Overview of Intestinal Drug Metabolism and Transport

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Proximal Intestinal Microanatomy

Figure 2
Key DMEs and Transporters in the Intestinal Epithelial Barrier

Won et al, Pharmacol Ther, 2012
• OATP1A2 protein not detected by MS analysis in any of the intestinal samples tested; conflicts with NP-drug interaction data?
OATP 1A2 and 2B1 mRNA Expression

OATP1A2, OATP2B1 Expression

Human Duodenum
Human Jejunum
Human Ileum
Human Colon
Human Rectum

RQ

OATP1A2
OATP2B1

Courtesy of J Wang
unpublished data
<table>
<thead>
<tr>
<th>Transporter/alias (Gene)</th>
<th>Selected substrates</th>
<th>Selected inhibitors</th>
<th>Organs/cells</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR1/P-gp, ABCB1 (ABCB1)</td>
<td>Digoxin*, loperamide*, berberine, irinotecan, doxorubicin, vinblastine, paclitaxel, fexofenadine</td>
<td>Cyclosporine*, quinidine*, tariquidar, verapamil</td>
<td>Intestinal enterocytes, kidney proximal tubule, hepatocytes (canalicular), brain endothelia</td>
<td>Has a role in absorption, disposition and excretion, Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>BCRP/MXR (ABCG2)</td>
<td>Mitoxantrone, methotrexate, topotecan, imatinib, irinotecan, statins*, sulphate conjugates, porphyrins</td>
<td>Oestrone, 17β-oestradiol, fumitremorgin C</td>
<td>Intestinal enterocytes, hepatocytes (canalicular), kidney proximal tubule, brain endothelia, placenta, stem cells, mammary glands (lactating)</td>
<td>Has a role in absorption, disposition and excretion, Has clinically relevant genetic polymorphisms, Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>MRP2/ABCC2, cMOAT (ABCC2)</td>
<td>Glutathione and glucuronide conjugates, methotrexate, etoposide, mitoxantrone, valsartan, olmesartan, glucuronidated SN-38</td>
<td>Cyclosporine, delavirdine, efavirenz, emtricitabine</td>
<td>Hepatocytes (canalicular), kidney (proximal tubule, luminal), enterocytes (luminal)</td>
<td>Has a role in absorption, disposition and excretion, Has clinically relevant genetic polymorphisms, Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>MRP3/ABCC3 (ABCC3)</td>
<td>Oestradiol-17β-glucuronide, methotrexate, fexofenadine, glucuronate conjugates</td>
<td>Delavirdine, efavirenz, emtricitabine</td>
<td>Hepatocytes (sinusoidal), intestinal enterocytes (basolateral)</td>
<td>Has a role in disposition</td>
</tr>
<tr>
<td>Transporter/alias (Gene)</td>
<td>Selected substrates</td>
<td>Selected inhibitors</td>
<td>Organs/cells</td>
<td>Comments</td>
</tr>
<tr>
<td>-------------------------</td>
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</tr>
<tr>
<td>OATP1A2/OATP-A (SLCO1A2)</td>
<td>Oestrone-3-sulphate, dehydroepiandrosterone sulphate, fexofenadine*, bile salts, methotrexate, bromosulphophthalein, ouabain, digoxin, levofloxacin, statins*</td>
<td>Naringin, ritonavir, lopinavir, saquinavir, rifampicin*</td>
<td>Brain capillaries endothelia, cholangiocytes, distal nephron</td>
<td>• Has role in disposition and excretion</td>
</tr>
<tr>
<td>OATP2B1/OATP-B (SLCO2B1)</td>
<td>Oestrone-3-sulphate, bromosulphophthalein, taurocholate, *statins, fexofenadine, glyburide, taurocholate</td>
<td>Rifampicin, cyclosporine*</td>
<td>Hepatocytes (sinusoidal), endothelia</td>
<td>• Has a role in disposition and excretion • Has a role in clinical drug–drug interactions</td>
</tr>
<tr>
<td>OCT1 (SLC22A1)</td>
<td>Tetraethylammonium, N-methylpyridinium, metformin*, oxaliplatin</td>
<td>Quinine, quinidine, disopyramide</td>
<td>Hepatocytes (sinusoidal), intestinal enterocytes</td>
<td>• Has a role in disposition and excretion • Has clinically relevant genetic polymorphisms • Has a role in clinical drug–drug interactions</td>
</tr>
</tbody>
</table>
PMAT (ENT4, SLC29A4) in Human Intestine

- Considering the apparent apical localization of PMAT (controversial), it may play a role in the intestinal availability of metformin and other OCs.
Intestinal Availability of Metformin Revisited

• Based on experimental results from Caco-2 cell incubations, Thakker and colleagues proposed a complex mechanism of enterocyte accumulation of metformin, that facilitates the eventual paracellular absorption of the drug.

• If true, multiple opportunities for NP-Drug and Drug-Drug DDIs.

Han et al, JPET, 2015
Intestinal Drug Metabolizing Enzymes

**PHASE I**
Cytochromes P450 (CYPs)
Carboxylesterases (CEHs)
Epoxide Hydrolases (EHs)

**PHASE II**
UDP-Glucuronosyl Transferases (UGTs)
Sulfotransferases (SULTs)
$N$-Acetyl Transferases (NATs)
Methyl Transferases (MTs)
CYP3A dominates P450 content in both the liver and small intestine, but its contribution is more significant in the intestine.

Gut CYP2C9/19 might be important for some drugs.

Paine et al, Drug Metab Disp, 2006
CYP3A4 (+) expression is confined to the “absorptive” enterocytes of the mucosal villi.

Courtesy of P.B. Watkins, MD
The extent of intestinal first-pass metabolism will depend on the relative values of the intrinsic clearance, the mucosal blood flow and the cellular permeability clearances.

Paired Liver-Duodenal Enterocyte Homogenates

<table>
<thead>
<tr>
<th></th>
<th>Enterocyte</th>
<th></th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP3A4</td>
<td>Cl&lt;sub&gt;int&lt;/sub&gt;</td>
<td>Verapamil N-demethylation</td>
<td>Cl&lt;sub&gt;int&lt;/sub&gt;</td>
</tr>
<tr>
<td>(pmol/mg)</td>
<td>(µl/min/mg)</td>
<td>(µl/min/mg)</td>
<td>(µl/min/mg)</td>
</tr>
<tr>
<td>76.0 ± 50.6</td>
<td>7.51 ± 4.29</td>
<td>23.6 ± 17.4</td>
<td>6.76 ± 2.93</td>
</tr>
<tr>
<td>(19.2 – 173.9)</td>
<td></td>
<td>(0.9 – 58.8)</td>
<td></td>
</tr>
</tbody>
</table>

Abstracted from von Richter et al., CPT 75:172-83, 2004
• 1 mg IV or 2 mg PO during anhepatic phase of a liver transplant operation.

*Paine et al, CPT, 1996*
## Intestinal Midazolam Extraction: Anhepatic Patients

<table>
<thead>
<tr>
<th>Intravenous Dose</th>
<th>Intraduodenal Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>E (%)</td>
</tr>
<tr>
<td>3</td>
<td>11.1</td>
</tr>
<tr>
<td>4</td>
<td>25.6</td>
</tr>
<tr>
<td>6</td>
<td>8.5</td>
</tr>
<tr>
<td>7</td>
<td>-2.6</td>
</tr>
<tr>
<td>10</td>
<td>-1.8</td>
</tr>
</tbody>
</table>

| Mean     | 8.2 | Mean     | 43.0* |
| S.D.     | 11.5| S.D.     | 18.1  |

*Paine et al., CPT 60:14-24 (1996)*
Fig. 1. Whole-body PBPK, with the liver and other lumped compartments (highly perfused, poorly perfused) being connected to the intestine model (TM and SFM), depicting the intestine (A) and intestine and liver (B) as the eliminating tissue(s)/organ(s). The intestine subcompartments are as follows: for TM, subscripts int and intB denote intestinal tissue and intestinal blood, respectively; for SFM, subscripts en and enB denote enterocyte and enterocyte blood, respectively; s and sB denote serosal tissue and serosal blood, respectively. For the liver, subscripts L and LB represent liver tissue and liver blood, respectively; subscript R denotes the reservoir or blood compartment. For TM, the intestine represents a well mixed enterocyte region and receives the entire intestinal blood flow, \( Q_l \) or \( Q_{PV} \). For SFM, the intestinal blood flow is segregated to perfuse the enterocyte and serosal regions; the flow to the enterocyte region is denoted as \( f_{PV}Q_{PV} \), and the serosal region, \( (1-f_{PV})Q_{PV} \). At the basolateral membrane, the drug influx and efflux clearances into or out of the intestine or enterocyte are characterized by the transport clearance parameters \( CL_{d1} \) and \( CL_{d2} \), respectively. For SFM, additional influx and efflux clearance into or out of the serosal tissue compartment are characterized by the transport clearance parameters, \( CL_{d3} \) and \( CL_{d4} \). The liver receives blood from hepatic blood artery (\( Q_{HA} \)) arising from the blood compartment and venous flow, \( Q_{PV} \), from the intestine; the summed blood flow exits the liver as \( Q_{L} \). The influx and efflux clearances of the drug into or out of the liver are \( CL_{l1}^H \) and \( CL_{l2}^H \), respectively. Intrinsic metabolic clearance of parent drug (P) to form the primary metabolites in the intestine are denoted by \( CL_{intmet1}^H \) and \( CL_{intmet2}^H \); and those in liver are \( CL_{intmet1}^H \) and \( CL_{intmet2}^H \); the intestine and liver secrete P out via secretory intrinsic clearances, \( CL_{intsec1}^H \) and \( CL_{intsec2}^H \), respectively. The bile flow rate is denoted as \( Q_{bile} \). Drug administered orally (solution form) is administered into the lumen and may be either absorbed into intestine with the rate constant, \( k_{in} \), or degraded in lumen by the rate constant, \( k_{g} \); drug given intravenously directly enters the blood compartment.
Simulations of Intestinal and Oral Bioavailability

Considers 3 types of PBPK models:

Traditional: Intestinal flow = portal flow

$Q_m$: hybrid of traditional and segmented;

Segmented: separate serosal and mucosal flow to small intestine

Pang & Chow, DMD, 2012
## Oral Bioavailability of Select CYP3A Substrates

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buspirone</td>
<td>anxiety</td>
<td>4 ± 4</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>antihistamine</td>
<td>&lt; 2</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>hypercholesterolemia</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Saquinavir</td>
<td>HIV</td>
<td>4 - 14</td>
</tr>
<tr>
<td>Felodipine</td>
<td>hypertension</td>
<td>15 ± 8</td>
</tr>
<tr>
<td>Verapamil</td>
<td>angina, arrhythmia</td>
<td>22 ± 8</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>immunosuppression</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>Midazolam</td>
<td>sedation/hypnosis</td>
<td>30 ± 10</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>bacterial infections</td>
<td>35 ± 2</td>
</tr>
<tr>
<td>Diltiazem</td>
<td>angina, hypertension</td>
<td>44 ± 10</td>
</tr>
</tbody>
</table>
Heterogeneous Distribution of CYP3A4 Protein in Small Intestine

Mary Paine et al, JPET, 1997

A common VDR signaling pathway for both calcium transport proteins (TRPV6, calbindin D9K) and CYP3A4?
Heterogeneous expression pattern consistent with primary site of calcium absorption.

Regulation of Intestinal CYP3A4 by Vitamin D

## Effect of Oral Vitamin D Supplementation on Oral Atorvastatin Clearance

<table>
<thead>
<tr>
<th>Targets</th>
<th>Baseline</th>
<th>Vitamin D supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1\alpha,25(OH)_2D$</td>
<td>$37 \pm 17$</td>
<td>$38 \pm 15$</td>
</tr>
<tr>
<td>25OHD (ng/ml)</td>
<td>$19 \pm 18$</td>
<td>$29 \pm 14 \ast$</td>
</tr>
<tr>
<td>Atorvastatin AUC</td>
<td>$5,190 \pm 4,557$</td>
<td>$3,250 \pm 3,037 \ast$</td>
</tr>
</tbody>
</table>

- Crossover study in healthy elderly adults (n= 16);
- During supplementation, people received 800 IU of vitamin D (per day for 6 weeks);
- Atorvastatin administered twice a day, to steady-state; plasma AUC determined for AM dose interval.

VDR Expression is Relatively Constant Along the Length of the Small Intestine

What about delivery of the ligand?
Hypothesis: Biliary Vitamin D Conjugates Regulate Intestinal CYP3A4 Expression

Major Metabolic Pathways of 25OHD₃

Kidney → Liver

- 1α,25(OH)₂D₃
- CYP27B1
- CYP24A1
- CYP3A4
- 24R,25(OH)₂D₃
- 4β,25(OH)₂D₃

Liver

- UGT1A4/1A3
- SULT2A1/1A1
- 25OHD₃-gluc
- M1
- M2
- M3
- d6-25OHD₃-3-S
- 25OHD₃-3-sulfate

(Shican Wang et al, Mol Pharmacol, 2012)
(Zhican Wang et al, Endocrinol, 2014)
(Tim Wong et al, In Preparation)
Hypothesis: Biliary Vitamin D Metabolites Regulate Intestinal CYP3A4 Expression

Vitamin D conjugates?

CYP3A4

Bile

e.g.
25OHD-3-glucuronide
25OHD-3-sulfate
1α,25(OH)₂D-25-glucuronide

Gut lumen
Implications for Drug Therapy

Demographic factors, concomitant medications, diet, season, and genetic variation in intestinal CYP3A genes and regulatory genes are all likely to contribute to inter-individual differences in CYP3A function and clinical response to CYP3A substrates.
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