Imaging Studies with the Transporter Probe
$^{99m}$Tc-Mebrofenin Reveal Altered Hepatic Exposure in Patients with Non-Alcoholic Steatohepatitis (NASH)

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Conflict of Interest Disclosure

- The Brouwer lab receives research funding from the National Institutes of Health, National Institute of General Medical Sciences [Grant R01 GM041935-24], Intercept Pharmaceuticals, and Otsuka Product Development & Commercialization
- 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
Outline

- **Background**
  - The Obesity Epidemic
    - Non-Alcoholic Fatty Liver Disease (NAFLD)
    - Non-Alcoholic Steatohepatitis (NASH)
  - NASH-mediated Alterations in Hepatic Transporters
  - $^{99m}$Tc-Mebrofenin
    - Clinical Probe to Assess Hepatic Transporter Function

- **Results**
  - Imaging Hepatic Exposure of $^{99m}$Tc-Mebrofenin in Patients with Biopsy-confirmed NASH

- **Conclusions**
The Obesity Epidemic

- Associated with metabolic syndrome
  - Includes: dyslipidemia, hypertension, type II diabetes, and obesity
  - 90% of NAFLD patients have at least one component

The Spectrum of NAFLD

- Steatosis and steatohepatitis comprise Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH).
- In the US, the prevalence of NAFLD is ~30%; NASH prevalence is ~3-5%.

Liver histopathology reveals progression from fatty liver to steatosis, hepatocyte ballooning and lobular inflammation.

Progression to NASH is associated with increased liver-related mortality and morbidity.

What is the Impact of NASH on:

- Hepatic transport protein expression?
- Hepatic transporter function?
- Hepatic exposure to drugs and metabolites?
Hepatic Uptake and Efflux Transporters

(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)
Hepatobiliary Transport Proteins as Underlying Factors in Hepatic Disease

Rotor Syndrome

Dubin-Johnson Syndrome

PFIC Type 2

van de Steeg et al., J Clin Inv, 122:519, 2012
Hepatic Disease-Associated Alterations in Hepatobiliary Transport Proteins

HCV-related Cirrhosis
Primary Biliary Cirrhosis
Obstructive Cholestasis

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

Protein

Clarke et al., J Hepatol, 61:139, 2014
Increased Expression of Hepatic Efflux Transporters in NASH

Aim #1

Human liver tissue

Normal | Steatosis | NASH (fatty) | NASH (not fatty)

- MRP1
- MRP3
- MRP4
- P-gp
- BCRP
- Pan-Cadherin

Human liver tissue

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
Altered MRP2 Localization and Expression in NASH

Hardwick et al., Drug Metab Dispos, 39:2395, 2011
Impact of NASH-Mediated Changes in Hepatic Transporter Function on Systemic and Hepatic Drug Exposure

(Adapted from Ho and Kim, Clin Pharmacol Ther, 78:260, 2005)
Increased M3G and M6G Serum Concentrations in NASH

<table>
<thead>
<tr>
<th>MG Parameters</th>
<th>Healthy (n=14)</th>
<th>NASH (n=7)</th>
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<tbody>
<tr>
<td>C_{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>
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<tr>
<td>Half-life (min)</td>
<td>187 (153-229)</td>
<td>146 (104-205)</td>
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</tbody>
</table>

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

Simulations Predict That MRP2 Substrates Have Increased Hepatic Exposure in NASH

$^{99m}$Tc-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

Ghibellini...Brouwer, Pharm Res, 25:1851, 2008
Gamma Scintigraphic Images (0-180 min) of $^{99m}$Tc-Mebrofenin Hepatic Disposition

- $^{99m}$Tc-mebrofenin rapidly distributes into the liver, is excreted into bile, and collects in the gall bladder

- Liver $t_{\text{max}} \sim 13$ min
Gamma Scintigraphic Images (0-180 min) of $^{99m}$Tc-Mebrofenin Hepatic Disposition

- $^{99m}$Tc-mebrofenin rapidly distributes into the liver, is excreted into bile, and collects in the gall bladder.
- Liver $t_{\text{max}} \approx 13$ min.

Ghibellini et al. AAPS Journal 6 (4) Article 33, 2004
Study Objectives

- Determine the systemic and hepatic exposure of $^{99m}$Tc-mebrofenin, an organic anion transporter probe, in patients with biopsy-confirmed NASH compared to healthy subjects.
- Utilize a pharmacokinetic model describing the systemic and hepatic disposition of $^{99m}$Tc-mebrofenin to evaluate NASH-mediated alterations in hepatic transporter function.
Clinical Study Design

- 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

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

Screen/Informed Consent

99mTc-mebrofenin PK

- Subjects discharged following exit exam

- Continuous γ-scintigraphy
- Urine collection
- Attenuation factor
- Safety questionnaire & discharge
## Demographics and Clinical Chemistries

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<tr>
<th></th>
<th>Control (n=14)</th>
<th>NASH (n=7)</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td>8 M; 6 F</td>
<td>4 M; 3 F</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>14 non-Hispanic</td>
<td>1 Hispanic; 6 Non-Hispanic</td>
</tr>
<tr>
<td>Race</td>
<td>11 Caucasian; 3 African-American</td>
<td>5 Caucasian; 1 Mexican; 1 Asian</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>38.9 ± 15.4</td>
<td>37.4 ± 17.4</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>72.1 ± 12.1</td>
<td>102 ± 16*</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.4 ± 2.2</td>
<td>33.3 ± 5.1*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.86 ± 0.17</td>
<td>0.83 ± 0.15</td>
</tr>
<tr>
<td>Bilirubin, total (mg/dL)</td>
<td>0.729 ± 0.237</td>
<td>0.957 ± 0.391</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.20 ± 0.20</td>
<td>4.49 ± 0.38</td>
</tr>
<tr>
<td>ALT (u/L)</td>
<td>28.7 ± 9.8</td>
<td>113 ± 60*</td>
</tr>
<tr>
<td>AST (u/L)</td>
<td>25.2 ± 8.0</td>
<td>72.9 ± 34.3*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.56 ± 0.53</td>
<td>8.18 ± 4.56*</td>
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<tr>
<td>ALP (u/L)</td>
<td>56.3 ± 17.8</td>
<td>68.1 ± 20.0</td>
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Mean ± SD; *p < 0.05 using 2-tailed Student’s t-test
Summary

- Hepatic transport protein expression and function are altered in patients with NASH, which may impact the systemic and/or hepatic exposure to substrates [drugs, metabolites, and endogenous compounds (e.g., bile acids)]

- **Impaired MRP2 function**
  - $^{99m}$Tc-Mebrofenin *hepatic* and systemic exposure were significantly increased in NASH

- **MRP3 upregulation**
  - Morphine glucuronide *systemic* exposure ($C_{\text{max}}$, AUC) and conjugated bile acid serum concentrations were significantly associated with NASH severity

- 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.
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