Interplay between Drug Metabolizing Enzymes and Drug Transporters

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Key Decision Points in Drug Development

Pritchard et. al., Nat Rev Drug Disc 2003, 3, 542-53
Drug interactions related to transporters are being documented with increasing frequency and may be addressed more fully in future guidances.

AGAH/ACCP 2nd Joint Meeting, Düsseldorf, Germany, Feb 19-21, 2006

Bernd Meibohm, PhD, FCP, University of Tennessee
Drug Disposition is a Multifactorial Process, with Close Interplay of Numerous Players!
Potential Sites of Drug Interaction

- **Transport**
  - Intestinal Lumen
  - Systemic Circulation
  - Biliary & Renal Elimination

- **Efflux**
  - Enterocyte
  - Drug
  - Drug/Metabolite

- **Uptake**
  - Hepatocytes, Renal tubular cells
  - Metabolism

- **Modified from RB Kim**
Drug Metabolizing Enzymes

Major Phase I & Phase II Enzymes

Evans & Relling, Science 1999, 286, 487-490
Drug Transporter Families

- **Export Transporters**
  - Human ATP-Binding Cassette Transporters
    - 48 human ABC transporters
  - ABCB (subfamily B)
    - MDR1 (P-gp, ABCB1), MDR3 (ABCB4), BSEP (ABCB11)
  - ABCC (subfamily C)
    - MRP1 – 6 (ABCC1 – 6)
  - ABCG (subfamily G)
    - BCRP (ABCG2)

- **Uptake Transporters**
  - Solute Carrier Family 10
    - NTCP (SCL10A1)
  - Solute Carrier Family 21
    - OATP-A (SLCO21A3), OATP-C (SLCO1B1), OATP-B (SLCO1B3), OATP-8 (SLCO1B3)
  - Solute Carrier Family 22
    - OCT1 & 3 (SLC22A1 & 3)
    - OAT2 & 4 (SLC22A7 & 11)
The CYP3A / P-gp System

Substrate Overlap

CYP3A substrates

Calcium antagonists
• Diltiazem (I), Nicardipine (I), Verapamil (I)

Hormones
• Dexamethasone, Hydrocortisone (I)

Imunosuppressants
• Cyclosporine (I), Tacrolimus (I), Sirolimus

HIV protease inhibitors
• Indinavir (I), Nelfinavir (I), Ritonavir (I), Saquinavir (I)

Others
• Digoxin, Erythromycin (I), Terfenadine (I)

(I) = also P-gp inhibitors

P-gp substrates
The CYP3A / P-gp System

- **Substrate selectivity**
  - CYP3A & P-gp have substantial substrate overlap

- **Tissue localization**
  - Both expressed in enterocytes and hepatocytes

- P-gp expression modulates metabolism (oral bioavailability and hepatic clearance) by limiting intracellular substrate availability
  - Spatial separation of P-gp (apical membrane) and CYP3A (ER):
    - High P-gp activity in the gut wall results in longer gut wall transit time (intracellular residence time) and, hence, increased gut wall CYP3A metabolism
    - High P-gp activity in the liver is suggested to result in decreased hepatic CYP3A metabolism for cosubstrates of CYP3A and P-gp

Lan et al., *Mol Pharmacol* 2000, 58, 863-9
The CYP3A / P-gp System

Increased Gut Wall Transit Time

The CYP3A / P-gp System

Enzyme/Transporter mRNA Expression at different locations of the Human GI Tract

Thörn et al., Br J Clin Pharmacol 2005, 60, 54-60
The CYP3A / P-gp System

Functional Collaboration of CYP3A & P-gp (I)

- P-gp keeps drug concentrations in CYP3A linear range
- CYP3A
- Drug
- P-gp
- Repeated exposure leads to more efficient metabolism

Functional Collaboration of CYP3A & P-gp (II)

- CYP3A
- Met
- P-gp
- Metabolites become better substrates for P-gp
- ABCx
- Metabolites become better substrates for other transporters

Effect of P-gp Inhibition on CYP3A-mediated Metabolism

- CYP3A
- Met
- P-gp
- P-gp inhibition: Drug overwhelms CYP3A
- Decreased exposure to CYP3A
  - Metabolism↓
  - Oral Bioavailability (F) ↑

Modified from Christians, Ther Drug Monit 2004, 26, 104-6
AGAH/ACCP 2nd Joint Meeting, Düsseldorf, Germany, Feb 19-21, 2006
Bernd Meibohm, PhD, FCP, University of Tennessee
Orally administered cyclosporine (Sandimmune® formulation) has F=27%.

Not an absorption problem: 86% absorbed.

Extensive presystemic metabolism in the gut:
- 14% unabsorbed, exported by P-gp, or degraded in the gut lumen.
- 51% metabolized in enterocytes in the gut wall (CYP3A).
- Only 8% lost due to first-pass metabolism in the liver.

Data from Benet et al., *J Control Release* 1996, 39, 139-43.
The CYP3A / P-gp System

Oral Bioavailability of Cyclosporine

- Selective inhibition of P-gp and/or CYP3A in the gut increases drug bioavailability while decreasing interindividual variability

- Examples:
  - Concomitant administration of grapefruit juice increases the bioavailability of oral cyclosporine by 38%, probably by selective inhibition of intestinal CYP3A
    Bistrup et al., Nephrol Dial Transplant 2001, 16, 373-7
  - Increased cyclosporine blood concentrations due to verapamil administration, probably due to selective inhibition of P-gp
    Jacob et al., Am J Kidney Dis 1999 Feb;33(2):301-3
The CYP3A / P-gp System

Functional Collaboration of CYP3A & P-gp (I)
- P-gp keeps drug concentrations in CYP3A linear range
- P-gp
- CYP3A
- Enterocyte Gut lumen
- Portal vein

Functional Collaboration of CYP3A & P-gp (II)
- Metabolites become better substrates for P-gp
- Metabolites become better substrates for other transporters
- P-gp
- CYP3A
- ABCx

Effect of P-gp Inhibition on CYP3A-mediated Metabolism
- P-gp inhibition: Drug overwhelms CYP3A
- Decreased exposure to CYP3A
  - Metabolism↓
  - Oral Bioavailability (F) ↑
- P-gp
- CYP3A

Co-regulation
- PXR
- Inducers

Repeated exposure leads to more efficient metabolism
- Drug
- P-gp

Modified from Christians, Ther Drug Monit 2004, 26, 104-6
Transcriptional Control of CYP3A & P-gp

- Transporters and DMEs as stress response genes
- Transactivated by Orphan Nuclear Receptors
  - Ligand-activated transcription factors
  - Xenobiotics as ligands (promiscuous binding domain)
  - Act as xenosensors for DMEs and transporters
  - Activated by rifampin, steroids, ritonavir, phenobarbital, hyperforin
  - Allow tissue-specific gene induction
  - Overlap and cross-talk
- Orphan nuclear receptors regulate multiple xenobiotic clearance pathways
# Transcriptional Control of CYP3A & P-gp

## Nuclear Receptor Activated Genes (Selection)

<table>
<thead>
<tr>
<th>Nuclear receptor</th>
<th>Tissue</th>
<th>Ligands</th>
<th>Target genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pregnane X receptor</strong></td>
<td>Liver</td>
<td>Rifampin</td>
<td>CYP3A4/7</td>
</tr>
<tr>
<td>(PXR)</td>
<td>Kidney</td>
<td>Clotrimazole</td>
<td>CYP2B6</td>
</tr>
<tr>
<td></td>
<td>Small intestine</td>
<td>Nifedipine</td>
<td>ABCB1(MDR1)</td>
</tr>
<tr>
<td></td>
<td>Colon</td>
<td>Hyperforin</td>
<td>ABCC2(MRP2)</td>
</tr>
<tr>
<td><strong>Constitutive androstan e receptor</strong></td>
<td>Liver</td>
<td>Phenobarbital</td>
<td>CYP3A4</td>
</tr>
<tr>
<td>(CAR)</td>
<td>Intestine</td>
<td>Androstanol</td>
<td>CYP2B6</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td></td>
<td>CYP2C9</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td></td>
<td>UGT1A1</td>
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<tr>
<td></td>
<td>Lung</td>
<td></td>
<td>ABCB1(MDR1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ABCC2(MRP2)</td>
</tr>
<tr>
<td><strong>Farnesoid X receptor</strong></td>
<td>Liver</td>
<td>Bile acids</td>
<td>ABCB11(BSEP)</td>
</tr>
<tr>
<td>(FXR)</td>
<td>Kidney</td>
<td></td>
<td>ABCC2(MRP2)</td>
</tr>
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<td></td>
<td>Small intestine</td>
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<td></td>
<td>Colon</td>
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</tbody>
</table>
Transcriptional Control of CYP3A & P-gp

Xenobiotic (Drug) → Uptake transporter (?) (e.g., OATP-C for rifampin)

Xenobiotic

NR = PXR, CAR, FXR, etc.

Target Genes

CYP3A4, CYP2C9, MDR1 (ABCB1), MRP2 (ABCC2), ...

NR = PXR, CAR, FXR, etc.
Transcriptional Control of CYP3A & P-gp

Overlapping Ligand Recognition and Nuclear Receptor-Mediated Gene Regulation

Ekins et al., Pharm Res 2004, 19,1788-800
The HIV-1 protease inhibitor indinavir is a substrate for CYP3A4 and P-gp.

The AUC resulting from 800 mg indinavir every 8 hours after pretreatment with 300 mg TID of a St. John’s Wort preparation standardized for 0.3% hypericin given for 14 days, was 57% lower than without pretreatment.

Hyperforin, the active ingredient of St. John’s Wort, is a potent activator of PXR.

The mechanism of the interaction is likely the induction of CYP3A4 and MDR1 expression by constituents of St. John’s Wort.

Piscitelli et al., Lancet 2000, 355, 547-8
Is the Interplay between Drug Transporters and Drug Metabolizing Enzymes Limited to CYP3A & P-gp?
Examples for Enzyme-Transporter Interplay

- **GSTA1-1 and MRP1**
  - Presence of MRP1 is essential for drug resistance of cancer cells against the alkylating agent chlorambucil by the phase II conjugating enzyme glutathione S-transferase (GSTA1-1)
  - Chlorambucil conjugate accumulates intracellularly and prevents further detoxification of the parent by product inhibition
  
  Paumi et al., J Biol Chem 2001, 276, 7952-6

- **UGT1A6 and MRP2**
  - Glucuronide transporter as essential component in the glucuronidation mechanism of drug resistance (SN38 and NU/ICRF 505)


- **SUT2A1 and MRP4**
  - SUT2A1: Transferase that preferentially sulfates steroid and bile acids
  - MRP4: Transporter of sulfated steroids and bile acids
  - Coordinately regulated via CAR

  Assem et al., J Biol Chem 2004, 279, 22250-7
Examples for Enzyme-Transporter Interplay

Irinotecan Metabolic Pathways in the Liver

- Enzymes: CES1/2, CYP3A, UGT1A1, UGT1A6, UGT1A9
- Transporters: ABCB1, ABCC1, ABCC2, ABCG2

From PharmGKB.org

AGAH/ACCP 2nd Joint Meeting, Düsseldorf, Germany, Feb 19
What are the Consequences of the Interplay of Drug Transporters and Drug Metabolizing Enzymes for Drug Development?
Biopharmaceutical Classification System

Solubility vs. Permeability

- **Class 1**: High solubility, High permeability
- **Class 2**: Low solubility, High permeability
- **Class 3**: High solubility, Low permeability
- **Class 4**: Low solubility, Low permeability
Biopharmaceutical Classification System

Relationship to Drug Disposition

Major Route of Elimination
- High solubility:
  - Class 1: Metabolism
  - Class 3: Renal and/or biliary excretion in unchanged form
- Low solubility:
  - Class 2: Metabolism
  - Class 4: Renal and/or biliary excretion in unchanged form

Transporters in Drug Disposition
- High solubility:
  - Class 1: Transporter effects minimal
  - Class 2: Efflux transporter effects predominate
- Low solubility:
  - Class 3: Absorptive transporter effects predominate
  - Class 4: Absorptive & efflux transporter effects could be important

Significant interactions after oral dosing are expected for Class 2 drugs, that are substrates for both intestinal enzymes and intestinal apical efflux transporters.

Modified from Wu & Benet, Pharm Res 2005, 22, 11-230

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The PDE5 inhibitor vardenafil is a BCS Class 2 drug.

- Metabolized mainly by CYP3A4/5, CYP2C9 & P-gp substrate
  - Erythromycin is moderate inhibitor for CYP3A & P-gp
  - Ketoconazole, Indinavir are strong inhibitors for CYP3A & P-gp
  - Ritonavir is a strong inhibitor for CYP3A4, CYP2C9 and P-gp

Gupta et al., J Clin Pharmacol 2005, 45, 987-1003
S-M Huang, 2004
Conclusions for Drug Development

- Drug-drug interactions are not limited to enzymatic processes, but can frequently involve transporters and particularly transporter-enzyme interplay.
- Drug-drug interactions on the transporter level can alter drug metabolism without direct interference with CYPs or other DMEs.
- Drug-drug interactions involving transporter-enzyme interplay is particularly likely for BCS Class 2 compounds.
- Strong interaction effects may be occur due to multiple interaction points.
Acknowledgement

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