An Overview of Drug Transporters in ADME & Safety

14 January 2010
Principles of Clinical Pharmacology
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Implications of Drug Transport in Drug Discovery and Development

- Impact of Drug Transport on ADME
  - Oral absorption of drug
  - Complex metabolism interaction(s)
  - Drug Distribution and elimination
  - Organ-selective delivery of drugs and prodrugs
- Impact of Drug Transport on Response and Toxicology
  - Emerging Role in Toxicology
    - Over expression of drug transporter may be a major factor in tumor, bacterial, and fungal multi-drug resistance (MDR).
- Drug Transporters as Targets
  - LY335979, Zosuquidar
Rosuvastatin Calcium (Crestor) Pharmacokinetics and Prescribing Information

![Graph showing plasma rosuvastatin levels over time for different ethnic groups.](image)

**FDA ALERT [03/2005]**

Rhabdomyolysis (serious muscle damage) has been reported in patients taking Crestor as well as other statin drugs. To date, it does not appear that the risk is greater with Crestor than with other marketed statins. However, the labeling for Crestor is being revised to highlight important information on the safe use of Crestor to reduce the risk for serious muscle toxicity (myopathy/rhabdomyolysis), especially at the highest approved dose of 40 mg. The labeling will also be revised to reflect the results of a large pharmacokinetic study involving a diverse population of Asian patients compared with a Caucasian control group that found drug levels to be elevated approximately 2-fold. Kidney failure of various types

**Impact: Start patients of Asian descent at lowest dose of Rosuvastatin (5 mg)**
Influence of *SLCO1B1* T521>C Genotype on Rosuvastatin AUC

CYP2C9 responsible for formation of N-desmethyl rosuvastatin (10%)
Rosuvastatin also substrate for BCRP (ABCG2)
Presentation Objectives

- Provide an Integrated approach to transporter biology
- Review when drug transport is the rate-limiting step of ADME
  - Absorption
  - Distribution
  - Metabolism and Transporter Interplay
  - Elimination (kidney and liver)
- Provide examples of drug-drug and drug-transporter interactions
- Inter-Individual variability as a determinant of drug transport
- Examples of when drug transport is a primary determinant of drug-induced toxicity.
# P-glycoprotein Substrates

- **Cancer Chemotherapy**
  - Doxorubicin
  - Daunorubicin
  - Vinblastine
  - Vincristine
  - Paclitaxel
  - Teniposide
  - Etoposide

- **Immunosuppressive Drugs**
  - Cyclosporine A
  - FK506

- **Antihistamine**
  - Terfenadine

- **Steroid-like**
  - Aldosterone
  - Hydrocortisone et al.

- **HIV Protease Inhibitors**
  - Amprenavir
  - Indinavir
  - Ritonavir
  - Saquinavir

- **Cardiac Drugs**
  - Digoxin
  - Quinidine
  - Posicor
  - Most statins

- **Anti-helmintics**
  - Ivermectin
  - Abamectin

- **Miscellaneous**
  - Loperamide
  - Colchicine
  - Ondansetron
  - Erythromycin
Clinical Translation of P-gp Inhibition at the BBB

• N=12 subjects
  $[^{11}\text{C}]$verapamil +/- CsA.
• Mean 88% increase in BBB exposure (range 62-148%).
• Clinical observation significantly less than mouse prediction.
Role of Mdr1a in the Blood-Brain Barrier and the Placenta

- Mdr1a/b (-/-) were found to be:
  - Viable
  - Fertile
  - Without observable phenotype until pharmacological challenge with IVM.
    - mdr1a -/- LD50 = 0.7 mg/kg
    - mdr1a +/- LD50 = 60 mg/kg

- CF-1 mice were found to be spontaneously mutant in mdr1a by MSD Scientists. The degree of chemical exposure of fetuses within each litter was inversely related to expression of placental P-gp and cleft palate susceptibility
  - mdr1a -/- 100% cleft palate
  - mdr1a +/- 50% cleft palate
  - mdr1a +/- 0%

Figure from A.H. Schinkel et al., Cell, Vol.77, 491-501, 1994
P-gp at the Blood-Brain Barrier

- Many Examples of Drugs whereby BBB Entry is Not Desirable
  - Ivermectin
  - Digoxin
  - Non-sedating antihistamines
    - Fexofenadine
    - Loratadine
    - Cetirizine

Ivermectin Toxicity in the Collie

- 50% of Collies display CNS toxicity when treated with normal doses of IVM (>60 μg/kg).
- Ivm-sensitive Collies lack functional P-gp at the blood brain barrier.
- ABCB1 cDNA sequencing
  - Sensitive Collies (7/7)
    - 4-base pair deletion
    - homozygous
  - Non-sensitive Collies (6/6)
    - heterozygous (mutant/normal)
  - Other breeds (4/4)
    - normal/normal


http://www.awca.net/drug.htm
P-glycoprotein (ABCB1) Cluster Evaluation

Clinical Study
- Human DDI

Lower Throughput
- abcb1a (KO)
- Preclinical DDI
- FACs Analyses
- Isolated Perfused Organ (brain/gut)
- Confocal studies

Medium Throughput
- Caco-2 or MDCK ABCB1
- Substrate/Inhibitor
- Radiochemical Uptake Assay
- Membrane vesicle assays

Higher Throughput
- CAM Inhibition
- P-gp ATPase
- PXR-Induction
- Cytotoxicity Assays
- In-silico
In Vitro Permeabilities

- **Mannitol**: Passive paracellular
  - Wild-type: \(0.90\)
  - MDR1: \(0.22\)
  - MDR1 + CsA: \(1.9\)

- **Testosterone**: Passive transcellular
  - Wild-type: \(0.28\)
  - MDR1: \(85\)
  - MDR1 + CsA: \(101\)

- **Vinblastine (P-gp substrate)**
  - Wild-type: \(15\)
  - MDR1: \(45\)
  - MDR1 + CsA: \(1.9\)
Caco-2 and MDCK cell comparison

Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub
### In Vitro P-gp IC$_{50}$ for Inhibition of Digoxin Efflux Data from Multiple Labs / Techniques

<table>
<thead>
<tr>
<th>Compound</th>
<th>Pfizer (Net Flux, Caco-2)</th>
<th>Pfizer (Efflux Ratio, Caco-2)</th>
<th>GSK (B to A flux, MDR-MDCK)</th>
<th>Borchardt (B to A flux, MDR-MDCK)</th>
<th>Borchardt (B to A Flux, Caco-2)</th>
<th>BI (B to A flux, MDR-MDCK)</th>
<th>Kim, Wilkinson (Net flux, Caco-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amiodarone</td>
<td>0.01</td>
<td>0.46</td>
<td>4.75-fold</td>
<td>2.5-fold</td>
<td>5.6-fold</td>
<td>14.5-fold</td>
<td>15.7-fold</td>
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<tr>
<td>Cyclosporin</td>
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<td>36</td>
<td>5.0-fold</td>
<td>14.5-fold</td>
<td>110</td>
<td>100</td>
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<td>0.8</td>
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<td>7.37-fold</td>
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<td>5.6-fold</td>
<td>28.2</td>
<td>9.1-fold</td>
<td>1.5-fold</td>
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<tr>
<td>Ketoconazole</td>
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<td>10</td>
<td>4.75-fold</td>
<td>2.5-fold</td>
<td>15.1</td>
<td>1.65</td>
<td>140</td>
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<tr>
<td>Nifedipine</td>
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<td>0.8</td>
<td>0.01</td>
<td>0.46</td>
<td>4.75-fold</td>
<td>5.6-fold</td>
<td>387</td>
</tr>
<tr>
<td>Quinidine</td>
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<td>14.5-fold</td>
<td>2.5-fold</td>
<td>15.7-fold</td>
<td>8.92</td>
<td>140</td>
</tr>
<tr>
<td>Ritonavir</td>
<td>0.01</td>
<td>10</td>
<td>9.1-fold</td>
<td>1.5-fold</td>
<td>8.92</td>
<td>1.5-fold</td>
<td>387</td>
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<tr>
<td>Talinolol</td>
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<td>7.37-fold</td>
<td>13.6-fold</td>
<td>5.6-fold</td>
<td>28.2</td>
<td>1.5-fold</td>
<td>140</td>
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<tr>
<td>Verapamil</td>
<td>0.01</td>
<td>7.37-fold</td>
<td>13.6-fold</td>
<td>5.6-fold</td>
<td>28.2</td>
<td>1.5-fold</td>
<td>387</td>
</tr>
</tbody>
</table>

**IC$_{50}$ Value (uM)**

- **Slide courtesy of M. Troutman/C. Lee Pfizer**

NIH Principles in Clinical Pharmacology: Transporter Biology 14 January 2010
2006 FDA Draft Guidance, International Transport Consortium and FDA Critical Path Workshop

2006 FDA Draft Guidance

- Knowledge of NME metabolic pathways, interactions, and influence of active transport on drug disposition with respect to DDI potential is key to benefit/risk assessment.

- Integrated approach may reduce number of unnecessary studies and optimize clinical pharmacology studies.

- Classification of CYP inhibitors and substrates can aid in study design and labeling.
  - Substrate (25% metabolism)
  - Inhibitor ([I]/Ki > 0.1)
  - Inducer (40% control)

International Transport Consortium

Slide adapted from Shiew-Mei Huang, Ph.D., FDA
Digoxin: Safety Concerns

- Therapeutic conc ~ 1.5 ng/mL
- 33% change in Digoxin Exposure (C<sub>max</sub>) ~ 2.0 ng/mL → Safety concerns
- 25% change in exposure might be clinically relevant

Clinical Pharmacology & Therapeutics (2009); 85, 173–181
P-gp Mediated Digoxin DDIs

• <2-fold change in digoxin Cmax or exposure were observed in the majority of published cases
  – I/IC50 > 0.1 is predictive of positive clinical digoxin DDI related to P-gp
  – I2/IC50 < 10 is predictive of no clinical digoxin DDI

• For Digoxin or NMEs that have a narrow T.I. (similar to digoxin), P-gp may be an important determinant of PK and response.

• Additional work is needed to fully understand the mechanism of false (-)’s observed with I/IC50 or false (+)’s with I2/IC50
Drug Metabolizing Enzyme - Drug Transporter Interplay

Substrate overlap with multiple CYPs and Drug Transporters complicates in vitro to in vivo predictions

However, if your drug is a substrate of CYP3A4 and P-gp, Ketoconazole or Itraconazole represents the worse case scenario for a Clinical DDI study

Mol. Pharmaceutics, 2009, 6 (6), pp 1766–1774
P-gp Summary

• For some compounds, P-gp may hinder drug absorption, moderately change AUC/Cmax and be moderate to major determinant of CNS exposure.

• P-gp may be a target for Drug-Drug Interactions, optimal in-vitro to in-vivo or in-vivo to in-vitro strategy is needed. No Single in-vitro assay appears to be durable enough to perform within diverse chemical libraries and yield consistent ‘predictable’ in-vivo performance.
  – Multi-tiered Assay Cluster Approach used to define NCE/Drug- P-gp interaction.

• Use of mdr1a KO mouse appears to be the most sensitive method to define P-gp substrates, however, cross-species differences in P-gp remains a concern

• Overlap in CYP3A4 and P-gp inhibition may produce ‘worse case scenario’ for some drugs that are substrates for CYP3A4 and P-gp
ABC Substrate/Inhibitor Overlap

Distinct but Overlapping Substrate Specificities

Figure adapted from Thomas Litman
ABCG2 (alias BCRP, MXR, ABCP, BMDP)

- Expressed endogenously in the intestine (small & large), liver, kidney, placenta, skeletal muscle, brain, and in hematopoietic stem cells
- In-vitro role in tumor drug resistance for Topo-1 and Topo-2 inhibitors (MXR, SN-38, Topotecan, J-107088)
- Emerging role in drug absorption of camptothecan analogues (Irinotecan and Topotecan).

- ABC subfamily 7 (G); member 2 (related to Drosophila White proteins)
- 655 amino acid protein
  - ABCP isolated from human placenta R482 WT (Allikmets, 1996)
  - BCRP breast cancer resistance protein R482 T (Doyle et al., 1998)
  - MXR: Mitoxantrone resistance protein R482G (Bates et al., 1999)
  - BMDP: Brain multidrug resistance protein (Eisenblatter et al., 2003)
# Substrates & Inhibitors of ABCG2

<table>
<thead>
<tr>
<th>Drugs/NMEs</th>
<th>Xenobiotics</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>– Topotecan</td>
<td>– PhIP</td>
<td>– FTC</td>
</tr>
<tr>
<td>– CPT-11/SN-38</td>
<td>– Pheophorbide A</td>
<td>• Ko134, 143</td>
</tr>
<tr>
<td>– J-107088</td>
<td>– Estrogen SO₄</td>
<td>– Tryprostatin A</td>
</tr>
<tr>
<td>– Mitoxantrone</td>
<td>– lysotracker (green)</td>
<td>– GF120918</td>
</tr>
<tr>
<td>– Flavoperidol</td>
<td>– H33342</td>
<td>– Lapatinib</td>
</tr>
<tr>
<td>– Diflomotecan</td>
<td>– Rhodamine 123</td>
<td>– Erlotinib</td>
</tr>
<tr>
<td>– Methotrexate</td>
<td>– Bodipy-prazosin</td>
<td>– Gefitinib</td>
</tr>
<tr>
<td>– Sulfasalazine</td>
<td></td>
<td>– CI-1033</td>
</tr>
<tr>
<td>– Prazosin</td>
<td>– Riboflavin (vitamin B2)</td>
<td>– Novobiocin</td>
</tr>
<tr>
<td>– Benzoylphenylurea</td>
<td></td>
<td>– Imatinib</td>
</tr>
<tr>
<td>– Cimetidine</td>
<td></td>
<td>– Ritonavir</td>
</tr>
<tr>
<td>– Imatinib</td>
<td></td>
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</tr>
</tbody>
</table>

- **Bcrp -/- ADME Phenotype**
  - Mice displayed diet-dependent phototoxicity
  - Protoporphyria
  - Enhanced oral absorption of topotecan
  - ABCG2 is expressed in bone marrow stem cells.

**Expression BCRP in mammary gland across species**

<table>
<thead>
<tr>
<th></th>
<th>Nonlactating</th>
<th>Lactating</th>
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</thead>
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<tr>
<td>Mouse</td>
<td><img src="image1" alt="Mouse Image" /></td>
<td><img src="image2" alt="Mouse Image" /></td>
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<tr>
<td>Cow</td>
<td><img src="image3" alt="Cow Image" /></td>
<td><img src="image4" alt="Cow Image" /></td>
</tr>
<tr>
<td>Human</td>
<td><img src="image5" alt="Human Image" /></td>
<td><img src="image6" alt="Human Image" /></td>
</tr>
</tbody>
</table>

**Literature:**
- BCRP substrates reported concentrated into milk of each of these species
- MRP1-5, P-glycoprotein not upregulated in lactating mouse mammary gland

*Slide from A.H. Schinkel, NKI*
Of mice and men: Topotecan:BCRP interaction

Jonker et al., JNCI, 2000

Jonker et al., PNAS, 2002

Jonker et al., JNCI, 2000

Kruijtzer et al., JCO, 2002
Absorption, metabolism, and excretion of salicylazosulfapyridine in man

Fig. 2. Serum concentrations of SASP after ingestion of a single 4 Gm. dose of SASP on Day 1 (10 subjects) and 4 × 1 Gm. of SASP on Days 2 to 10 (9 subjects).

Hasse Schröder and Dag E. S. Campbell  Uppsala, Sweden
Department of Zoophysiology, University of Uppsala, Pharmacia AB, Box 604, 751 25
Permeability is an important determinant of \textit{In vitro-in vivo} extrapolation for both Metabolism and Transport

\textbf{Amidon et al., Pharm. Res. 12:413 (1995)}

\textbf{Wu and Benet, Pharm. Res. 22:11 (2005)}
Sulfasalazine (SASP) Hypothesis

Inter-individual differences in intestinal expression and function of ABCG2 (BCRP) contribute to variability in drug bioavailability, exposure and pharmacological response to SASP.
ABCG2 Polymorphisms and Ethnic Distribution of SNPs.

- The ABCG2 Q141K genotype significantly affected the pharmacokinetics of diflomotecan (Clin Pharmacol Ther. 2004).
- Gefitinib-induced diarrhea correlates with Q141K (J Natl Cancer Inst. 2006).
- ABCG2 expression correlates with flavopiridol-induced myelotoxicity.

<table>
<thead>
<tr>
<th>Allelic Variant</th>
<th>Caucasians</th>
<th>African-Americans</th>
<th>Asians</th>
<th>Hispanics</th>
<th>Africans</th>
<th>Middle Easterns</th>
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<tbody>
<tr>
<td>V12M</td>
<td>2</td>
<td>4</td>
<td>20–45</td>
<td>40</td>
<td></td>
<td>5</td>
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<tr>
<td>Q141K</td>
<td>11–14</td>
<td>2.3–5.0</td>
<td>15–35</td>
<td>10</td>
<td>1.0</td>
<td>13</td>
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<tr>
<td>I206L</td>
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<td>0</td>
<td>0</td>
<td>10</td>
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<td>N590Y</td>
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<td></td>
</tr>
</tbody>
</table>

Figg et al., Anticancer Drugs. 2007
Sulfasalazine (SASP) Disposition

- Indications: Rheumatoid arthritis (RA), Long term therapy of ulcerative colitis, and Crohn’s disease
- Bioavailability (F) of SASP in humans is low (F< 15%) and highly variable
- Low %F primarily attributed to SASP’s low permeability and poor solubility (thus, poor absorption)
- Azo-reduction is the primary route of metabolic clearance
- Metabolism occurs in distal small intestine and large intestine via bacterial flora
- Studies in T-cells (CEM) demonstrate SASP is an ABCG2 (BCRP) substrate

![Chemical structure of Sulfasalazine (SSZ), 5-Aminosalicylic Acid (5-ASA), Sulfapyridine (SP)]

N-acetylation (NAT)  |  Hydroxylation  |  N-acetylation (2 phenotypes)  |  O-glucuronidation
Rapid  |  Slow  |  

Bacterial hydrolysis
Abcg2 is Major Determinant of SASP Absorption and Elimination in the Mouse

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
Abcb1 (mdr1a) does not contribute to SASP Bioavailability or Clearance

Zaher et al., Molecular Pharmaceutics, epub January 4, 2006
<table>
<thead>
<tr>
<th>Mice</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>AUC (ng.hr/mL)</th>
<th>Relative exposure, AUC&lt;sub&gt;KO&lt;/sub&gt;/AUC&lt;sub&gt;WT&lt;/sub&gt;</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WT</td>
<td>KO</td>
<td>Duration (hr)</td>
</tr>
<tr>
<td>Bcrp1</td>
<td>IV</td>
<td>5</td>
<td>1827</td>
<td>13570</td>
<td>0-4</td>
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<tr>
<td></td>
<td>PO</td>
<td>20</td>
<td>233</td>
<td>16176</td>
<td>0-24</td>
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<td>Mdr1a</td>
<td>IV</td>
<td>5</td>
<td>2749</td>
<td>2266</td>
<td>0-6</td>
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<td>PO</td>
<td>20</td>
<td>349</td>
<td>440</td>
<td>0-24</td>
</tr>
</tbody>
</table>

* IV (intravenous) = C<sub>max</sub> at time zero was extrapolated from the model; PO (Oral) = visual C<sub>max</sub> from raw data

SASP C<sub>max</sub> and exposure (AUC) in Bcrp1 (abcg2) and mdr1a (WT and KO) mice following intravenous (IV) and oral (PO) administration.

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
SASP Disposition in North American Healthy Volunteers

Altered SASP Exposure in Q141K Subjects

421C>A SNP Changes Surface ABCG2 Expression

SASP Disposition in Healthy Japanese Volunteers

Figure 2  Effect of ABCG2 genotype on pharmacokinetics of sulfasalazine (SASP). Plasma concentration-time profiles of SASP after oral administration of a 2,000 mg conventional SASP tablet to 421C/C subjects (closed circles, n = 12), 421C/A subjects (open triangles, n = 16), and 421A/A subjects (closed diamonds, n = 9).

Yamasaki et al., CPT January 2, 2008
**ABCG2 Pharmacogenomic Studies**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Dose, Route</th>
<th># Patients</th>
<th>Ethnic Group, Gender</th>
<th>Result</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfasalazine</td>
<td><img src="image" alt="Structure" /></td>
<td>1000 mg po</td>
<td>17^</td>
<td>Caucasian Both</td>
<td>1.7-2.4X increase in AUC, Cmax</td>
<td>Urquhart et al (2008) Pharmacogen &amp; Genomics, ePub</td>
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<tr>
<td>Sulfasalazine</td>
<td><img src="image" alt="Structure" /></td>
<td>500 mg po</td>
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<td>Chinese Both</td>
<td>No effect on AUC, Cmax</td>
<td>Adisson et al (2008) ASCPT mtg poster</td>
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<td>Gefitinib (IRESSA)</td>
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<td>250 mg po</td>
<td>124^</td>
<td>Caucasian Both</td>
<td>44% with mutation had diarrhea vs. 12% with WT</td>
<td>Cusatis et al (2007) JNCI 98(23):1739</td>
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<tr>
<td>Topotecan</td>
<td><img src="image" alt="Structure" /></td>
<td>&lt;2.5 mg po, w</td>
<td>18^</td>
<td>Caucasian Both</td>
<td>1.35X increase in oral bioavailability</td>
<td>Sparreboom et al (2005) Canc Biol Ther 4:650</td>
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<tr>
<td>Diflomotecan</td>
<td><img src="image" alt="Structure" /></td>
<td>&lt;0.5 mg po, iv</td>
<td>22^</td>
<td>Caucasian Both</td>
<td>3X increase in AUC and Cmax for iv only</td>
<td>Sparreboom et al (2004) Clin Pharmacol Ther 76:38</td>
</tr>
<tr>
<td>Imatinib (GLEEVEC)</td>
<td><img src="image" alt="Structure" /></td>
<td>100-1000 mg po</td>
<td>82^</td>
<td>Caucasian Both</td>
<td>No difference</td>
<td>Gardner et al (2006) Clin Pharmacol Ther 80:192</td>
</tr>
</tbody>
</table>

**Formulation**
- IR
- susp
- SR
Gefitinib (Iressa)-enhanced SASP Bioavailability

Plasma concentrations versus time curve after oral administration of SASP (20 mg/kg) alone or combined with gefitinib (50 mg/kg) gavage 2 hrs prior to SASP administration in wt-type mice.
Curcumin increases SASP Bioavailability

ABCG2 Summary

• ABCG2 (BCRP/ABCP) has a role in the absorption and the elimination of a growing list of drugs, endobiotics, and xenobiotics.
• Additional probe substrates and inhibitors are needed to investigate cross-species to human comparisons and to improve *in-vitro* to *in-vivo* predictions.
  – SASP dose and formulation are important determinants of ABCG2’s influence on F.
• ABCG2-transfected LLC-PK1 or MDCK cells may be useful to evaluate the interaction of this transporter with NCEs or Drugs, however, many BCRP (ABCG2) substrates require a basolateral uptake transporter.
• The abcg2 KO mouse in combination with ABCG2 (BCRP) assay cluster may be best way to define ABCG2 substrates and inhibitors.
The SLC Superfamily

- Solute Carrier (SLC) superfamily contains
  - 43 families
  - 298 genes

- HUGO database (see http://www.gene.ucl.ac.uk/nomenclature/)
  - SLC root symbol
  - Followed by numeral (family)
  - Followed by letter
  - Followed by numeral (ie SLC22A1)
  - Further elaborated in the SLC21/SLCO

Major Renal Transporters

Blood Flow

Filtration (GFR) *fu

\[ \text{CL}_r = \text{GFR} + \text{secretion} - \text{reabsorption} \]

\[ \text{CL}_r = \text{GFR} \]

Filtration only

secretion = reabsorption

\[ \text{CL}_r < \text{GFR} \text{ (net reabsorption)} \]

\[ \text{CL}_r > \text{GFR} \text{ (net secretion)} \]
Renally-Mediated DDIs

Penicillin/Probenecid one of the earliest examples of ATS (Active Tubular Secretion) inhibition.

Drugs that have labeling precautions relating to renally-mediated drug transport:

- **Dofetilide (Tikosyn™)**
  - Concomitant administration OCT inhibitors increase potential for cardiac toxicity
- **Cidofovir (Vistide™)**
  - Concomitant administration of OAT inhibitors decrease potential for nephrotoxicity
When is it Important to Study Renal Transporters?

- Does scientific evidence suggest that it is necessary to investigate renal transport DDI potential for NMEs?
  - Toxicologic significance
  - Primary determinant of systemic CL
  - NME inhibits the $\text{CL}_{R}$ of compound with narrow TDI

- What is the optimal in vitro and in vivo strategy that will bridge preclinical to Clinical Development Plan?

- Is there a need to perform both probenecid and cimetidine studies in healthy volunteers if in vitro and preclinical data support that compound is a prototypical transport substrate?
Package Inserts: Clinical Studies and DDI Potential

<table>
<thead>
<tr>
<th>Drug (CL&lt;sub&gt;R&lt;/sub&gt;)</th>
<th>Results (Bedside)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mirapex (400 mL/min) + cimetidine + probenecid</td>
<td>N=12 subjects/treatment arm. 50% ↑ in AUC; 40% ↑ in T 1/2 No effect on PK</td>
</tr>
<tr>
<td>Tikosyn (420 mL/min) + cimetidine + probenecid</td>
<td>Narrow TDI 40% ↑ in AUC; CLR ↓ 33%; QTc ↑ 17-19 ms No effect</td>
</tr>
<tr>
<td>Oseltamivir +cimetidine +probenecid</td>
<td>N=12-18/treatment (see Hill et al.) No change on PK 2.5-fold AUC of Ro64-0802 (active metab)</td>
</tr>
<tr>
<td>Axid (500 mL/min)</td>
<td>Not currently defined, however TDI very high</td>
</tr>
</tbody>
</table>
### Transporter Nomenclature

<table>
<thead>
<tr>
<th>SLC Family</th>
<th>ABC Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basolateral</strong></td>
<td><strong>Apical</strong></td>
</tr>
<tr>
<td>– OCT2 = SLC22A2</td>
<td>– MDR1 = ABCB1</td>
</tr>
<tr>
<td>– OAT1 = SLC22A6</td>
<td>– MRP2 = ABCC2</td>
</tr>
<tr>
<td>– OAT3 = SLC22A8</td>
<td>– MRP4 = ABCC4</td>
</tr>
<tr>
<td>– System L = SCL7A5/8</td>
<td>– BCRP = ABCG2</td>
</tr>
<tr>
<td><strong>Apical</strong></td>
<td></td>
</tr>
<tr>
<td>– PepT2 = SLC15A2</td>
<td></td>
</tr>
<tr>
<td>– OCTTN1 = SLC22A4</td>
<td></td>
</tr>
<tr>
<td>– OCTN2 = SLC22A5</td>
<td></td>
</tr>
<tr>
<td>– OAT4 = SLC22A11</td>
<td></td>
</tr>
</tbody>
</table>
**Hepatic Transporters**

| Question 1. Is uptake transport the rate-Limiting Step of total clearance (assume low/no metabolism). |
| Question 2. Is it possible to predict the DDI potential mediated through hepatic uptake or efflux or are we only able to define potential mechanisms of a PK observation? |
| Question 3. Toxicological significance of bile acid uptake, synthesis, or efflux inhibition |
Hepatic Uptake/Efflux Transporters

Hepatic permeability

Basolateral membrane

Nucleus

Nucleus

Bile canaliculus

Canalicular membrane

Taurocholate, bile acids

Vinblastine, taxol, doxorubicin, large-hydrophobic MW drugs

Etoposide-glucuronide

BCC

ATP1B

NTCP

OATP2B1

DATP1B3

ABCB1

BCBG2

ABCB3

PC (flippase)

NTCP

Na+

OATP1B1

OATP1B3

OATP2B1

Nucleus

Hepatic permeability

NIH Principles in Clinical Pharmacology: Transporter Biology 14, January 2010
Hepatic Transport and Liver Injury


## OATP Substrates

<table>
<thead>
<tr>
<th>OATP1B1 (OATP-C, LST-1, OATP2)</th>
<th>OATP1B3 (OATP8, LST-2)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endogenous Substrates:</strong></td>
<td><strong>Endogenous Substrates:</strong></td>
</tr>
<tr>
<td>Estrone Sulfate, PGE₂, Bilirubin, thyroid hormone (T₃, T₄) Bilirubin-glucuronides Estradiol 17β-d-glucuronide, bile acids</td>
<td>CCK-8, PGE₂ Thyroid hormone (T₃, T₄) Estradiol 17β-d-glucuronide, Bile acids, Deltorphin, DPDPE,</td>
</tr>
<tr>
<td><strong>Drug Substrates:</strong></td>
<td><strong>Drug Substrates:</strong></td>
</tr>
<tr>
<td>Atorvastatin, Cerivastatin, Pravastatin</td>
<td>Pravastatin, Pitavastatin, Rosuvastatin,,</td>
</tr>
<tr>
<td>Rosuvastatin, Pitavastatin, Caspofungin,</td>
<td>Fexofenadine, BQ-123, Oubain,, Digoxin,</td>
</tr>
<tr>
<td>Troglitazone-sulfate, Rifampin, Arsenic,</td>
<td>Doxorotaxel, Paclitaxel,, Rifampin, MTX, Bilirubin,</td>
</tr>
<tr>
<td>Atrasentan, Valsartan, Olmesartan, Enalapril,</td>
<td>Repaglinide, Telmisartan, Valsartan,</td>
</tr>
<tr>
<td>MTX, Temocaprilat, SN-38</td>
<td>Olmesartan, Enalapril, Temocaprilat, SN-38</td>
</tr>
<tr>
<td><strong>Toxins:</strong></td>
<td><strong>Toxins:</strong></td>
</tr>
<tr>
<td>Phalloidin, Microcystin-LR</td>
<td>Phalloidin, Microcystin-LR</td>
</tr>
</tbody>
</table>

©Richard B. Kim M.D.
The NEW ENGLAND JOURNAL of MEDICINE

SLCO1B1 Variants and Statin-Induced Myopathy —
A Genomewide Study

The SEARCH Co-Investigators Group

ABSTRACT

INTRODUCTION

Looing for adverse drug events, such as neuropathy in statin therapy, is challenging with transporter expression and function. In one study, neuropathy occurs in patients with renal insufficiency, especially when the statin is administered with a hydroxy-substituted benzene ring. However, the mechanism and pathogenesis of statin-induced myopathy are not fully understood.

METHODS

We conducted a genomewide association study using 2,764 cases of statin-induced myopathy and 9,950 controls, all of whom were taking statin therapy daily as part of a trial involving 12,000 participants. Replication was tested in a subset of 114 of 105 of the 12,000 participants.

RESULTS

The genomewide scan revealed a single marker association of myopathy with the nonsynonymous single-nucleotide polymorphism rs4149056, which was strongly associated with the myopathy phenotype (odds ratio, 1.38; 95% confidence interval, 1.13 to 1.68), and has a minor allele frequency of 0.05. The odds ratio for myopathy was 4.5 (95% confidence interval, 2.6 to 7.7) per copy of the risk allele, and 16.9 (99% CI, 4.7 to 63.1) in 52.5% of patients with myopathy. More than 60% of those reproducible cases were not attributed to the chromosomes. The association of rs4149056 with myopathy was replicated in the trial of 1,249 patients with statin therapy, which also showed an association between rs4149056 and the development of myopathy. The findings were consistent with the association of this variant with statin-induced myopathy.

CONCLUSION

We have identified a common variant in SLCO1B1 that is strongly associated with an increased risk of statin-induced myopathy. Further studies are needed to explore the mechanism of the association, and to develop effective management strategies.

**SLC01B1 Variants and Statin-Induced Myopathy**

Figure 1. Results of Tests for a Trend in the Association between Myopathy and Each SNP Measured in the Genome-Wide Association Study.

P-values are shown for each SNP measured among 85 participants with myopathy and 90 matched controls who were taking 80 mg of simvastatin daily. Analyses are based on 316,184 of the 318,237 SNPs (99.4%) on the Sentrix HumanHap300-Duo BeadChip (Illumina). A result above the horizontal red line indicates strong evidence of an association (P<5x10^-6).

Hepatic Drug-Drug and Drug Transporter Interaction Potential

• Is Drug eliminated unchanged in the bile and is a substrate of uptake transporter or transporters?
  – Permeability
  – Multiplicity
  – Affinity and Capacity
    • Relative abundance of OATP1B1, OATP1B3, OAT2B1, NTCP
    • Selective vs pan-inhibitors (ie CsA)
• Is Drug a substrate of uptake and efflux transporters
  – Multiplicity (ABCB1, ABCC2, and ABCG2)
• Uptake/efflux synergy
Rifampicin Inhibits Atorvastatin through OATP

- 600 mg rifampicin IV increases atorvastatin acid AUC 7-fold.
- Acutely, single dose rifampicin may inhibit OATP1B3, CYP3A4, and CYP2C8.

(Lau YY et al., Clin Pharmacol Ther, 81, 194-204 (2007), slide courtesy of Dr. L.Z. Benet)
**Rifampicin**

- Antibiotic used in treatment of tuberculosis
- Known for its ability to induce drug metabolizing enzymes and transporters through activation of pregnane X receptor (PXR)
- Recently identified as an inhibitor of OATPs and entry into human hepatocytes mediated by OATP1B1

Rifampacin Disposition in WT vs Slco1b2/- KO Mice

Pravastatin Css Disposition in WT vs Slco1b2−/− Mice


Ongoing work with Oatp1b2 KO

- Understand the physiologic role of Oatp1b2
- Further characterize translatability of murine Oatp’s to human ADME and disease

Figure from Henriette E. Meyer zu Schwabedissen
Future Direction of Drug Transport in Preclinical Development and Clinical Pharmacology

- DDIs mediated through drug transporter(s) have received increased attention, however, at present one can define the likelihood of a DDI for well characterized transporters only qualitatively (Likely, Possible, and Not Likely).
- Significant overlap exists between drug metabolizing enzymes and drug transporters.
- Evaluation of in-vitro screens to predict in-vivo drug-drug interactions is an area of increased regulatory awareness. Therefore, the accuracy of the predicted DDI is dependent on the Quality of the in-vitro assay.
- Greater emphasis on Clinical Translation with respect to PK/PD of select transport probes is needed.
- Preclinical and clinical differences in transporter expression may be a determinant of drug-induced toxicity and a developing area of research for drug-induced diseases.
  - Additional KO and Tg mice to investigate the in-vivo contribution of drug transporters are needed.
Acknowledgment(s) and Contributors

Genentech Development Sciences Clinical Pharmacology, ED-PK/PD, SA, and DMPK

Collaborators: Richard Kim, Yuichi Sugiyama, Tim Tracy, Thomas Litman & Suresh Ambudkar and Hani Zaher


PHA Legacy Collaborators: AZO: Tom Raub, Phil Burton, Larry Schaaf, Mark Grillo, Wade Adams, Jeff Stevens, Jim Bourdage, John Easter, Brad Maxwell, and Greg Winterrowd. Nerviano Medical Sciences (Congregazione dei Figli dell’Immacolata Concezione, CFIC): Pietro Grossi, Mario Monshouwer, Marina Ciomei, Erminia Fontana, Chris James, and Cinzia Pellizzoni

Legacy Pfizer PDMLT: Suri Surendran, Steven Michael, Simon Ball, Terry Smolarek, Madhu Cherukury, Phil Worboys, Cathy Knupp, Rob
Renally-Mediated DDIs

\[ \text{CLR} = \text{GFR} + \text{ATS} - \text{TR} \]

- **CLR**
- **GFR**
- **ATS**
- **TR**

†**Tubular Reabsorption (TR)**
 Sys-L et al.,

- *Flow/pH (No vol effect)*
- *Km/Vmax*

Nonclinical species and/or select DDI & model PBPK

- Cell lines expressing human transporters
  What [ ]?

Validating Transporter Model & Aid Clin PK DDI Design

---

- Very low potential for a clinically significant inhibitor of Sys-L

- *GFR should be determined in some studies*

†few reported DDIs mediated via TR (Lithium and amiloride)
Interspecies Comparison of Oxazolidinone CL\textsubscript{R}/GFR in Rat, Dog, Monkey & Humans
Inhibition of PHA-288034 Clearance via Probenecid or Cimetidine in the Rat

Probenecid (LD= 70 mg/kg) followed by MD=40 mg/kg q40 min or Cimetidine 50 mg/kg q40 min

<table>
<thead>
<tr>
<th>Rat</th>
<th>288034 CLR</th>
<th>288034 + PBCD CLR</th>
<th>GFR-1 (control)</th>
<th>GFR-2</th>
<th>GFR-3</th>
<th>CLR/GFR-2</th>
<th>CLR/GFR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22.96</td>
<td>14.46</td>
<td>12.55</td>
<td>12.19</td>
<td>13.22</td>
<td>1.88</td>
<td>1.29</td>
</tr>
<tr>
<td>2</td>
<td>21.96</td>
<td>17.33</td>
<td>10.55</td>
<td>12.40</td>
<td>14.51</td>
<td>1.77</td>
<td>1.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rat</th>
<th>288034 CLR</th>
<th>288034 + Cimetidine CLR</th>
<th>GFR-1</th>
<th>GFR-2</th>
<th>GFR-3</th>
<th>CLR/GFR-2</th>
<th>CLR/GFR-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>22.18</td>
<td>16.67</td>
<td>11.53</td>
<td>11.40</td>
<td>12.22</td>
<td>1.95</td>
<td>1.36</td>
</tr>
<tr>
<td>4</td>
<td>24.11</td>
<td>17.28</td>
<td>11.35</td>
<td>11.81</td>
<td>11.36</td>
<td>2.04</td>
<td>1.52</td>
</tr>
</tbody>
</table>
Monkey PHA288034 Probenecid Interaction Study

Probenecid 30 mg/kgPO q6h starting 24 h prior to IV PNU-288034

Study by WJ Adams et al.
Model Systems to Study Renal Transport

- Isolated Perfused kidney
- Kidney Slices
- Isolated Renal Tubules (PCTs)
- Isolated BBMVs
- Individual Transporter Clones
  - Transient
  - Stable
- GeMMs
In Vitro Uptake Models

- Transport of PHA-288034 in human proximal tubules.
  - Drug uptake in cell suspension of hPTs.
  - Determine kinetics, substrate specificity, energy & ion dependence
  - Preliminary study suggested no metabolism in hPTs
Na⁺-dependent Uptake of PHA288034

Human Proximal Tubule Studies

- Uptake (Na⁺)
- Na-free buffer

Time (min)

nmol/min/mg
PHA-288034 Uptake in HeLa cells Transfected with Transporter cDNAs

Richard Kim and Brenda Leake
Experimental Protocol: Interaction Assay in Stable Transfectants

Result/calculation = Inhibition of [3H]-ES uptake (% of control) in presence of NME
PHA-288034 Interaction with hOAT1-HEK, hOAT3-HEK, hOCT2-HEK, hOCTN1-HEK and hOCTN2-HEK Cells.
PHA-288034 uptake in hOAT3 cells

Michaelis-Menten Model fitted to individual responses using OLS

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
<th>Standard Error</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Km (uM)</td>
<td>18</td>
<td>6.9</td>
<td>7.42</td>
</tr>
<tr>
<td>Vmax (pmol/mg/min)</td>
<td>31.5</td>
<td>2.90</td>
<td>25.1 38.0</td>
</tr>
</tbody>
</table>

NIH Principles in Clinical Pharmacology: Transporter Biology 14 January 2010
Cross-species Homology of OAT3 (SLC22A8) vs PHA288034 CL$_R$

The diagram shows a graph with the x-axis labeled as \% Protein identity OAT3 (SLC22A8) and the y-axis labeled as Renal Clearance/GFR. The graph includes data points for different species:

- Mus (Mouse): Renal Clearance/GFR = 0.5
- Rat: Renal Clearance/GFR = 1.0
- Dog: Renal Clearance/GFR = 1.5
- Human: Renal Clearance/GFR = 2.0
- Monkey: Renal Clearance/GFR = 3.0

The graph illustrates a trend where the Renal Clearance/GFR increases with increasing \% Protein identity OAT3 (SLC22A8).
Summary of PHA288034 Studies

Multi-tier approach appears to be the best way to identify substrates/inhibitors of uptake/efflux drug transporters.

Active Tubular Secretion

• PHA-288034 appears to be a substrate and an inhibitor of hOAT3 (SLC22A8).
• PHA-288034 does not appear to be a substrate for hOAT1, OCT2, OCTN1, or OCTN2.
• Additional work is needed to fully appreciate OAT3 cross-species differences.
• Cimetidine inhibits OAT3-mediated transport as well as OCT-2 mediated transport.
Drug Interactions: CYP Mediated

- Significant CYP mediated drug interactions based on AUC ratio

N= 115 Studies
CYP2C9, 2D6, 3A4

AUCi/AUC related to P-gp DDI

CYP Summary

• CYP interactions were complex when first recognized
• Largest CYP-mediated DDIs
  – Increase AUC 20X, $C_{\text{max}}$ 12X
• Mechanism of CYP inhibition
  – Competitive or non-competitive
  – Potent inhibitors in sub-nanomolar range
• Many CYP liabilities are thought to be ‘screened’ out at an early stage of preclinical development, however, what liabilities are we selecting for?
The rate determining process

“To understand the transporter-mediated drug-drug interaction, we have to know the rate determining process of a substrate in the overall clearance.”

uptake, basolateral efflux, apical excretion, metabolism

Professor Sugiyama, Keynote address AAPS, November 2007