport by Troglitazone Sulfate (TS)}\]

Data generated using membrane vesicles from HEK293 cells stably transfected with MRP4

\( \text{Noncompetitive inhibition} \)
\( K_m = 3.26 \mu M \)
\( V_{max} = 800 \text{ pmol/min/mg P} \)
\( K_i = 7.98 \mu M \)

TS inhibited MRP4-mediated DHEAS transport independent of GSH (Ki = 8.0 \mu M)

Yang…Brouwer, J Pharmacol Exp Ther, 353:415, 2015
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
Scheme Depicting the Uptake and Efflux Protocol for Estradiol-17β-Glucuronide (E₂₁₇G) in Sandwich-Cultured Human Hepatocytes (SCHH)

Standard HBSS condition (Maintain bile compartment)

Pre-incubation phase

- Standard HBSS 10 min

Uptake phase

- Sampling: Cells+bile (e.g., 1, 2, 4, 10 min)

Efflux phase

- Sampling: Cells+bile, Buffer (e.g., 1, 2, 4, 10 min)

Ca²⁺-free HBSS condition (Open bile compartment)

Pre-incubation phase

- Ca²⁺-free HBSS 10 min

Uptake phase

- Sampling: Cells (e.g., 1, 2, 4, 10 min)

Efflux phase

- Sampling: Cells, Buffer (e.g., 1, 2, 4, 10 min)

Pfeifer...Brouwer, J Pharmacol Exp Ther, 347:727, 2013
Estradiol-17β-Glucuronide (E$_2$17G) Accumulation and Efflux in SCHH

Cell lysate: Standard HBSS (Cells + bile)

Cell lysate: Ca$^{2+}$-free HBSS (Cells)

Buffer: Standard HBSS

Buffer: Ca$^{2+}$-free HBSS
Parameter Estimates Based on Mechanistic Model Describing E$_2$17G Disposition in SCHH

<table>
<thead>
<tr>
<th>Hepatocyte Lot</th>
<th>$\text{CL}_{\text{Uptake}}$</th>
<th>$\text{Cl}_{\text{int, BL}}$</th>
<th>$\text{Cl}_{\text{int, bile}}$</th>
<th>$\text{K}_{\text{flux}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lot 1</td>
<td>0.69 (15%)</td>
<td>0.16 (68%)</td>
<td>0.18 (47%)</td>
<td>0.31 (41%)</td>
</tr>
<tr>
<td>Lot 2</td>
<td>1.2 (9%)</td>
<td>0.17 (19%)</td>
<td>0.083 (35%)</td>
<td>0.21 (22%)</td>
</tr>
<tr>
<td>Lot 3</td>
<td>0.98 (9%)</td>
<td>0.21 (14%)</td>
<td>0.073 (28%)</td>
<td>0.17 (22%)</td>
</tr>
<tr>
<td><strong>Mean±SD</strong></td>
<td><strong>0.96 ± 0.26</strong></td>
<td><strong>0.18 ± 0.03</strong></td>
<td><strong>0.11 ± 0.06</strong></td>
<td><strong>0.23 ± 0.07</strong></td>
</tr>
</tbody>
</table>

$\text{Cl}_{\text{int, BL}}$ = Basolateral Efflux Clearance

$\text{Cl}_{\text{int, bile}}$ = Biliary Clearance

Basolateral efflux contributes significantly to E$_2$17G excretion from human hepatocytes
Impact of Transporter-Mediated Drug Interactions: Inhibition of $\text{Cl}_{\text{int, bile}}$ and $\text{Cl}_{\text{int, BL}}$ Increased Hepatic $\text{E}_{217G}$ Exposure in SCHH
Beyond BSEP: Compensatory Basolateral Efflux Transporters

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

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

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

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); *p<0.0005; 10-min preincubation with 100 μM inhibitors

Malinen…Brouwer, _Mol Pharmaceutics_ 16:238, 2019
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
6-α ethyl substitution

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
+ Ca^{2+}

- Ca^{2+}

Mechanistic PK Modeling

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

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

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)

CDCA Decreased TCA Uptake Clearance and Increased TCA Efflux Clearance in SCHH

**Parameter (µL/min/mg protein)** | **Control Mean (CV%)** | **CDCA-Treated Mean (CV%)**
--- | --- | ---
$CL_{\text{Uptake}}$ | 1.3 (21%) | 0.78 (23%)
$CL_{\text{Bile}}$ | 0.77 (10%) | 1.3 (28%)
$CL_{\text{BL}}$ | 0.24 (12%) | 1.5 (10%)

Guo…Brouwer, *J Pharmacol Exp Ther*, **365**:413, 2018
Organic Solute Transporter (OSTα/β) SLC51A/B is Upregulated in Liver Disease

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

Chai et al, Plos One, 2015

Malinen…Brouwer, Am J Physiol Gastrointest Liver Physiol 314:G597, 2018
Does Altered Hepatic Transporter Function In Disease (e.g., NASH) Predispose Patients to DILI?

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
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 lead to drug-induced liver injury

• Induction of basolateral efflux transporters (e.g., OSTα/β) 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
UNC-Duke Collaborative Clinical Pharmacology Postdoctoral Training Program (NIH T32)

**Goal**: Prepare a new generation of clinician-scientists to address critical problems in clinical pharmacology.

Program includes didactic and experiential training, with an emphasis in one of the following core research focus areas:

- Drug disposition and action
- Pharmacogenomics
- Drug-induced organ toxicity
- Quantitative pharmacology and clinical trial design

Training is provided in the methodology and conduct of basic and clinical research necessary to investigate the effects and mechanisms of drug actions in humans.

For more information please visit: [https://pharmacy.unc.edu/academics/fellowships/unc-duke-collaborative-clinical-pharmacology-t32-postdoctoral-training-program/]