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

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Associate Dean for Research and Graduate Education
UNC Eshelman School of Pharmacy
The University of North Carolina at Chapel Hill
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)
In Vitro Potency of BSEP Inhibition Correlates with Cholestatic Drug-Induced Liver Injury (DILI) Dawson et al., Drug Metab Dispos, 40:130, 2012

BSEP inhibition alone cannot accurately predict hepatotoxic potential of drugs
The inhibitory potency of non-cholestatic (n=40) and cholestatic (n=48) drugs on MRP3-mediated E₂₁₇G transport was examined in membrane vesicles prepared from MRP3-overexpressing HEK293T cells.
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

Controls

Cholestatic

Patients with Obstructive Cholestasis

OSTα monomer

OSTα dimer

OSTα/β is a Bidirectional Heteromeric Transporter that is Upregulated in Liver Disease

Boy 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®)

Day 0

Overlay 16 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

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.
Standard Buffer (cells+bile)

Buffer: $C_{Buffer}^+$, $V_{Buffer}$

Cell: $C_{cell}^+$, $V_{cell}$

Bile: $X_{bile}$

CL_up: $CL_{up}$

CL_BL: $CL_{BL}$

K_flux: $K_{flux}$

Loading phase: Cells

Efflux phase: Wash

Accumulation (pmol/mg protein)

Time (min)

Ca²⁺-free Buffer (cells)

Buffer: $C_{buffer}^-$, $V_{Buffer}$

Cell: $C_{cell}^-$, $V_{cell}$

Bile: $X_{bile}$

CL_up: $CL_{up}$

CL_BL: $CL_{BL}$

CL_Bile: $CL_{Bile}$

Loading phase: Cells

Efflux phase: Wash

Accumulation (pmol/mg protein)

Time (min)

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

RSV is a substrate of human MRP4

**Human SCH**

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

Increased Expression of Hepatic Efflux Transporters in Patients with Non-alcoholic Steatohepatitis (NASH)

Aim #1

<table>
<thead>
<tr>
<th></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>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP4</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>P-gp</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BCRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pan-Cadherin</td>
<td></td>
<td></td>
<td></td>
<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

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>$AUC_{0-last}$ (µM*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 for geometric mean); ** 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
Increased Hepatic Exposure to $^{99m}$Tc-Mebrofenin, an MRP2 Probe, in Patients with NASH

**PK Parameter** | **Control (n=14)** | **NASH (n=7)**
---|---|---
$t_{\text{max, liver}}$ (min) | 13 (12-15) | 13 (11-17)
$X_{\text{max}}$ (counts/s) | 2350 (2180-2530) | 3230* (2640-3970)
$\text{AUC}_{0-\infty, \text{liver}}$ (kcounts* min/s) | 105 (88-124) | 210* (178-248)
$k_e$ (min$^{-1}$) | 0.0309 (0.0275-0.0345) | 0.0190* (0.0127-0.0284)

Mean ± SD | Control (n = 14) | NASH (n = 7)

2.0-fold ↑ in exposure

Geometric mean (95% CI); $t_{\text{max, liver}}$ (median and range); * $p < 0.05$, Student’s two-tailed $t$-test of log transformed healthy subjects vs. NASH

Increased Systemic Exposure to $^{99m}$Tc-Mebrofenin, an MRP2 Probe, in Patients with NASH

<table>
<thead>
<tr>
<th>PK Parameter</th>
<th>Control (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nCi/mL)</td>
<td>132 (117-148)</td>
<td>246* (188-322)</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty,blood}$ (nCi*min/mL)</td>
<td>2150 (1840-2600)</td>
<td>3760* (2590-5450)</td>
</tr>
<tr>
<td>$\text{CL}_{\text{blood}}$ (mL/min/kg)</td>
<td>16.1 (13.9-18.8)</td>
<td>6.49* (4.16-10.1)</td>
</tr>
<tr>
<td>$V_{\text{ss}}$ (L)</td>
<td>194 (147-254)</td>
<td>156 (95-256)</td>
</tr>
<tr>
<td>$k_{e,blood}$ (min$^{-1}$)</td>
<td>0.00286 (0.00243-0.00337)</td>
<td>0.00221 (0.00161-0.00303)</td>
</tr>
<tr>
<td>$\text{CL}_{\text{renal}}$ (mL/min/kg)</td>
<td>0.117 (0.088-0.154)</td>
<td>0.082 (0.063-0.109)</td>
</tr>
</tbody>
</table>

Mean ± SD
- Control (n = 14)
- NASH (n = 7)

Geometric mean (95% CI); $t_{\text{max, liver}}$ (median and range); * p < 0.05, Student’s two-tailed t-test of log transformed healthy subjects vs. NASH

**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

- FXR: nuclear receptor regulating bile acid

- 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

https://www.sec.gov/Archives/edgar/data/1270073/000114420415068713/v425958_ex99-1.htm
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⁺

Mechanistic PK Modeling

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

SCH: Sandwich-cultured Human Hepatocytes
TCA: taurocholate
FXR Agonists Treatment with Human SCH

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

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

Donor 1
Donor 2
Donor 3

Control
OCA (1 µM)
CDCA (100 µM)

Time-course of TCA concentration in cell lysate and medium
Clearance: Uptake, Efflux, Simulation

Mechanistic PK Modeling

Donor 2
Donor 3
Uptake and Efflux Study of TCA in Human SCH

Standard Buffer (Cell+Bile)

Loading phase

Accumulation (pmol/mg protein)

Time (min)

+Ca

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

Ca²⁺-free Buffer (Cell)

Loading phase

Accumulation (pmol/mg protein)

Time (min)

- Ca

ution and Efflux Study of TCA in Human SCH

Uptake and Efflux Study of TCA in Human SCH

Standard Buffer (Cell+Bile)

-Cells-

loading phase

Accumulation (pmol/mg protein)

Time (min)

-Cells-

efflux phase

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

-Cells-

loading phase

Accumulation (pmol/mg protein)

Time (min)

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

Mechanistic PK Modeling

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

Medium \quad Cell \quad Bile canaliculi

- $C_{\text{Buffer}^+}$
- $C_{\text{Cell}^+}$
- $X_{\text{Bile}}$

- $V_{\text{Buffer}}$
- $V_{\text{Cell}}$

- $CL_{\text{Uptake}}$
- $CL_{\text{Bile}}$
- $CL_{\text{BL}}$

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

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

Medium \quad Cell

- $C_{\text{Buffer}^-}$
- $C_{\text{Cell}^-}$

- $V_{\text{Buffer}}$
- $V_{\text{Cell}}$

- $CL_{\text{Uptake}}$
- $CL_{\text{BL}}$
- $CL_{\text{Bile}}$

Fitted vs. Observed PK Profile in Human SCH

Guo et al., *ITCW3 Abstract*, 2017
Fitted vs. Observed PK Profile in Human SCH

Donor 1

Donor 2

Donor 3

Guo et al., ITCW3 Abstract, 2017
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Donor</th>
<th>CL\textsubscript{Uptake}</th>
<th>CV%</th>
<th>CL\textsubscript{BL}</th>
<th>CV%</th>
<th>CL\textsubscript{Bile}</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>1.6</td>
<td>6.4</td>
<td>0.29</td>
<td>33</td>
<td>0.80</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.95</td>
<td>5.8</td>
<td>0.25</td>
<td>52</td>
<td>0.80</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.4</td>
<td>4.0</td>
<td>0.19</td>
<td>58</td>
<td>0.70</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.3</td>
<td>21</td>
<td>0.24</td>
<td>12</td>
<td>0.77</td>
<td>10</td>
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<tr>
<td>OCA</td>
<td>1</td>
<td>1.9</td>
<td>18</td>
<td>1.71</td>
<td>32</td>
<td>1.3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1.0</td>
<td>24</td>
<td>1.65</td>
<td>41</td>
<td>1.3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.5</td>
<td>17</td>
<td>1.38</td>
<td>38</td>
<td>1.5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.5</td>
<td>23</td>
<td>1.58</td>
<td>9</td>
<td>1.4</td>
<td>23</td>
</tr>
<tr>
<td>CDCA</td>
<td>1</td>
<td>1.0</td>
<td>25</td>
<td>1.71</td>
<td>40</td>
<td>1.1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.54</td>
<td>36</td>
<td>1.65</td>
<td>64</td>
<td>1.4</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.79</td>
<td>24</td>
<td>1.3</td>
<td>56</td>
<td>1.4</td>
<td>24</td>
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<tr>
<td></td>
<td>Mean</td>
<td>0.78</td>
<td>23</td>
<td>1.5</td>
<td>10</td>
<td>1.3</td>
<td>28</td>
</tr>
</tbody>
</table>

Functional increases in basolateral and canalicular efflux of TCA were consistent with induction of OST\(\alpha/\beta\) and BSEP, respectively.

Guo et al., *ITCW3 Abstract*, 2017
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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- Amgen Predoctoral Fellowships in PK and Drug Disposition

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- Hugh Barton
- Kenneth Brouwer
- Jeffrey Edwards
- Carl LaCerte
- Paul Stewart
- Peter Swaan
Basolateral Efflux in SCH

Standard Buffer
(cells + bile)

\[ \text{CL}_{\text{BL}} \quad \text{CL}_{\text{Bile}} \]

\[ K_{\text{Flux}} \]

Efflux (mass/well)

Accumulation (mass/well)

Uptake phase

Efflux phase

Wash

Time (min)

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

\[ \text{CL}_{\text{BL}} \quad \text{CL}_{\text{Bile}} \]

Wash

Uptake phase

Efflux phase

Accumulation (mass/well)

Efflux (mass/well)

Time (min)

Pfeifer…Brouwer, Annul Rev Pharmacol Toxicol, **54**:509, 2014
PK Model for Rosuvastatin in SCH

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

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

Observations: $X_{Cells+Bile}$, $X_{Cells}$, $X_{Buffer^+}$, $X_{Buffer^-}$

Parameters: $CL_{Uptake}$, $CL_{BL}$, $CL_{Bile}$, $K_{Flux}$

0.1 µM Rosuvastatin in Rat SCH

**Cell Lysate**

WT (Control)  
BEI = 60%

WT + GF120918  
BEI = 24%

TR⁻ (Control)  
BEI = 33%

TR⁻ + GF120918  
BEI = 8%

**Legend**

- +Ca²⁺
- -Ca²⁺

**Buffer (Efflux Phase)**

- [³H]RSV Accumulation (pmol/well)
- [³H]RSV Efflux (pmol/well)

Pfeifer…Brouwer, J Pharmacol Exp Ther 347:727, 2013
Example of incomplete extraction: carboxydichlorofluorescein (CDF) (Chandra...Brouwer, Drug Metab Dispos 33:1238, 2005)

Perfusate

Bile

Outflow Perfusate Rate (nmol/min)

Time (min)

0 15 30 45 60 75 90

CL_{BL} = 1.4 \pm 0.3 \text{ mL/min/g liver}

CL_{BL} = 0.83 \pm 0.29 \text{ mL/min/g liver}

CL_{BL} = 0.55 \pm 0.19* \text{ mL/min/g liver}

CL_{BL} = 0.51 \pm 0.13 \text{ mL/min/g liver}

Perfusate

WT

TR

Control +GF120918

P<0.05, adjusted: * WT v. TR (±GF120918, respectively)
† + v. – GF120918 (WT or TR, respectively)

Pfeifer...Brouwer, J Pharmacol Exp Ther 347:737, 2013
Rosuvastatin metabolism in rat:


![Diagram showing metabolism of Rosuvastatin](image)

Parent: * WT v. TR⁻ (±GF120918, respectively)
† + v. – GF120918 (WT or TR⁻, respectively)

Metabolite: ‡ WT v. TR⁻ (±GF120918, respectively)

P<0.05, adjusted:

* WT v. TR⁻ (±GF120918, respectively)
† + v. – GF120918 (WT or TR⁻, respectively)

Pfeifer…Brouwer, J Pharmacol Exp Ther 347:737, 2013
Rosuvastatin Transport in Human SCH

Human SCH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{[3]}$HRSV Mass (pmol/well)</td>
<td><img src="#" alt="Graph showing uptake and efflux phases for cell lysate and buffer" /></td>
</tr>
<tr>
<td>Time (min)</td>
<td></td>
</tr>
</tbody>
</table>

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>$\text{CL}_{\text{Uptake}}$ ($\mu$L/min)</th>
<th>$\text{CL}_{\text{BL}}$ ($\mu$L/min)</th>
<th>$\text{CL}_{\text{Bile}}$ ($\mu$L/min)</th>
<th>$K_{\text{Flux}}$ ($\text{min}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human SCH</td>
<td>1.4 ± 0.3</td>
<td>0.10 ± 0.02</td>
<td>0.037 ± 0.015</td>
<td>0.044 ± 0.035</td>
</tr>
</tbody>
</table>

Legend

- $+$ Ca$^{2+}$

- $-$ Ca$^{2+}$

Pfeifer...Brouwer, J Pharmacol Exp Ther 347:727, 2013
Species Differences in Relative Contribution of Efflux Pathways to Hepatic RSV Excretion

<table>
<thead>
<tr>
<th></th>
<th>Human SCH</th>
<th>Rat SCH</th>
<th>Rat IPL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CL\textsubscript{Uptake}</strong> (mL/min/g liver)</td>
<td>1.4 ± 0.3</td>
<td>9.5 ± 2.7</td>
<td>40</td>
</tr>
<tr>
<td><strong>CL\textsubscript{Bile}</strong> (mL/min/g liver)</td>
<td>0.037 ± 0.015</td>
<td>0.23 ± 0.04</td>
<td>0.26 ± 0.12</td>
</tr>
<tr>
<td><strong>CL\textsubscript{BL}</strong> (mL/min/g liver)</td>
<td>0.10 ± 0.02</td>
<td>0.21 ± 0.07</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

Pfeifer…Brouwer, J Pharmacol Exp Ther 347:727, 2013
Rosuvastatin

- HMG-CoA reductase inhibitor
- Primarily hepatic elimination
- Minimal metabolism
- Low passive permeability; transport dominates

Rosuvastatin (RSV, Crestor®)

Ho et al., Gastroenterol, 130:793, 2006
Kitamura et al., Drug Metab Dispos, 36:2014, 2008
Rosuvastatin

- HMG-CoA reductase inhibitor
- Primarily hepatic elimination
- Minimal metabolism
- Low passive permeability; transport dominates

Rosuvastatin (RSV, Crestor®)
Rosuvastatin

- HMG-CoA reductase inhibitor
- Primarily hepatic elimination
- Minimal metabolism
- Low passive permeability; transport dominates

Rosuvastatin (RSV, Crestor®)

![Rosuvastatin molecule]

<table>
<thead>
<tr>
<th>Transporters</th>
<th>Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1, 1B3, 2B1</td>
<td>HMG-CoA, bile</td>
</tr>
<tr>
<td>NTCP</td>
<td>Na+</td>
</tr>
<tr>
<td>MRP2</td>
<td>ATP</td>
</tr>
<tr>
<td>MRP3</td>
<td>ATP</td>
</tr>
<tr>
<td>MRP4</td>
<td>ATP</td>
</tr>
</tbody>
</table>

**Rosuvastatin (RSV, Crestor®)**

• HMG-CoA reductase inhibitor
• Primarily hepatic elimination
• Minimal metabolism
• Low passive permeability; transport dominates
Sandwich-Cultured Hepatocytes

Standard Buffer (cells+bile)

- Uptake phase
- Wash

Accumulation (pmol/mg protein)

Time (min)

+Ca

Std HBSS 10 min

UPTAKE std HBSS, 20 min

Sampling (2, 5, 10 and 20 min)

-Ca

-Ca HBSS 10 min

UPTAKE std HBSS, 20 min

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

- Uptake phase
- Wash

Accumulation (pmol/mg protein)

Time (min)

Sandwich-Cultured Hepatocytes

**Standard Buffer (cells+bile)**

- Uptake phase
- Efflux phase
- Wash

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

- Uptake phase
- Efflux phase
- Wash

<table>
<thead>
<tr>
<th>Condition</th>
<th>Buffer</th>
<th>Duration</th>
<th>Action</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>+Ca</td>
<td>Std HBSS</td>
<td>10 min</td>
<td>UPTAKE</td>
<td>Std HBSS, 20 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wash (~1 min)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sampling (2, 5, 10 and 20 min)</td>
</tr>
<tr>
<td>-Ca</td>
<td>- Ca HBSS</td>
<td>10 min</td>
<td>UPTAKE</td>
<td>Std HBSS, 20 min</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Efflux - Ca HBSS, 15 min</td>
</tr>
</tbody>
</table>

Sandwich-Cultured Hepatocytes (SCH)

Standard Buffer
(cells + bile)

$K_{\text{Flux}}$

Day 2 - Rat SCH - 40 minute time series

Bile Canalicular Contractions

Basolateral Efflux in SCH

Standard Buffer (cells+bile)

\[ K_{\text{Flux}} \]

loading phase

efflux phase

Accumulation (pmol/mg protein)

Time (min)

+Ca

- Ca HBSS 10 min

UPTAKE std HBSS, 20 min

Sampling (2, 5, 10 and 20 min)

Wash (~1 min)

Sampling (2, 5, 10, 15 min)

- Ca HBSS, 15 min

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

loading phase

efflux phase

Accumulation (pmol/mg protein)

Time (min)

Basolateral Efflux in SCH

Standard Buffer (cells+bile)

- Buffer: $C_{Buffer}^{+}$, $V_{Buffer}^{-}$
- Cell: $C_{cell}^{+}$, $V_{cell}^{-}$
- Bile: $C_{bile}^{+}$, $V_{bile}^{-}$

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

- Buffer: $C_{buffer}^{+}$, $V_{buffer}^{-}$
- Cell: $C_{cell}^{+}$, $V_{cell}^{-}$

Taurocholate (³H-TCA) Disposition in Human and Rat Sandwich-Cultured Hepatocytes

**Human**

**Cell Lysate**

BEI (%) = 70 ± 4

**Buffer**

**Rat**

BEI (%) = 82 ± 3

**Legend**

- +Ca²⁺
- -Ca²⁺

Mean ± SEM of n=3 SCH preparations

Yang…Brouwer, in submission
Rosuvastatin Efflux in SCH

- 0.1 and 1 µM RSV, ± 0.5 µM GF120918 (GF918) in WT and TR⁻ Rat SCH
- n=3 liver preparations, each in triplicate

Rosuvastatin Uptake and Efflux in Rat SCH

\[ \begin{align*}
\text{Parameter} & \quad \text{Value} \\
\text{WT} & \quad \text{CL}_{\text{Uptake}} \\
\text{TR} & \quad \text{CL}_{\text{Uptake}} \\
\text{WT+GF} & \quad \text{CL}_{\text{Uptake}} \\
\text{TR+GF} & \quad \text{CL}_{\text{Uptake}} \\
\end{align*} \]

P<0.05, adjusted:
* WT v. TR⁺ (±GF120918, respectively)
† + v. − GF120918 (WT or TR⁺, respectively)

**Single-Pass Rat Isolated Perfused Liver (IPL)**

**Excretion Rate**
- Excretion Rate (nmol/min) vs. Time (min)
- Loading phase vs. Efflux phase

**Equilibration**
- 15 min

**Loading**
- 0.5 µM Rosuvastatin, 60 min

**Efflux / Washout**
- Blank Buffer, 30 min

**Bile**
- Portal vein

---

<table>
<thead>
<tr>
<th></th>
<th>Rat IPL</th>
<th>Rat SCH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CL_{Bile}</strong></td>
<td>(mL/min/g liver) 0.26 ± 0.12</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td><strong>CL_{BL}</strong></td>
<td>(mL/min/g liver) 1.4 ± 0.3</td>
<td>0.21 ± 0.07</td>
</tr>
</tbody>
</table>

N=3 livers

Pfeifer...Brouwer, J Pharmacol Exp Ther, 347:737, 2013
Rosuvastatin Basolateral Efflux in SCH: Species Differences

<table>
<thead>
<tr>
<th></th>
<th>Human SCH</th>
<th>Rat SCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRP2/BCRP</td>
<td>27%</td>
<td>53%</td>
</tr>
<tr>
<td>MRP3/4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mrp3/4</td>
<td>73%</td>
<td>47%</td>
</tr>
<tr>
<td>CL&lt;sub&gt;Bile&lt;/sub&gt;</td>
<td>0.037±0.015</td>
<td>0.23±0.04</td>
</tr>
<tr>
<td>CL&lt;sub&gt;BL&lt;/sub&gt;</td>
<td>0.10±0.02</td>
<td>0.21±0.07</td>
</tr>
</tbody>
</table>

Importance of Basolateral Efflux Transporters in Rosuvastatin Hepatobiliary Disposition

- A novel uptake and efflux protocol was developed in SCH to quantify basolateral efflux and biliary excretion

- Basolateral efflux contributed significantly to rosuvastatin hepatocellular elimination in rat and human sandwich-cultured hepatocytes and rat isolated perfused livers

- Rosuvastatin is a substrate of human MRP4, which likely mediates basolateral efflux in human liver

Mechanisms of Drug-Induced Liver Injury: Transporter-Mediated Bile Acid-Drug Interactions

BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)
Questions:
1. What are the relative contributions of biliary vs. basolateral efflux to hepatic bile acid excretion in rats vs. humans?
2. What is the impact of transporter-mediated drug-bile acid interactions on hepatocellular bile acid exposure?
Species Differences in Relative Contribution of Efflux Pathways to Hepatic Taurocholate Excretion

**Human SCH**

- NTCP
  - BSEP 77%
  - MRP3/4
  - OSTα/β 23%

**Human SCH**

- MRP3/4

**Rat SCH**

- Ntcp
  - Bsep 57%
  - Mrp3/4
  - Ostα/β 43%

<table>
<thead>
<tr>
<th>Species</th>
<th>Efflux Pathways</th>
<th>Taurocholate Excretion %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>NTCP</td>
<td>23%</td>
</tr>
<tr>
<td>Rat</td>
<td>Ntcp</td>
<td>43%</td>
</tr>
</tbody>
</table>

**Human SCH**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL_{Uptake}</td>
<td>2.2 ± 0.4 mL/min/g liver</td>
</tr>
<tr>
<td>CL_{Bile}</td>
<td>0.14 ± 0.04 mL/min/g liver</td>
</tr>
<tr>
<td>CL_{BL}</td>
<td>0.042 ± 0.019 mL/min/g liver</td>
</tr>
</tbody>
</table>

Yang...Brouwer, J Pharmacol Exp Ther, 353:415, 2015
Species Differences in Relative Contribution of Efflux Pathways to Hepatic Taurocholate Excretion

<table>
<thead>
<tr>
<th></th>
<th>Human SCH</th>
<th>Rat SCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL_{Uptake}</td>
<td>2.2 ± 0.4</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>CL_{Bile}</td>
<td>0.14 ± 0.04</td>
<td>0.34 ± 0.07</td>
</tr>
<tr>
<td>CL_{BL}</td>
<td>0.042 ± 0.019</td>
<td>0.26 ± 0.07</td>
</tr>
</tbody>
</table>

Yang…Brouwer, J Pharmacol Exp Ther, 353:415, 2015
Impact of Transporter-Mediated Drug-Bile Acid Interactions on Hepatic Taurocholate Exposure

Human SCH

Rat SCH

Yang...Brouwer, J Pharmacol Exp Ther, 353:415, 2015
Impact of Transporter-Mediated Drug-Bile Acid Interactions on Hepatic Taurocholate Exposure

Human SCH

Yang...Brouwer, J Pharmacol Exp Ther, 353:415, 2015
Impact of Transporter-Mediated Drug-Bile Acid Interactions on Hepatic Taurocholate Exposure

Yang…Brouwer, J Pharmacol Exp Ther, 353:415, 2015
Hepatic Disease-Associated Alterations in Hepatobiliary Transport Proteins

Disease-mediated changes in hepatic transport function may impact drug exposure.

Hepatitis-
Cirrhosis

Obstructive Cholestasis

Hepatitis-
Cirrhosis

Obstructive Cholestasis

MRP4

MRP3

MRP2

MRP1

BCRP

OSTα/β

MDR3

BSEP

NTCP

MATE1

OCT1

OAT2

OATP2B1

OATP1B3

OATP1B1

Bile canaliculus

Bloodstream

Chai et al, Hepatology, 55:1485, 2012; Ogasawara et al., Drug Metab Pharmacokinet, 25:190, 2010

Zollner et al., Liver Intl, 2007; Takeyama and Sakisaka, Hepatology Res, 42:120, 2012
Altered Expression of Hepatic OATPs in NASH

mRNA

Clarke et al., J Hepatol, 61:139, 2014
Altered Expression of Hepatic OATPs in NASH

**mRNA**

[Graphs showing mRNA levels of OATP1B1, OATP1B3, and OATP2B1 in Normal, Steatosis, and NASH stages.]

**Protein**

[Graphs showing protein levels of OATP1B1, OATP1B3, and OATP2B1 in Normal, Steatosis, and NASH stages, with up and down arrows indicating changes in expression.]
Clinical Study Design: $^{99m}$Tc-Mebrofenin

- Subjects admitted on morning of study after an overnight fast
- Attenuation correction obtained with a cobalt-57 flood source
- Subjects positioned supine under gamma camera

Subjects discharged following exit exam
# Demographics and Clinical Chemistries

<table>
<thead>
<tr>
<th></th>
<th>Control (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>8 M; 6 F</td>
<td>4 M; 3 F</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>14 non-Hispanic</td>
<td>1 Hispanic; 6 Non-Hispanic</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>11 Caucasian; 3 African-American</td>
<td>5 Caucasian; 1 Mexican; 1 Asian</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>38.9 ± 15.4</td>
<td>37.4 ± 17.4</td>
</tr>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td>72.1 ± 12.1</td>
<td><strong>102 ± 16</strong>*</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.4 ± 2.2</td>
<td><strong>33.3 ± 5.1</strong>*</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dL)</strong></td>
<td>0.86 ± 0.17</td>
<td>0.83 ± 0.15</td>
</tr>
<tr>
<td><strong>Bilirubin, total (mg/dL)</strong></td>
<td>0.729 ± 0.237</td>
<td>0.957 ± 0.391</td>
</tr>
<tr>
<td><strong>Albumin (g/dL)</strong></td>
<td>4.20 ± 0.20</td>
<td>4.49 ± 0.38</td>
</tr>
<tr>
<td><strong>ALT (u/L)</strong></td>
<td>28.7 ± 9.8</td>
<td><strong>113 ± 60</strong>*</td>
</tr>
<tr>
<td><strong>AST (u/L)</strong></td>
<td>25.2 ± 8.0</td>
<td><strong>72.9 ± 34.3</strong>*</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>1.56 ± 0.53</td>
<td><strong>8.18 ± 4.56</strong>*</td>
</tr>
<tr>
<td><strong>ALP (u/L)</strong></td>
<td>56.3 ± 17.8</td>
<td>68.1 ± 20.0</td>
</tr>
</tbody>
</table>

Mean ± SD; *p < 0.05 using 2-tailed Student’s t-test
Hepatic Metabolism of Morphine

Morphine

Morphine-6-Glucuronide (M6G)

Morphine-3-Glucuronide (M3G)

Hepatic UGT2B7 Glucuronidation
Vectorial Transport of Morphine and Metabolites

Morphine

**OCT1**  **OATP1B1, 1B3**  *(SLC22A1) (SLCO1B1 and 1B3)*

**bile**

**MRP2**  *(ABCC2)*

**ATP**

**Morphine Glucuronides**

**blood flow**

**bile**

**MRP3**  *(ABCC3)*

**Morphine Glucuronides**

**blood flow**
Simulations Predict That MRP3 Substrates Have Increased Systemic Exposure in NASH
Clinical Study Design: Morphine / Glucuronides

- Healthy subjects without insulin resistance: n=14
- Biopsy confirmed NASH patients [NAFLD activity score (NAS)>3]: n=7

## Demographics

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td>7 males, 7 females</td>
<td>3 males, 4 females</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td>1 Hispanic</td>
<td>1 Hispanic</td>
</tr>
<tr>
<td></td>
<td>13 Non-Hispanic</td>
<td>6 Non-Hispanic</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td>12 Caucasian</td>
<td>7 Caucasian</td>
</tr>
<tr>
<td></td>
<td>2 African-American</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>42 (13)</td>
<td>48 (10)</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>26 (3)</td>
<td>32* (5)</td>
</tr>
<tr>
<td><strong>ALT (u/L)</strong></td>
<td>33 (11)</td>
<td>75* (36)</td>
</tr>
<tr>
<td><strong>ALP (u/L)</strong></td>
<td>63 (13)</td>
<td>80* (14)</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>2 (1)</td>
<td>12* (9)</td>
</tr>
</tbody>
</table>

Mean (SD); * p<0.05 using Student’s two-tailed t-test comparing healthy subjects to NASH Ferslew…Brouwer, Clin Pharmacol Ther, 97:419, 2015
Mrp3 Mediates Hepatic Efflux of Morphine-3-Glucuronide (M3G) into Plasma

van de Wetering K et al. Mol Pharmacol, 72:387, 2007
Morphine Pharmacokinetics in Patients with NASH

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (nM)</td>
<td>296 (237-369)</td>
<td>332 (200-551)</td>
</tr>
<tr>
<td>$AUC_{0-\text{last}}$ ($\mu\text{M*min}$)</td>
<td>4.1 (3.1-5.3)</td>
<td>3.5 (2.4-5.0)</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>88 (66-117)</td>
<td>95 (49-183)</td>
</tr>
</tbody>
</table>

Geometric mean (95% CI); * p<0.05 t-test on log transformed parameters

# Increased Serum Concentrations of Morphine Glucuronide, an MRP3 Probe, in Patients with NASH

**MG Parameters**

<table>
<thead>
<tr>
<th>Parameter</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$_{0\text{-last}}$ (µM*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

Impact of NASH-Mediated Changes in Hepatic Efflux Transporters on Systemic and Hepatic Drug Exposure

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
MRP3 and MRP4 Inhibitor Screening Based on a Structurally Diverse Dataset

88 FDA-Approved Drugs

BSEP Non-Inhibitors [50]  
BSEP Inhibitors [38]

Cholestatic [26]  
Non-Cholestatic [24]

Cholestatic [22]  
Non-Cholestatic [16]

Bayesian Models for MRP4 and MRP3 Inhibition

Favorable molecular features for interactions with MRP4:


Favorable molecular features for interactions with MRP3:

In Vitro Verification of Predicted MRP3 Inhibitors

Mean±SEM (N=3)

## Number of Drugs with Evidence of Liver Injury/Total Number of Drugs Fitting Column and Row Criteria

<table>
<thead>
<tr>
<th>Transporter Assay</th>
<th>BSEP $C_{ss}/IC_{50}$ Ratio &lt; 0.1</th>
<th>BSEP $C_{ss}/IC_{50}$ Ratio ≥ 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP2 stimulation or stimulation/inhibition</td>
<td>$8/10 \ (80%)$</td>
<td>$6/6 \ (100%)$</td>
</tr>
<tr>
<td>MRP2 $C_{ss}/IC_{50}$ ratio &lt; 0.1</td>
<td>$6/10 \ (60%)$</td>
<td>$3/3 \ (100%)$</td>
</tr>
<tr>
<td>$C_{ss}/IC_{50}$ ratio ≥ 0.1</td>
<td>None found</td>
<td>$1/1 \ (100%)$</td>
</tr>
<tr>
<td>MRP3 $C_{ss}/IC_{50}$ ratio &lt; 0.1</td>
<td>$10/16 \ (63%)$</td>
<td>$7/7 \ (100%)$</td>
</tr>
<tr>
<td>$C_{ss}/IC_{50}$ ratio ≥ 0.1</td>
<td>$0/1 \ (0%)$</td>
<td>$5/5 \ (100%)$</td>
</tr>
<tr>
<td>MRP4 $C_{ss}/IC_{50}$ ratio &lt; 0.1</td>
<td>$16/29 \ (55%)$</td>
<td>$10/10 \ (100%)$</td>
</tr>
<tr>
<td>$C_{ss}/IC_{50}$ ratio ≥ 0.1</td>
<td>$2/4 \ (50%)$</td>
<td>$12/13 \ (92%)$</td>
</tr>
</tbody>
</table>

### Flowchart

- **In vitro BSEP vesicle assay**
  - Prioritize least potent compounds for advancement
  - No Effect
  - Proceed

- **Css/BSEP IC$_{50}$ ratio**
  - $<< 0.1$ and little or no effect on MRPs
    - Proceed with minimal risk
  - ≥ 0.1 and no effect on MRPs
    - Proceed with moderate risk
  - ≥ 0.1 and some effect on one or more of the MRPs
    - Proceed with high risk

---

Estimated Probability of Cholestatic Potential Based on Percent MRP4 Inhibition Among BSEP Non-Inhibitors

Logistic regression curve for estimated probability of cholestasis with 95% confidence intervals (shaded areas). Observed % MRP4 inhibition for cholestatic (0 = 1.00) and non-cholestatic (0 = 0.00) drugs. Inset: estimated odds ratios (OR) for different MRP4 inhibition values.

Köck…Brouwer, Drug Metab Dispos, 42: 665, 2014
Hepatic Uptake and Efflux Transporters

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
Importance of Hepatic Efflux Transporters in Disease…

Impaired BSEP Function

Bile Acids


(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
Hepatic MRP3 and MRP4 are Upregulated in Patients with Obstructive Cholestasis

Multidrug Resistance Protein 3

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP3</td>
<td></td>
</tr>
</tbody>
</table>

Multidrug Resistance Protein 4

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP4</td>
<td></td>
</tr>
</tbody>
</table>

Chai et al, Hepatol, 55:1485, 2012

Chai et al, J Gastrointest Surg, 15:996, 2011
OSTα/β-mediated Taurocholate (TCA) Uptake was Modulated by Numerous Compounds.

No Pre-incubation (10 min) with the Compounds, Uptake (30 sec) with TCA and Compound

Pre-incubation (10 min) with the Compounds, Uptake (30 sec) with TCA

Malinen…Brouwer, *ITCW3 Abstract*, 2017
OSTα/β-mediated Taurocholate (TCA) Uptake was Modulated by Numerous Compounds

No Pre-incubation (10 min) with the Compounds, Uptake (30 sec) with TCA and Compound

Pre-incubation (10 min) with the Compounds, Uptake (30 sec) with TCA
Obeticholic Acid (OCA)

- First-in-class Farnesoid X Receptor (FXR) agonist
- OCA approved for primary biliary cirrhosis (PBC)
- OCA treatment for non-alcoholic steatohepatitis (NASH) is in Phase III clinical trials
Gene Expression Changes in Human SCH in Response to CDCA

- **No Change** in NTCP expression, responsible for bile acid uptake
- Expression of OATPs (uptake) and MRP3/4 (basolateral efflux) was not altered

**Increased** expression of BSEP (responsible for canalicular excretion of bile acids)
Farnesoid X Receptor (FXR): Key Regulator of Bile Acid Homeostasis

Camilleri et al., Am J Physiol Gastrointest Liver Physiol, 2014
Mechanism of Action for OCA: Improved Bile Acid Homeostasis

Human Hepatocytes

- BSEP (Bile Salt Export Pump)
- NTCP (Sodium-Taurocholate Cotransporting Polypeptide)
- MRP (Multidrug Resistance–Associated Protein)
- OST (Organic Solute Transporter)
- OATP (Organic Anion-transporting Polypeptides)
- FXR
- OCA
- CYP7A1
- Cholesterol

Blood flow
Dependent Variable (DV) vs. Individual Predicted Value (IPRED)

Cell+Bile

Cell

Standard HBSS

Ca^{2+}-free HBSS
Conclusions

- TCA basolateral efflux clearance was significantly increased by OCA and CDCA (> 6 fold), which contributed to decreased cellular accumulation of TCA in SCHH.
- Functional changes in basolateral efflux of TCA are consistent with increased gene expression of OSTα/β.