Altered Hepatic Transport due to Liver Disease: Implications for Drug Development

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Conflict of Interest Disclosure

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• 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, recently acquired by BioIVT.
Outline

• Hepatic Transporters: What Every Drug Development Scientist Needs to Know

• The Obesity Epidemic
  – Non-Alcoholic Fatty Liver Disease (NAFLD)
  – Non-Alcoholic Steatohepatitis (NASH)

• NASH-mediated Alterations in Hepatic Transporter Expression

• Clinical Probes to Assess Hepatic Transporter Function in NASH
  – $^{99m}$Tc-Mebrofenin
  – Morphine/Morphine Glucuronides

• Bile Acids as Endogenous Markers of Hepatic Uptake and Efflux Transporters in Patients with NASH
Hepatic Uptake and 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)
Hepatic Disease-Associated Alterations in Hepatobiliary Transport Proteins

Evers…Brouwer, *Clin Pharmacol Ther*, in press (Oct), 2018
The Obesity Epidemic

- Associated with metabolic syndrome
  - Dyslipidemia, hypertension, type II diabetes, and non-alcoholic fatty liver disease (NAFLD)

*Sample size <50 or the relative standard error (dividing the standard error by the prevalence) ≥ 30%.*
The Obesity Epidemic

- Associated with metabolic syndrome
  - Dyslipidemia, hypertension, type II diabetes, and non-alcoholic fatty liver disease (NAFLD)

*Sample size <50 or the relative standard error (dividing the standard error by the prevalence) ≥ 30%.*
Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

• Worldwide prevalence of NAFLD is 25% and increasing with highest prevalence in:
  – 32% Middle East
  – 31% South America
  – 27% Asia
  – 24% USA
  – 23% Europe
  – 14% Africa

• ~10-20% of patients with NAFLD present with the more progressive form, NASH
  – characterized by hepatocyte ballooning, steatosis, and inflammation

What is the Impact of NASH on:

- Hepatic transport protein expression?
- Hepatic transporter function?
- Hepatic exposure to drugs and metabolites?
Altered Protein Expression of Hepatic Transporters in Patients with NASH

Hepatic Uptake Transporters

OATP1B1
Cadherin

OATP1B3
Cadherin

OATP2B1
Cadherin

Hepatic Efflux Transporters

MRP1

MRP3

MRP4

P-gp

BCRP

Pan-Cadherin

Human Liver Tissue

Clarke et al., *J Hepatol*, 61:139, 2014
Altered MRP2 Expression and Localization in Liver Tissue from Patients with NASH

Clarke et al., Liver Int, 00:1-8, 2017

p<0.05 compared to Normal

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
Altered Hepatic Transporter Function in Patients with NASH

(Adapted from Ho and Kim, *Clin Pharmacol Ther*, 78:260, 2005)
Simulations Predict That MRP2 Substrates Have Increased Hepatic Exposure in NASH

99mTc-Mebrofenin (Choletec®): Probe for Transporter-Mediated Hepatobiliary Excretion

- Used clinically as a hepatobiliary imaging agent
- Liver uptake ~98%; negligible metabolism
- Urinary excretion <2% of dose
- Transporter-mediated hepatobiliary disposition
  - Hepatic uptake via OATP1B1 and OATP1B3
  - Biliary excretion via MRP2
  - Basolateral excretion via MRP3

Gamma Scintigraphic Images (0-180 min) of $^{99m}$Tc-Mebrofenin Human Hepatic Disposition

Ghibellini…Brouwer, AAPS Journal, 6 (4) Article 33, 2004
Clinical Study Design: $^{99m}$Tc-Mebrofenin

- Subjects (healthy and biopsy-confirmed NASH) admitted on morning of study after overnight fast
- Attenuation correction obtained with a cobalt-57 flood source
- Subjects positioned supine under gamma camera

2.5 mCi i.v. dose

blood sampling

| 0 | 2.5 | 5 | 7.5 | 10 | 15 | 20 | 40 | 60 | 80 | 100 | 120 | 140 | 160 | 180 | 210 | 240 | 270 | 300 min |

- Subjects discharged following exit exam
Hepatic $^{99m}$Tc-Mebrofenin Exposure was Increased in Patients with NASH

Mean ± SD

- Control (n = 14)
- NASH (n = 7)

Ali…Brouwer, Clin Pharmacol Ther, in press, 2018
Model Scheme Describing $^{99m}$Tc-Mebrofenin Disposition and Parameter Estimates

Observed Data and Model Predictions for Individual Subjects

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{CL}_{\text{uptake}}$ (L/min)</td>
<td>1.14 (0.73-2.27)</td>
<td>0.731 ** (0.382-1.04)</td>
</tr>
<tr>
<td>$\text{CL}_{\text{efflux}}$ (L/min)</td>
<td>0.00800 (0.00481-0.0139)</td>
<td>0.00579 (0.00475-0.00903)</td>
</tr>
<tr>
<td>$\text{CL}_{\text{bile}}$ (L/min)</td>
<td>0.0354 (0.0157-0.0728)</td>
<td>0.0171 ** (0.0110-0.0207)</td>
</tr>
<tr>
<td>$V_{\text{central}}$ (L)</td>
<td>11.1 (9.55-12.5)</td>
<td>6.32 ** (5.69-9.69)</td>
</tr>
<tr>
<td>$V_{\text{liver}}$ (L)</td>
<td>0.958 (0.527-1.39)</td>
<td>0.891 (0.648-1.43)</td>
</tr>
</tbody>
</table>

Median (range); **p<0.001

Ali…Brouwer, Clin Pharmacol Ther, in press, 2018
**SLCO1B1 Genotype and Hepatic Uptake Clearance of $^{99m}$Tc-Mebrofenin**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Healthy</th>
<th>NASH</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>*15/*15</td>
<td>1</td>
<td></td>
<td>Low (LF)</td>
</tr>
<tr>
<td>*1A/*15</td>
<td>3</td>
<td></td>
<td>Intermediate (IF)</td>
</tr>
<tr>
<td>*1A/*14</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*14/*14</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1A/*1A</td>
<td>2</td>
<td>3</td>
<td>Normal (NF)</td>
</tr>
<tr>
<td>*1B/*1B</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1B/*14</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1A/*1B</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Increased Hepatic MRP3 Expression in Liver Tissue from Patients with NASH

<table>
<thead>
<tr>
<th>Normal</th>
<th>Steatosis</th>
<th>NASH (fatty)</th>
<th>NASH (not fatty)</th>
</tr>
</thead>
</table>

~3-fold increase

Hardwick et al., *Drug Metab Dispos*, 39:2395, 2011
Hepatic Disposition of Morphine and Metabolites

Morphine

OCT1 (SLC22A1)

OATP1B1, 1B3 (SLCO1B1, 1B3)

MRP2 (ABCC2)

UGT2B7

Morphine-3- (M3G) and Morphine-6- (M6G) Glucuronide

MRP3 (ABCC3)

Morphine Glucurononides

blood flow
Clinical Study Design: Morphine / Glucuronides

- Healthy subjects without insulin resistance: n=14
- Biopsy confirmed NASH patients [NAFLD activity score (NAS)>3]: n=7
Increased M3G and M6G Serum Concentrations in Patients with Nonalcoholic Steatohepatitis (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$_{0\text{-last}}$ (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

Increased Conjugated Bile Acids in Serum of Patients with NASH

![Graph showing the comparison of total, glycocholate, and taurocholate bile acids between healthy and NASH individuals.]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Bile Acids</th>
<th>Glycocholate</th>
<th>Taurocholate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P-value</td>
<td>β</td>
</tr>
<tr>
<td>NAS+Fibrosis</td>
<td>0.43 (0.10)</td>
<td>0.004</td>
<td>0.03 (0.01)</td>
</tr>
</tbody>
</table>

Serum and Urine Bile Acids at Screening

Mean ± SEM, Healthy: n=15 (13 for urine), NASH: n=7

CDCA = Chenodeoxycholic Acid
CA = Cholic Acid
DCA = Deoxycholic Acid
LCA = Lithocholic Acid
UDCA = Ursodeoxycholic Acid

Ferslew et al., Dig Dis Sci, 60:3318-3328, 2015
Serum Bile Acid vs. Time Profiles

CDCA = Chenodeoxycholic Acid
CA = Cholic Acid
DCA = Deoxycholic Acid
LCA = Lithocholic Acid
UDCA = Ursodeoxycholic Acid

Mean ± SEM (Healthy n=15, NASH n=7)

Ferslew et al., Dig Dis Sci, 60:3318-3328, 2015
Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) Scores Plot Discriminates the Serum Bile Acid Profiles in NASH Patients

Ferslew et al., Dig Dis Sci, 60:3318-3328, 2015
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

ATP driven transporter
Electrochemical gradient
Coupled transporter

Organic Solute Transporter (OST\(\alpha/\beta\)) SLC51A/B is Upregulated in Liver Disease

Patients with Obstructive Cholestasis

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cholestatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>OST(\alpha)</td>
<td></td>
</tr>
<tr>
<td>OST(\beta)</td>
<td></td>
</tr>
</tbody>
</table>

Chai et al, *Plos One*, 2015

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

![Image of protein expression levels and immunofluorescence staining](image)

Bile Acid Regulation of Hepatic Transporter Function

BSEP (Bile Salt Export Pump);
NTCP (Sodium-Taurocholate Cotransporting Polypeptide);
MRP (Multidrug Resistance–Associated Protein);
OST (Organic Solute Transporter)
Hepatobiliary Disposition in Sandwich-Cultured Hepatocytes (SCH): B-CLEAR®

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

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.
Chenodeoxycholic Acid (CDCA) Alters Taurocholate (TCA) Uptake, Basolateral Efflux and Biliary Clearance in Human Sandwich-Cultured Hepatocytes (SCH)

CDCA (100 µM) or Vehicle Control Treatment for 72 hr

Uptake and Efflux of TCA in SCH

+ Ca²⁺

- Ca²⁺

SCH: Sandwich-cultured Human Hepatocytes
TCA: Taurocholate

Mechanistic PK Modeling

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

Guo…Brouwer, J Pharmacol Exp Ther, 365:413, 2018
CDCA, an Endogenous FXR Agonist, Upregulates OSTα/β and BSEP Expression in Human SCH
(100 µM treatment x 72 hr; n=3)

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

Data expressed as relative fold change

CDCA Decreases TCA Uptake Clearance and Increases TCA Efflux Clearance in Human SCH

**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
Summary: Altered Hepatic Transporter Function in Patients with NASH

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

- Alterations in hepatic transporters in patients with NASH may impact the systemic and/or hepatic exposure to substrates [drugs, metabolites, endogenous compounds (e.g., bile acids)]
- Impaired OATP1B1/1B3 and MRP2 function
  - $^{99m}$Tc-Mebrofenin hepatic and systemic exposure were significantly increased in NASH
- MRP3 upregulation
  - Morphine glucuronide systemic exposure and conjugated bile acid serum concentrations were significantly associated with NASH severity
- OSTα/β upregulation
- Patients with NASH have higher fasting and post-prandial exposure to bile acids, including the more hydrophobic and cytotoxic species. Bile acid profiles may be useful in the diagnosis of NASH
Perspectives on Drug Transport in Drug Development

• Our understanding of the role of drug transporters in humans is primarily based on experience in healthy subjects.

• Emerging data indicate that transporter activity may be altered in various diseases, which could impact drug efficacy and/or toxicity in patients.

• Information about disease-mediated alterations in transporters generated in preclinical species may not translate to clinical practice.
Perspectives on Drug Transport in Drug Development

• Delineate factors that regulate transport protein expression and function (e.g., disease, genetics, age, diet, environment, drugs, species differences) and mechanisms of regulation

• Develop more human-like *in vitro* model systems

• Develop broader, quantitative, systems-based approaches to integrate and mechanistically link the subcellular/cellular data with the biologic complexity of the whole organ, the *in vivo* system, and ultimately clinical outcomes

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