An Overview of Drug Transporters in ADME & Drug Action

10 January 2013
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
  - Drug Distribution and elimination
  - Drug-Drug Interaction
  - Influence of Pharmacogenomics (PGx) on Drug Transport

- Impact of Drug Transport on Response & Toxicology
  - Emerging Role in Toxicology
  - Over expression of drug transporter may be a major factor in tumor, bacterial, and fungal multi-drug resistance (MDR).

- Transporters as Targets
  - Zosuquidar and Tariquidar
  - SGLT2 Na-Glucose contransporter
When Is an Interaction Clinically Significant?

Wide Therapeutic Range

Narrow Therapeutic Range

Efficacy Curve

Safety Curve (Adverse Effect)

Drug Response

Dose, AUC, or Concentration [Exposure]

Adapted from S-M. Huang/FDA
Rosuvastatin Calcium (Crestor) Pharmacokinetics and Prescribing Information

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 \textit{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
  – Absorption
  – Distribution
  – Metabolism and Transporter Interplay
  – Elimination (kidney and liver)

• Examples of when drug transport is a primary determinant of drug action and drug-induced toxicity.

• Provide examples of significant drug-drug interactions mediated through drug transporter

• Influence of Drug Transport PGx on ADME


By the International Transporter Consortium (ITC): Academia, FDA, Industry

Key Issues Addressed:

- **Which transporters are clinically important and should be considered for evaluation during drug development for induction and/or inhibition studies?**

- **Which methods for studying transporters should be used?**

- **When are evaluations recommended (decision trees)?**
Transporters of Interest in DDI’s

International Transporter Consortium Recommended (ITC 7)

- P-gp (MDR1, ABCB1) – P-Glycoprotein
- BCRP (MXR, ABCG2) – Breast Cancer Resistance Protein
- OATP1B1 and OATP1B3 (SLCO1B1 and SLCO1B3) – Organic Anion Transporting Polypeptide 1B1 and 1B3
- OAT1 and 3 (SLC22A6 and SLC22A8) – Organic Anion Transporter 1 and 3
- OCT 2 (SLC22A2) – Organic Cation Transporter 2

Other Emerging

- OCT1 (SLC22A1) – Organic Cation Transporter 1
- MATEs (SLC47) – Multidrug and Toxin Extrusion
- MRPs (ABCC#) – Multidrug Resistance Proteins
- BSEP (ABCB11) – Bile Salt Export Pump
Transporters covered

- Efflux: P-gp, BCRP
- Renal: OAT/OCT
- Hepatic uptake: OATPs

Diagram descriptions:

a) Intestinal epithelia
- Blood
- Intestine
- OCT1
- OATP
- PEPT1
- ASBT
- MCT1
- MRP2
- BCRP
- P-gp

b) Hepatocytes
- Blood
- BSEP
- MRP3
- MRP4
- MRP6
- CYP3A4
- NTCP
- OATP
- OAT2
- OAT1
- OCT transporters

Transporters covered:

- Efflux: P-gp, BCRP
- Renal: OAT/OCT
- Hepatic uptake: OATPs
Emerging Drug Transporters

a Intestinal epithelia

<table>
<thead>
<tr>
<th>Blood</th>
<th>Intestine</th>
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<tbody>
<tr>
<td>OCT1</td>
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<tr>
<td>MRP3</td>
<td>BCRP</td>
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b Hepatocytes

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<td>OCTN2</td>
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<td>MRPS</td>
<td>MRP4, MRP5</td>
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</table>

Nature Reviews | Drug Discovery
Transporters in Drug Absorption

*Intestinal Epithelial Transporters*
Transporters in the Intestinal Epithelia

Efflux (efflux into lumen): P-gp (MDR1), BCRP

PK consequences of induction/inhibition of intestinal transporters
- Inhibition of intestinal uptake transporters or induction of efflux transporters may decrease intestinal absorption of drugs
- Inhibition of intestinal efflux transporters may increase the oral bioavailability of drugs
P-glycoprotein Substrates

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

- Imunosuppressive 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-thelmintics
  - Ivermectin
  - Abamectin

- Miscellaneous
  - Loperamide
  - Colchicine
  - Ondansetron
  - Erythromycin
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
Consequences of Inducing Intestinal Efflux Transporters

Blood

Intestine

P-gp

P-gp

P-gp

Absorption

Efflux

AUC
Expression of P-gp in Human Duodenal Biopsy

P-gp expression before rifampin administration.

P-gp expression after rifampin administration.

Consequences of Inhibiting Intestinal Efflux Transporters
Effect of P-gp Inhibitors on Plasma Digoxin Concentrations

Mean digoxin plasma concentration time curves in 28 patients before (closed circles) and at least 14 days after the start (open circles) of an antiretroviral therapy containing 400 mg lopinavir + 100 mg ritonavir twice daily. The patients received 0.5 mg digoxin orally at both occasions. Error bars indicate standard deviations.

*Clinical Pharmacology & Therapeutics* (2008); 84, 1, 75–82
Digoxin: Safety Concerns

- Therapeutic conc \( \sim 1.5 \) ng/mL
- 33\% change in Digoxin Exposure \( (C_{\text{max}}) \sim 2.0 \) ng/mL \( \rightarrow \) Safety concerns
- 25\% change in exposure might be clinically relevant

Fenner et al., Clinical Pharmacology & Therapeutics (2009); 85, 173–181
In Vitro Permeabilities

\[
\begin{align*}
\text{mannitol} & \\
B & \quad 0.28 \\
A & \quad 0.26 \\
\text{passive paracellular} & \\
\text{testosterone} & \\
B & \quad 101 \\
A & \quad 85 \\
\text{passive transcellular} & \\
\text{vinblastine (P-gp substrate)} & \\
B & \quad 1.9 \\
A & \quad 0.22 \\
\text{wild-type} & \\
B & \quad 15 \\
A & \quad 0.90 \\
\text{MDR1} & \\
B & \quad 45 \\
A & \quad 0.22 \\
\text{MDR1 + CsA} & \\
B & \quad 1.9 \\
A & \quad 1.9
\end{align*}
\]
Caco-2 and MDCK cell comparison

Figure courtesy from Phil Burton/Allen Hilgers/ Thomas Raub
In Vitro P-gp IC₅₀ for Inhibition of Digoxin Efflux Data from Multiple Labs / Techniques

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

Clinical Pharmacology & Therapeutics (2005) 77, 503–514
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 -/- $LD_{50} = 0.7$ mg/kg
    - mdr1a +/- $LD_{50} = 60$ 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

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
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>Endobiotics</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topotecan</td>
<td>PhIP</td>
<td>Pheophorbide A</td>
<td>FTC</td>
</tr>
<tr>
<td>CPT-11/SN-38</td>
<td>Estrogen SO₄</td>
<td>lysotracker (green)</td>
<td>Ko134, 143</td>
</tr>
<tr>
<td>J-107088</td>
<td>H33342</td>
<td>Rhodamine 123</td>
<td>Tryprostatin A</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>Riboflavin (vitamin B2)</td>
<td>Bodipy-prazosin</td>
<td>GF120918</td>
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<tr>
<td>Flavoperidol</td>
<td></td>
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<td>Lapatinib</td>
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<tr>
<td>Diflomotecan</td>
<td></td>
<td></td>
<td>Erlotinib</td>
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<tr>
<td>Methotrexate</td>
<td></td>
<td></td>
<td>Gefitinib</td>
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<tr>
<td>Sulfasalazine</td>
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<td>CI-1033</td>
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<tr>
<td>Prazosin</td>
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<td>Novobiocin</td>
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<td>Benzoylphenylurea</td>
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<td>Imatinib</td>
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<td>Cimetidine</td>
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<td>Ritonavir</td>
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<tr>
<td>Imatinib</td>
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</table>
The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria.


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

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**Expression BCRP in mammary gland across species**

<table>
<thead>
<tr>
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<th>Nonlactating</th>
<th>Lactating</th>
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<tbody>
<tr>
<td>Mouse</td>
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<td>Cow</td>
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<tr>
<td>Human</td>
<td><img src="Human_Nonlactating.png" alt="Image" /></td>
<td><img src="Human_Lactating.png" alt="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

Kruijzer 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 804, 751 25
Permeability is an important determinant of In vitro-in vivo extrapolation for both Metabolism and Transport

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.
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**
Bcrp is Major Determinant of SASP Absorption in the Mouse

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
P-gp does not contribute to SASP Bioavailability or Clearance in the mouse

Zaher et al., Molecular Pharmaceutics epub January 4, 2006
### Sulfasalazine PK in the Mouse

<table>
<thead>
<tr>
<th>Mice</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>C\textsubscript{max} (ng/mL)*</th>
<th>AUC (ng.hr/mL)</th>
<th>Relative exposure, AUC\textsubscript{KO}/AUC\textsubscript{WT}</th>
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<td>20</td>
<td>349 440 0-24 1098 1781</td>
<td>2</td>
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</table>

* IV (intravenous) = C\textsubscript{max} at time zero was extrapolated from the model; PO (Oral) = visual C\textsubscript{max} from raw data.

SASP C\textsubscript{max} 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>
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<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) Pharmacogenetics &amp; Genomics, ePub</td>
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<td>Sulfasalazine</td>
<td><img src="image" alt="Structure" /></td>
<td>500 mg po</td>
<td>36^</td>
<td>Chinese Both</td>
<td>No effect on AUC, Cmax</td>
<td>Adkinson 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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<td>Topotecan</td>
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<td>&lt;2.5 mg po, iv</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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<td>Diflomotecan</td>
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<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>
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<td>Imatinib (GLEEVEC)</td>
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<td>100-1000 mg po</td>
<td>82^</td>
<td>Caucasian Both</td>
<td>No difference</td>
<td>Gandtner et al (2006) Clin Pharmacol Ther 80:192</td>
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<td>Pitavastatin</td>
<td><img src="image" alt="Structure" /></td>
<td>2 mg po</td>
<td>38^</td>
<td>Japanese Male</td>
<td>No difference</td>
<td>Itoh et al (2007) Clin Pharmacol Ther. 82:541</td>
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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>
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<tr>
<th>Allelic variant</th>
<th>Caucasians</th>
<th>African-Americans</th>
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<th>Hispanics</th>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Figg et al., Anticancer Drugs. 2007*
Gefitinib-enhanced SASP Bioavailability in the mouse

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 in the Mouse

Clinical SASP/Curcumin Interaction

- SASP absorption enhanced with curcumin
- Greater curcumin interaction after higher dose of SASP

Kusuhara et al., Br J Pharmacol. 2012 Jul;166(6):1793-803
Non-linear PK was observed between Micro-Dose (MD) and Therapeutic Dose (TD) of SASP

Kusuhara et al., Br J Pharmacol. 2012 Jul;166(6):1793-803
Mechanism of non-linear SASP Absorption explained by the interaction with OATP2B1

Slide from Prof. Yuichi Sugiyama
Kusuhara et al., Br J Pharmacol. 2012 Jul;166(6):1793-803
Co-administration of proton pump inhibitors delays elimination of plasma methotrexate in high-dose methotrexate therapy

Kunihiro Suzuki,1,2 Kosuke Doki,2 Masato Homma,1,2 Hirofumi Tamaki,1 Satoko Hori,3 Hisakazu Ohtani,3 Yasufumi Sawada1 & Yukinao Kohda1,2

Proton pump inhibitors co-administration 2.65 (1.03-6.82)
Pleural effusion and ascites 2.10 (0.78-5.69)
Methotrexate infusion time 1.03 (0.99-1.08)
Abnormality in laboratory data
Serum creatinine 4.60 (1.31-16.16)
Aspartate aminotransferase 4.12 (1.19-14.28)
Alanine aminotransferase 1.22 (0.42-3.53)

Adjusted odds ratio
Proton Pump Inhibitor (PPI) Pharmacology (*aka* Nexium Nation)

- All PPI’s are substituted benzimidazoles
  - Undergo chemical activation within parietal Cell
  - Only active parietal cells are inhibited (approximately 70-80% following meal)
  - Maximum inhibition occurs in 3-4 days

- Activated molecule irreversibly inhibits Proton Pump (H⁺/K⁺ ATPase)
  - Long off rate (may take 1 week to wash out)

When PPI’s are discontinued rebound acid hypersecretion occurs

Altered intestinal pH is known to impair drug absorption and has been reported in multiple therapeutic areas
  - CV, anti-viral, antifungal, oncology
PPI-High Dose MTX DDI cont.

- PPI are an important risk factor for delayed elimination of MTX
- PPI inhibition of BCRP IC50 >> fu Cmax PPIs
- Can interaction be solely attributed to BCRP inhibition?

<table>
<thead>
<tr>
<th>Dose (mg)</th>
<th>Cmax (μmol l^-1)</th>
<th>f_u</th>
<th>f_u-Cmax (μmol l^-1)</th>
<th>IC50 (μmol l^-1)</th>
<th>f_u/Cmax</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole 20</td>
<td>1.8 [11]</td>
<td>0.050 [15]</td>
<td>0.080</td>
<td>17.6</td>
<td>0.0051</td>
</tr>
<tr>
<td>Lansoprazole 30</td>
<td>2.7 [12]</td>
<td>0.030 [15]</td>
<td>0.081</td>
<td>14.4</td>
<td>0.0056</td>
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<tr>
<td>Rabeprazole 20</td>
<td>1.2 [13]</td>
<td>0.037 [13]</td>
<td>0.045</td>
<td>8.5</td>
<td>0.0093</td>
</tr>
<tr>
<td>Pantoprazole 40</td>
<td>5.5 [14]</td>
<td>0.020 [15]</td>
<td>0.109</td>
<td>5.5</td>
<td>0.0200</td>
</tr>
</tbody>
</table>

f_u: plasma unbound fraction.
Questions in Oncology Drug Discovery and Development

• Can we minimize the clinical variability of drug exposure in oncology patients?
  – Personalized medicine helps to identify which patient tumor mutations will respond to molecular targeted agents

• Do con-meds and herbal supplements taken by Cancer patients meaningfully alter targeted therapeutic drug levels?

• What is the best DDI-mitigation strategy when a patient requires 12-20 different con-meds?
Many Molecular Targeted Agents Display pH-dependent solubility

• Approximately 50-70% of recently approved orally administered targeted cancer therapies display pH-dependent solubility.

• Acid-reducing agents (ARAs), most notably proton pump inhibitors (PPIs), are the most commonly prescribed medications in North America and Western Europe.

• We hypothesize that a decrease in the overall exposure of an orally administered cancer therapy may occur due to concomitant ARA use and this could lead to compromised efficacy and overall patient outcomes.
Theoretical pH solubility curve

BCS Class II substrate
  – pH-dependent solubility and absorption
    • Patients with increased age may have achlorhydria due to H. pylori
      – Ethnic distribution (Japanese ~ 60%, European ~ 10%)
    • Some Cancer patients have had significant gastric surgery
    • Many Cancer patients take PPIs and other acid reducing agents
Drug Absorption Interactions Between Orally-administered Targeted Anti-Cancer Agents and Acid-Reducing Agents: When does pH-dependent Solubility Represent the Rate-Limiting Step of Drug Absorption?
Prevalence of Acid-Reducing Agent Use in Different Cancer Populations - Results

- Among all cancer patients, the total prevalence of ARA use was 20% and 33% for the MS and VA databases, respectively.
- Highest prevalence observed in GI, pancreatic, glioblastoma multiforme, lung and GIST cancer patients.
- PPIs were the most commonly prescribed ARA among cancer patients.
- Prevalence of PPI use across all cancer types was 17% and 23% for the MS and VA databases, respectively.

GS Smelick 2012 ASCPT meeting, Washington D.C.
Dasatinib exposure was reduced by ~85% with PPI-alone treatment (B).

Dasatinib exposure with rabeprazole and betaineHCl treatments (C) recovered much of the lost exposure in the PPI-alone treatment (B).

Marc Yago, Adam Frymoyer et al., ASCPT 2013
pH-dependent DDI Summary

- Absorption (solubility-mediated) DDIs not currently addressed in 2012 FDA DDI guidance.
  - The FDA recognizes the potential significance of this PPI-DDI and is more frequently requesting additional studies to explore the extent of this interaction on small molecule orally-administered molecular targeted agents with pH-dependent solubility

- How (and when) should pH-dependent solubility and PPI-DDI risks be addressed?
  - Target Candidate Profile, IND, EoP2, NDA, or PMC
    - Can pH-dependent DDI be predicted with GastroPlus/Simcyp?
  - Substitute H2-blocker for PPI and try to time-stagger treatments to avoid a clinically significant DDI
    - Limited potential given 1) the extent of the interaction for certain drugs (ex. dasatinib and erlotinib) and 2) unlikely patient compliance given the frequent and chronic use for palliative relief and complexity of dosing strategy.
    - Temporary gastric re-acidification at time of dosing may serve as a potential strategy to avoid a clinically significant DDI.
The SLC Superfamily

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

- HUGO database (see [http://www.gene.ucl.ac.uk/nomenclature/](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

<table>
<thead>
<tr>
<th>Transporter/Atlas (Gen)</th>
<th>Selected substrates</th>
<th>Selected inhibitors</th>
<th>Organs/cells</th>
<th>Comments</th>
</tr>
</thead>
</table>
| OAT1 (SLC22A8)          | Non-sodium-dependent, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolytes, weak, non-electrolyt
Major Renal Transporters

Blood Flow

Filtration (GFR) *fu

CL\textsubscript{r} = GFR + secretion – reabsorption

CL\textsubscript{r} = GFR
  Filtration only
  secretion = reabsorption

CL\textsubscript{r} < GFR (net reabsorption)

CL\textsubscript{r} > GFR (net secretion)

Urine
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 $CL_R$ of compound with narrow TDI

• 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?
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
## 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 TI 40% ↑ in AUC; CLR ↓ 33%; QTc ↑17-19 ms No effect</td>
</tr>
<tr>
<td>Metformin (600 mL/min) + cimetidine + probenecid</td>
<td>Narrow TI 40% ↑ in AUC and 60% ↑ in Cmax 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>
</tbody>
</table>
Metformin – 1st line therapy for newly diagnosed Type II Diabetics (T2D)

- The only oral antidiabetic agent proven to reduce diabetes-related and total mortality in obese T2D (UK Prospective Diabetes Study Group, 1998)
- Metformin is eliminated unchanged in the urine (CLR>>>GFR)
- Adverse reactions:
  - Most common: GI effects (~50%)
  - Lactic acidosis (extremely rare: 3/100,000 patients)
- Recent evidence suggests an anti-cancer benefit

Slide from Kari Morrissey, Ph.D. Candidate UCSF KM Giacomini Lab.
Metformin is Predominately Eliminated in the Proximal Tubule of the Kidney

Slide from Kari Morrissey, Ph.D. Candidate UCSF KM Giacomini Lab.
Consequences of Blocking Organic Cation Elimination

Blood → OCT2 → MATE1 → Urine
Blood → OCT2 → MATE2K → Urine

AUC ↑, C_{kidney} ↓, CL_R ↓

Elimination

Slide from Kari Morrissey, Ph.D. Candidate UCSF KM Giacomini Lab.
Consequences of Blocking Organic Cation Elimination

Slide from Kari Morrissey, Ph.D. Candidate UCSF KM Giacomini Lab.
Impact of Cimetidine on the PK of Metformin Depends on OCT2 Genotype

Fig 1

- GS genotype
- GT genotype
- TT genotype

Fig 2

- Group: GG
- Group: GT
- Group: TT

Pharmacogenetics and Genomics 2008, Vol 18 No 7
OCT1 transports metformin into the liver, the major site of its hypoglycemic activity.

Transport into the liver:
- OCT1 and OCT3 transport metformin into the liver.
- MATE1 transports 
- Blood flow to the liver.
- Hepatocytes are the target cells.
- Bile is the product for elimination.

Pharmacologic Activity:
- Metformin uptake
- AMPK activation
- Gluconeogenic enzymes
- Uptake of gluconeogenic substrates
- Glucose uptake transporter expression
- Glycolytic enzyme activation
- Gluconeogenesis
- Glucose uptake
- Glucose metabolism

Slide from Kari Morrissey, Ph.D. Candidate, UCSF KM Giacomini Lab.
Evaluation of OCT or OAT inhibitors requires determination of an IC50 in an *in vitro* study

Is NME an inhibitor of OCT2, OAT1 or OAT3?
Criteria: Determine the IC50 of NME against MPP*, for OCT2, PAH for OAT1 or ES for OAT3 or other model substrates

- Yes
  - Unbound \( \frac{C_{\text{mcp}}}{C_{\text{p}}} \) of the NME \( \geq 0.1 \)
  - Clinical DDI study with a sensitive substrate (see Box 5 footnote)

- No
  - Unbound \( \frac{C_{\text{mcp}}}{C_{\text{p}}} \) of the NME \( < 0.1 \)
  - DDI study is not needed
  - Poor or not a inhibitor of OCT2, OAT1 or OAT3

MPP*: 1-Methyl-4-phenylpyridinium
PAH: Para-aminobiphenyl
ES: Estrone-3-sulphate

*Note: DDI = Drug-Drug Interaction*
Hepatic Uptake/Efflux Transporters

- Glucuronide-, sulfate-, GS-conjugates, anionic
- ABCB1, ABCC11, ABCB3, ABCC2, ABCC3
- Bile canaliculus
- Vinblastine, taxol, doxorubicin, large-hydrophobic MW drugs
- Taurocholate, bile acids

Basolateral membrane
Nucleus
NTCP
OATP1B1
OATP2B1
OATP1B3
PC (flippase)
Bile canaliculus
Canalicular membrane
ABCB11
ABCC2
ABCG2
ABCB3
Hepatic Transporters

Uptake (from blood into hepatocytes): OATP1B1, OATP1B3
Efflux (excretion to bile): P-gp, BCRP, MRP2

- PK consequences of induction/inhibition of hepatic transporters
  - Inhibition of hepatocellular uptake transporters increase area under concentration curve and maximal plasma concentration
  - Inhibition of drug efflux transporters at the canalicular membrane may decrease the secretion of drug into the bile and significantly increase hepatic drug levels
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 Substrate Decision Tree

Is hepatic elimination an important route of elimination of NME?
Criteria: Cl_H > 0.3 Cl_total

Yes

Does the compound have active hepatocyte uptake, do the drugs physiological properties (e.g. low passive membrane permeability*, high hepatic concentrations relative to other tissues, organic anion/charged at physiological pH) support importance of active uptake into liver?

Likely a poor or not a substrate for OATPs

No

Likely a poor or not a substrate for OATPs

Yes

Investigate uptake transporters expressed in hepatocytes with inhibitions and/or transfected cell lines.

If an OATP substrate, consider a clinical DDI study with single dose rifampicin or cyclosporin as perpetrator. Further consideration could be given to review clinical PK based on OATP genotyping.

* Low permeability needs to be defined by each lab based on standards, such as atenolol (BCS reference drug). A general guide would be 10⁻⁶ cm/sec (10 nm/sec) or lower is ‘low’ permeability.

Nature Reviews Drug Discovery 9, 215-236 (March 2010)
SLCO1B1 Variants and Statin-Induced Myopathy — A Genome-wide Study

The SEARCH Collaborative Group*  

ABSTRACT

LEARNING HOW DIVERSE INTERACTIONS RESULT IN CLUSTERED MYOPATHIES IN DIFFERENT CLINICAL SETTINGs, AND LARGER INHIBITORS TO GENETIC VARIABILITY IN CLINICAL OUTCOMes TO STATIN TREATMENTS. IN THIS CASE, THE MYOPATHY IS ASSOCIATED WITH SLCO1B1, EXPRESSED INTERACTS WITH SLCO1B1 IN GENETICALLY MODIFIED MICE.

METHODS

WE CARRIED OUT A GENOMES-wide association study using approximately 700,000 marker genotypes in over 100,000 participants and 15,000 controls. All of whom were taking 40 mg of simvastatin daily as part of a trial involving 12,000 participants. Replication was tested in a trial of 40 mg of pravastatin daily involving 14,000 participants.

RESULTS

The genome-wide search identified a single strong association of myopathy with the rs4149077 single-nucleotide polymorphism (SNP) located within the 11.0kb region containing the transcription-activating gene (SLCO1B1) which has been shown to have a deleterious effect on the protein. The cDNA encoding rs4149077 was sequenced in 150 patients with aortic stenosis and 99 patients with diabetes mellitus. None of the patients had a variant in the rs4149077 SNP. The prevalence of the rs4149077 C allele was 13%. The odds ratio for myopathy was 4.599% compared to normal 0.98% per 3% of the C allele, and 16.9% in normal 30.1% in compared with TVR that were given 30% of their haplotypes have a same haplotype as the control. The association of rs4149077 with myopathy was replicated in a trial of 40 mg of pravastatin daily, which also showed an association between rs4149077 and the substantial dose of 40 mg of pravastatin daily.

CONCLUSION

We have identified common variants in SLCO1B1 that are strongly associated with an increased risk of statin-induced myopathy. Understanding these variants may help to achieve a better understanding of the genetic basis of statin-induced myopathy and its clinical implications.
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 30 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^-8).
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)
- Identified as an inhibitor of OATPs and entry into human hepatocytes mediated by OATP1B1
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)
Rifampacin Disposition in WT vs Slco1b2-/- KO Mice

In multivariate analyses, the rifampin AUC0-24 was significantly affected by rifampin dosage (in mg/kg), SLCO1B1 c.463C>A polymorphism.

Hepatic Transport and Liver Injury

- Troglitazone,
  $IC_{50} = 3.9 \pm 0.6 \mu M$
- Troglitazone-sulfate,
  $IC_{50} = 0.4 \pm 0.06 \mu M$
- Cyclosporin,
  $IC_{50} = 0.8 \pm 0.23 \mu M$
- Glibenclamide,
  $IC_{50} = 8.6 \pm 1.9 \mu M$

Transporter Interaction Redundancy:

- Drugs that are shown to interact with one transporter typically interact with multiple transporters.
- Thus, multiple pathways for clearance are possible for transporter substrates.
When Should You Look and at What!

If a compound is cleared primarily through bile identify the transporters responsible (BCRP, P-gp, MRPs, BSEP)

If $\text{Clr} > \text{fu} \times GFR_{\text{free}}$
Have active tubule secretion
Identify transporter responsible (OCT2, OAT1, OAT3, MATE’s)

If >25% of drug is cleared hepatically determine if it is actively taken up into hepatocytes (OATP1B1, OAPT1B3, OCT1)


Slide from K. Hilgren, 2012 CACO
Future Direction of Drug Transport in Preclinical Development and Clinical Pharmacology

- Drug-Drug Interactions mediated through drug transporter(s) have received increased attention and are recognized as important contributors of ADME.

- Significant substrate 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 awareness during drug development. Therefore, the accuracy of the predicted DDI is dependent on the Quality of the in-vitro assay and our ability to translate the interaction into the Clinic.
  - Clinical Translation with respect to physiologic PK of transport probe substrates and inhibitors is needed.

- Preclinical and clinical differences in transporter expression remain important determinants of drug-induced toxicity and an important consideration in drug development.
  - Additional KO and Tg models to investigate the in-vivo contribution of drug transporters are needed.
Acknowledgment(s) and Contributors

Genentech Research and Early Development, Development Sciences, Clinical Pharmacology (Mark Dresser, Sharmila Rajan, Eric Reyner, Gillian Smelick, Bert Lum), ED-PK/PD, SA, and DMPK (Laurent Salphati, Harvey Wong and Marcel Hopp)

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Lilly

Shiew Mei Huang  
Lei Zhang  
FDA  
FDA
References

• Transporter mediated drug uptake and efflux
  Clinical Pharmacology & Therapeutics (2011) 89 6, 798–805
  Clinical Pharmacology & Therapeutics (2012) 92 5,

• Transporter-mediated drug-drug interactions

• Membrane transporters in drug development

• UCSF-FDA Drug Transporter Portal (website)
  http://bts.ucsf.edu/fdatransportal/

• Transporter Mediated Drug-Drug Interactions (DDIs)
  Presentation slides by Lei Zhang, PhD (OCP, FDA)
  Clinical Pharmacology Advisory Committee (March 2010)
Thank-you !!
Examples of Mechanisms Underlying Adverse Drug Reactions Due to Modifications in Transport Processes

Clinical Pharmacology & Therapeutics (2011) 89 6, 798–805.
## Transporter Nomenclature

### SLC Family

- **Basolateral**
  - OCT2 = SLC22A2
  - OAT1 = SLC22A6
  - OAT3 = SLC22A8
  - System L = SCL7A5/8

- **Apical**
  - PepT2 = SLC15A2
  - OCTTN1 = SLC22A4
  - OCTN2 = SLC22A5
  - OAT4 = SLC22A11
  - hMATE1 = SLC47A1
  - hMATE2 = SLC47A2

### ABC Family

- **Apical**
  - MDR1 = ABCB1
  - MRP2 = ABCC2
  - MRP4 = ABCC4
  - BCRP = ABCG2
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
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
ABC Substrate/Inhibitor Overlap

Distinct but Overlapping Substrate Specificities

Figure adapted from Thomas Litman
Pravastatin Css Disposition in WT vs Slco1b2\(^{-/-}\) Mice

Many drugs that are efflux substrates are extensively absorbed (fa >80%).

Factors that contribute to efflux limited absorption are high Km, Vmax, low solubility, low permeability, metabolic stability and low dose.

However, fraction absorbed in humans and preclinical species is >90%. Thus a clinical study not required.
Pgp/BCRP Inhibitor Decision Tree

- Need to ‘calibrate’ in vitro systems using clinical data

### Bi-directional transporter assay with a probe P-gp substrate. For example, in Caco-2 or P-gp-overexpressing polarized epithelial cell lines

- Net flux ratio of a probe substrate decreases with increased concentrations of the investigational drug
- Probably a P-gp inhibitor

- Determine \( K_I \) or \( IC_{50} \) of the inhibitor

- Net flux ratio of the probe substrate is not affected with increased concentrations of the investigational drug
- Poor or non-inhibitor

\[
\frac{[I]}{IC_{50}} \text{ (or } K_I) \geq 0.1 \text{ or } \frac{[I]}{IC_{50}} \text{ (or } K_I) = 10
\]

- An in vivo drug interaction study with a P-gp substrate such as digoxin is recommended

\[
\frac{[I]}{IC_{50}} \text{ (or } K_I) < 0.1 \text{ and } \frac{[I]}{IC_{50}} \text{ (or } K_I) ~ 10
\]

- An in vivo drug interaction study with a P-gp substrate may not be needed

### Special Cases

- False Positives (unnecessary clinical studies)
- Alert for \( \frac{[I]}{IC_{50}} ~ 0.1 \text{ or } \frac{[I]}{IC_{50}} ~ 10 \),
  - \([I]_1\) is steady-state total Cmax at the highest clinical dose
  - \([I]_2\) is the GI concentration calculated as dose (mg)/250 mL
- \([I]_2/IC_{50} > 10 \) will be exceeded at a dose of ~12 mg for a drug with inhibition potency of ~10 µM in vitro (MW ~ 500).
- False Negatives (safety concerns for NTI drugs like digoxin and topotecan)

Slide courtesy from Joe Polli and ITC.
OATP Inhibitor Decision Tree

Is the IC_{50} of the NME ≤ 10 times unbound Cmax?

Yes

Is the AUC or Cmax of statin (e.g. rosuvastatin, pravastatin, pitavastatin) predicted to increase > 2 fold in presence of the NME using extrapolation (e.g. R-value > 2)?

Yes

Clinical DDI study with sensitive substrate (e.g. rosuvastatin, pravastatin, pitavastatin)

No

Clinical study may not be needed

No

NME likely not to be an in vivo inhibitor of OATP.

\[ \text{R-value} = 1 + \left( \frac{fu \cdot C_{\text{in, max}}}{IC_{50}} \right), \]

where, \( C_{\text{in, max}} \) is the estimated maximum inhibitor concentration at the inlet to the liver and is equal to \( C_{\text{in, max}} = \left( F_i \cdot \text{Dose} \cdot k_a \cdot Q_s \right) \). \( C_{\text{in, max}} \) is the maximum systemic plasma concentration of inhibitor; \( F_i \) is the fraction of the dose of inhibitor, \( \text{Dose} \), which is absorbed; \( k_a \) is the absorption rate constant of the inhibitor and \( Q_s \) is the hepatic blood flow (e.g., 1500 mL/min)

Nature Reviews Drug Discovery 9, 215-236 (March 2010)
2006/2012 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)

New Molecular Entity (NME)
International Transport Consortium (ITC)

Slide adapted from Shiew-Mei Huang, Ph.D., FDA