Role of Transporters in Drug Exposure during Lactation

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Babies were born to be breastfed.

1-800-994-WOMAN  www.4woman.gov

Or talk to your healthcare provider.

BREASTFEED FOR 6 MONTHS. YOU MAY HELP REDUCE YOUR CHILD’S RISK FOR CHILDHOOD OBESITY.

Recent studies show babies who are breastfed for six months have lower childhood obesity rates than those who are not breastfed for six months. Call 1-800-994-WOMAN or visit www.4woman.gov to learn more. Or talk to your healthcare provider.

BREASTFEEDED FOR SIX MONTHS, HELP REDUCE YOUR CHILD’S RISK FOR EAR INFECTIONS.

Recent studies show you can lower your child’s risk of ear infections by breastfeeding exclusively for six months. Call 1-800-994-WOMAN or visit www.4woman.gov to learn more. Or talk to your healthcare provider.

Babies were born to be breastfed.
US Breastfeeding Trends

- Numerous benefits for baby and mother
- Strong advocacy for breastfeeding

Ross Labs (Abbott) Breastfeeding Statistics
Breast-Feed Or Else

Is choosing bottle over breast like smoking during pregnancy?
A controversial new public health effort suggests it.

27 simple ways to save on everything

surprising news: helping your baby learn

5-minute fitness quickie routines that wo
Societal Concerns

• HIV passage in milk
  – HIV breastfeeding contraindicated in developed world
  – Major concern in underdeveloped world
  – Aids clinical trials, drug in breast milk might be of benefit
    “...affordable, feasible, and culturally acceptable interventions to decrease the risk of breast milk transmission of HIV-1 are urgently needed.”

  JS Read, Ped Branch NIH in Pediatr Infect Dis J 2008

• Drug or environmental exposure
  – Infant risk versus maternal benefit
  – Special concerns for developing infant

• Drugs of abuse
  – Methamphetamine
    • Bakersfield, CA; AP, 1994
  – Heroin, methadone
    • Tuscon, AZ; AP, 1997
Pharmacotherapy
Dilemma

Limited knowledge of drug effects from lactation exposure
All Drugs will appear in Milk!

• What is maternal exposure?
  – \[ \text{Dose}_{\text{Maternal}}, \text{AUC}_{\text{Maternal}}, \text{Cl}_{s, \text{Maternal}} \]

• What is infant dose?
  – \[ \text{AUC}_{\text{Maternal}}, \text{M/S}, \text{F}_{\text{infant}}, \text{Dosing rate (suckling)} \]

• What is infant exposure?
  – \[ \text{Dose}_{\text{Infant}}, \text{AUC}_{\text{Infant}}, \text{Cl}_{s, \text{Infant}} \]

• What is infant risk?
  – Response relative to exposure

• What is the impact of variation?
  – Pharmacogenetics / pathophysiology

\[
\bar{C}_{\text{Infant}} = \frac{FD_{\text{Infant}}}{\text{Cl}_{s,\text{Infant}}}
\]

\[
\bar{C}_{\text{Infant}} = \frac{F \left( \frac{M}{S} \right) C_{\text{maternal}}^{\text{serum}} \left( \frac{V_{\text{milk}}}{\tau} \right)}{\text{Cl}_{s,\text{Infant}}}
\]
American Academy of Pediatrics (AAP) Committee on Drugs

- **Cytotoxic Drugs** That May Interfere With Cellular Metabolism of the Nursing Infant (n=4)
  - Cyclophosphamide, Cyclosporine, Doxorubicin, Methotrexate

- **Drugs of Abuse** for Which Adverse Effects on the Infant During Breastfeeding Have Been Reported (n=5)
  - Amphetamine, Cocaine, Heroin, Marijuana, Phencyclidine

- **Drugs That Have Been Associated With Significant Effects on Some Nursing Infants and Should Be Given to Nursing Mothers With Caution** (n=12)
  - Acebutolol, 5-Aminosalicylic acid, Atenolol, Bromocriptine, Aspirin (salicylates), Clemastine, Ergotamine, Lithium, Phenindione, Phenobarbital, Primidone, Sulfasalazine
AAP Committee on Drugs

• Drugs for Which the Effect on Nursing Infants Is Unknown but May Be of Concern (n=28)
  – Alprazolam, Amitriptyline, Bupropion, Chlorpromazine, Desipramine, Diazepam, Doxepin, Fluoxetine, Fluvoxamine, Paroxetine, Sertraline

• Maternal Medication Usually Compatible With Breastfeeding (n=173)
  – Acetaminophen, Cimetidine, Ciprofloxacin, Codeine, Erythromycin, Gentamicin, Ibuprofen, Morphine, Phenytoin, Procainamide, Propranolol, Sumatriptan, Theophylline, Valproic acid, Verapamil, Warfarin

• Food & Environmental Agents: Effects on Breastfeeding (n=20)
  – Cadmium, Chlordane, Chocolate, DDT, Dieldrin, Aldrin, Hexachlorobenzene, Hexachlorophene, Lead, Mercury, Monosodium glutamate, PCBs, PBBs
Codeine and Breastfeeding

Safety of codeine during breastfeeding
Fatal morphine poisoning in the breastfed neonate of a mother prescribed codeine

Parvaz Madadi  Gideon Koren, MD, FRCPC  James Cairns, MD  David Chitayat, MD  Andrea Gaedigk, PhD
J. Steven Leeder, PHARM, PHD  Ronni Teitelbaum, MSC  Tatyana Karaskov, MD  Katarina Aleksa, PhD

VOL 53: JANUARY • JANVIER 2007  Canadian Family Physician • Le Médecin de famille canadien

FDA Public Health Advisory
Use of Codeine By Some Breastfeeding Mothers May Lead To Life-Threatening Side Effects In Nursing Babies (8/17/07)

Pharmacogenetics of Neonatal Opioid Toxicity Following Maternal Use of Codeine During Breastfeeding: A Case–Control Study

P Madadi1,2, CJD Ross3, MR Hayden3, BC Carleton4, A Gaedigk5, JS Leeder5 and G Koren1,2,6

CLINICAL PHARMACOLOGY & THERAPEUTICS | VOLUME 85 NUMBER 1 | JANUARY 2009
Case report: morphine milk concentration seven times higher than ever described from maternal codeine use.

Retrospective study concluded: “Breastfed infants of mothers who are CYP 2D6 UMs (gene duplication) combined with the UGT2B7*2/*2 are at increased risk of potentially life-threatening CNS depression.”
Analysis of FDA Drug Labeling

All Marketed Drugs  
n = 1625

New Molecular Entities  
1995-2002; n = 252

- Very limited information is available

(FDA - CDER) Kathleen Uhl
DRAFT GUIDANCE

Guidance for Industry
Clinical Lactation Studies – Study Design, Data Analysis, and Recommendations for Labeling

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)
February 2005
Clinical Pharmacology
Nursing vs Dosing Interval

• **Acute Dosing**
  – Banking milk
  – Timing of exposure

• **Chronic Dosing**
  – Strategies to minimize exposure impractical
    • Longer $t_{1/2}$ longer $\tau$
    • Longer $\tau$ less able to “control” exposure by timing the breastfeeding interval
**Neonatal Exposure**

\[
\frac{\text{Dose Rate}_{\text{neonate}}}{\text{Dose Rate}_{\text{maternal}}} = F_{\text{maternal}} \left( \frac{M}{S} \right) \left( \frac{V_{\text{milk}}}{\tau} \right) \quad \quad \frac{C_{\text{neonate}}}{C_{\text{maternal}}} = \frac{F_{\text{neonate}} F_{\text{maternal}} \left( \frac{M}{S} \right) \left( \frac{V_{\text{milk}}}{\tau} \right)}{C_{\text{systemic}}}
\]

<table>
<thead>
<tr>
<th>M/S</th>
<th>Cls, mother ml/min/kg</th>
<th>Cls, neonate ml/min/kg</th>
<th>Dose Ratio % of Maternal</th>
<th>Conc Ratio % of Maternal</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>20</td>
<td>20</td>
<td>5.6%</td>
<td>5.6%</td>
</tr>
<tr>
<td>1</td>
<td>20</td>
<td>20</td>
<td>0.56%</td>
<td>0.56%</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>5.6%</td>
<td>5.6%</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.2</td>
<td>5.6%</td>
<td>55.6%</td>
</tr>
</tbody>
</table>

F(s) assumed to be 1; \( V_{\text{milk}}/\tau \) assumed to be 800 ml/d for 5 kg infant

- Dose exposure easier to predict than concentration
- Higher exposure
  - Higher M/S
  - Lower Cls
    - Maternal (Dose exposure risk)
    - Neonate (Concentration exposure risk)
  - Absorption, First Pass, Active Metabolites may also contribute
Pharmacotherapy Dilemma

- What is maternal exposure?
  - $Dose_{Maternal}$, $AUC_{Maternal}$, $Cl_{s, Maternal}$

- What is infant dose?
  - $AUC_{Maternal}$, $M/S$, $F_{infant}$, Dosing rate (suckling)

- What is infant exposure?
  - $Dose_{Infant}$, $AUC_{Infant}$, $Cl_{s, Infant}$

- What is infant risk?
  - Response relative to exposure

- What is the impact of variation?
  - Pharmacogenetics/pathophysiology

\[
\overline{C}_{Infant} = \frac{FD_{infant}}{Cl_{s, Infant}}
\]

\[
\overline{C}_{Infant} = F \left( \frac{M}{S} \right) \frac{C_{maternal \ serum}}{\tau} \left( \frac{V_{milk}}{Cl_{s, Infant}} \right)
\]
Developmental Expression of Cytochrome P450 in Human Liver

- Metabolic pathways change and overall clearance changes with time
- "Exposure" changes with stage of development

Alcorn, and McNamara, Clin Pharmacokinet, 2002
Pharmacotherapy Dilemma
Focus of the Talk

- What is maternal exposure?
  - $\text{Dose}_{\text{Maternal}}$, $\text{AUC}_{\text{Maternal}}$, $\text{Cl}_s$, Maternal

- What is infant dose?
  - $\text{AUC}_{\text{Maternal}}$, $\text{M/S}$, $\text{F}_{\text{infant}}$, Dosing rate (suckling)

- What is infant exposure?
  - $\text{Dose}_{\text{Infant}}$, $\text{AUC}_{\text{Infant}}$, $\text{Cl}_s$, Infant

- What is infant risk?
  - Response relative to exposure

- What is the impact of variation?
  - Pharmacogenetics/pathophysiology

\[
\overline{C}_{\text{Infant}} = \frac{\text{FD}_{\text{infant}}}{\text{Cl}_{s, \text{Infant}}} \quad \text{or} \quad \overline{C}_{\text{Infant}} = \frac{F \left( \frac{M}{S} \right) \text{C}_{\text{maternal}} \left( \frac{V_{\text{milk}}}{\tau} \right)}{\text{Cl}_{s, \text{Infant}}}
\]
Mammary Physiology

- Complex set of signals prepare the breast for lactation
- Function unit is the **secretory alveolar epithelium**

Modified from Hennighausen, 2001

Modified from Neville, 2001
Milk Distribution

- Initial approach
  - Simple Diffusion

\[
C_{\text{Unbound, unionized, Milk}} = C_{\text{Unbound, unionized, Serum}}
\]

Passive diffusion model:

\[
\frac{M}{S_{\text{Predicted}}} = \begin{bmatrix}
  f_{s}^{\text{un}} & f_{s} & W \\
  f_{m}^{\text{un}} & f_{m} & Sk
\end{bmatrix}
\]

Fleishaker et al. 1988; Fleishaker et al. 1988 McNamara et al. 1991; McNamara et al. 1992
Xanthines in Human Milk

- Metabolites in milk.
- Diffusion model predicted M/S for caffeine (CA) and its metabolites paraxanthine (PA), theobromine (TB) and theophylline (TP)

[Oo, 1995 #1924]
Milk Distribution

- Initial approach
  - Use a simple diffusion model to explain the data

\[ C_{\text{Milk}}^{\text{Unbound,unionized}} = C_{\text{Serum}}^{\text{Unbound,unionized}} \]

Passive diffusion model:

\[ \frac{M}{S_{\text{Predicted}}} = \begin{bmatrix} f_s^{\text{un}} & f_s & W \\ f_m^{\text{un}} & f_m & Sk \end{bmatrix} \]

Fleishaker et al. 1988; Fleishaker et al. 1988
McNamara et al. 1991; McNamara et al. 1992
Cimetidine and Nitrofurantoin M/S

- Human
  - Observed Cimetidine M/S is 6 times diffusion prediction
  - Observed Nitrofurantoin M/S is 20 times diffusion prediction
- Rats even more pronounced (NF is 100X)

<table>
<thead>
<tr>
<th>Cimetidine</th>
<th>Nitrofurantoin</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/S</td>
<td>M/S</td>
</tr>
<tr>
<td>Predicted</td>
<td>1.0 (0.2)</td>
</tr>
<tr>
<td>Observed</td>
<td>5.8 (1.2)</td>
</tr>
</tbody>
</table>


ABC2G and M/S

- Knockout mice suggest Abcg2 is responsible for CM and NF accumulation

<table>
<thead>
<tr>
<th>M/S</th>
<th>Wild-Type</th>
<th>Bcrp1-/-</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrofurantoin</td>
<td>45.7</td>
<td>0.6</td>
<td>76.2</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>13.6</td>
<td>2.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Topotecan</td>
<td>6.75</td>
<td>0.8</td>
<td>8.4</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>1.3</td>
<td>0.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Folic Acid</td>
<td>0.023</td>
<td>0.021</td>
<td>1.1</td>
</tr>
<tr>
<td>PhIP</td>
<td>12.7</td>
<td>0.3</td>
<td>42.3</td>
</tr>
<tr>
<td>Alfatoxin B1</td>
<td>0.7</td>
<td>0.18</td>
<td>3.9</td>
</tr>
<tr>
<td>Q1</td>
<td>0.95</td>
<td>0.28</td>
<td>3.4</td>
</tr>
<tr>
<td>Trp-P-1</td>
<td>1.15</td>
<td>0.43</td>
<td>2.7</td>
</tr>
<tr>
<td>DHEAS</td>
<td>0.15</td>
<td>0.13</td>
<td>1.2</td>
</tr>
</tbody>
</table>

ABCG2 Expression in Mammary

- Western analysis of Abcg2 in mammary glands of virgins and lactating female wild-type mice
- Apical expression mouse, cow and human

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Animal Model: Rat Abcg2

- Abcg2 & not P-gp expressed in rat LMEC
- Abcg2 is located on apical surface of rat LMEC

Wang, (2008)
Cell Culture Model

- Stably transfect MDCKII cell line
- Transcellular flux experiments
- Western Blot Analysis for ABCG2-MDCKII clones
- Polarized monolayer with BXP-21+Alexa488/DAPI imaged by laser scanning confocal microscopy.

![Diagram of vector showing locations of bla, Amp(R), CMV promoter, T7 promoter, pUC origin, Neo(R), SV40 pA, SV40 early promoter, and BGH pA.]

![Western Blot images showing ABCG2 and β-actin expression levels in different clones: Saos ABCG2, Empty Vector, ABCG2 Clone 40, ABCG2 Clone 46, ABCG2 Clone 50.]

![Laser scanning confocal microscopy image showing apical and basolateral staining.]
Animal Model: Rat Abcg2

In vitro model
- Cell culture model using rAbcg2 in MDCKII
  - Dye flux (panel A & B)
- NF is substrate for rAbcg2
  - Directional flux (panel C)
- GF120918 can inhibit rAbcg2 mediated flux of NF (panel D)

Wang, (2008)
Animal Model: Rat Chemical Knockout


Five SD lactating rats
- Balanced crossover design
- Infused with NF (0.5 mg/h) for 5 h and a bolus dose 10 min before the NF infusion of either:
  - GF120918 (10mg/kg in DMSO IV)
  - DMSO alone

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cls</td>
<td>Control</td>
<td>4.12</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>GF120918</td>
<td>2.78</td>
<td>0.5</td>
</tr>
<tr>
<td>M/S</td>
<td>Control</td>
<td>41.4</td>
<td>19.1</td>
</tr>
<tr>
<td></td>
<td>GF120918</td>
<td>3.04</td>
<td>2.27</td>
</tr>
</tbody>
</table>
ABCG2 (BCRP)

- 655 amino acid protein
- A single ATP binding cassette
- Six transmembrane domains
- Functions as homodimer
- Wide distribution – thought to have protective role
- Role in Mammary Gland?
  - Nutrient transport (riboflavin)

Milk Distribution

- Simple diffusion model worked for some drugs
- Others appear to be more consistent with active transport – NF, CM, RN
- Cell biology indicates that the simple model is inadequate

Simple diffusion model cannot explain the data
Basolateral Transport

- Do we need a BL transporter?
- Does it need to be present to concentrate substrates in milk?
- If we do need it, what is the BL transporter?

in vivo ≈ in vitro

\[ C_{D} \approx P_{D} \]
Implications for BL Transporter

- Do not need $PS_{B,U}$ for high M/S or to accumulate to same conc.
  
  $\frac{M}{S} = \left[ \frac{(Cl_D + Cl_{B,U}) (Cl_D + Cl_{A,E})}{Cl_D} \right] \frac{M}{S_{Diffusion}}$

- However, less efficient
  - 5000 “units” vs 1144 for both

- Moreover, time course is prolonged!

Implications for lactation biology!
- suckling every 2-4 hr

"units" = $PS_{A,E} \approx \frac{T_{Max}}{K_M}$
Do we need a BL transporter?

- Steady State Apical concentrations equal for either transporter situation
- Clearly transporters have a dramatic effect on intercellular dynamics
  - Initial MEC conc for high $PS_{B,U}$ simulation are 1000 times $PS_{A,E}$
  - SS MEC conc also differ for identical Apical

Implications for lactation biology!

Single Transporters
- High $PS_{B,U}$ => cellular tox?
- High $PS_{A,E}$ => slower transfer
Transporters on Lactating MECs

• What other transporter genes are up-regulated in lactation?
  – Basolateral Uptake?

• A number of reports in the literature, but
  – Mostly animal data – whole mammary gland
  – Incomplete human data set

• Utilize microarray of human isolated cells
MECs: Extensive Cell Proliferation / Death

Implications for gene expression studies
Cell proliferation vs Up-regulation in expression
LMEC/MEC Purification

Breast Milk

MUC1 (EMA)
MFGM/5/11 (ICR.2)
(Harlan Seralab)

FACS

Immunohistochemical validation of separation (cytokeratin 18)

Reduction Mammaplasty

RNA Isolation
Microarray
qPCR

P Empey Thesis
Validation of Pure Populations

- Immunocytostaining of luminal epithelial cell specific cytokeratins in the presorted and sorted populations to verify purity
Screening Paradigm

**Groups**
- LMEC (n=3)
- MEC (n=3)
- Liver (n=6)
- Kidney (n=6)

- Genes up-regulated
- Genes expressed compared to liver / kidney

Probeset (Transporter) is of interest because up in lactation or of an expression level equivalent to, or higher than, an expression level known to be relevant in the liver or kidney.
Microarray Results

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Lactating MEC Mean</th>
<th>NonLactating MEC Mean</th>
<th>Fold Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCG2</td>
<td>17536</td>
<td>107</td>
<td>164.04</td>
</tr>
<tr>
<td>SLC15A2</td>
<td>868</td>
<td>89</td>
<td>9.72</td>
</tr>
<tr>
<td>SLC22A12</td>
<td>184</td>
<td>24</td>
<td>7.84</td>
</tr>
<tr>
<td>SLC6A14</td>
<td>3988</td>
<td>1773</td>
<td>2.25</td>
</tr>
<tr>
<td>SLCO4C1</td>
<td>3362</td>
<td>48</td>
<td>69.7</td>
</tr>
</tbody>
</table>

Gene expression lactating vs non-lactating

Up-regulated during lactation:
- ABCG2: 164.04 fold
- SLCO4C1: 69.7 fold

- Transporter expression profile in lactating mammary gland secretory epithelial cells.

P Empey Thesis
SLCO4C1 (OATP-H) Expression

- RT-PCR confirmation of expression
  - Human, mouse and rat (data not shown)

McNamara Lab, unpublished
rSlco4c1 in LMEC

- Protein expression in rat Kidney
- Present in rat lactating mammary gland, but less abundant than kidney
Organic Anion Transporting Polypeptides, OATPs (SLCO)

Adapted from Tamai, AAPS workshop 2005
### Organic Anion Transporting Polypeptides (OATPs)

- **Important hepatic uptake function**

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Alias</th>
<th>Substrates</th>
<th>Drugs</th>
<th>Tissue expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLCO1A2</td>
<td>OATP1A2</td>
<td>Bile salts, organic anions, hormone conjugates, eicosanoids</td>
<td>Saquinavir, methotrexate, fexofenadine, ouabain, indomethacin</td>
<td>Brain, kidney, liver, lung, small intestine</td>
</tr>
<tr>
<td>SLCO1B1</td>
<td>OATP1B1</td>
<td>Bile salts, hormone conjugates, eicosanoids</td>
<td>Benzylpenicillin, pravastatin, rifampin, methotrexate,</td>
<td>Liver</td>
</tr>
<tr>
<td>SLCO1B3</td>
<td>OATP1B3</td>
<td>Bile salts, organic anions</td>
<td>Digoxin, methotrexate, rifampin</td>
<td>Liver, cancer cell lines</td>
</tr>
<tr>
<td>SLCO1C1</td>
<td>OATP1</td>
<td>T4, rT3, BSP</td>
<td>Atorvastatin</td>
<td>Brain, testis (Leydig cells)</td>
</tr>
<tr>
<td>SLCO2A1</td>
<td>OATP2A1</td>
<td>Eicosanoids</td>
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<td>Ubiquitous</td>
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<tr>
<td>SLCO2B1</td>
<td>OATP2B1</td>
<td>Bile salts, organic anions, hormone conjugates</td>
<td>Digoxin, benzylpenicillin</td>
<td>Liver, placenta, brain, heart, kidneys, intestine</td>
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<td>SLCO3A1</td>
<td>OATP3A1</td>
<td>E-3-S, prostaglandin</td>
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<td>Ubiquitous</td>
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<td>SLCO4A1</td>
<td>OATP4A1</td>
<td>Taurocholate, T3, prostaglandin</td>
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<td>Ubiquitous</td>
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<tr>
<td>SLCO4C1</td>
<td>OATP4C1</td>
<td>T3</td>
<td>Digoxin, ouabain</td>
<td>Kidney</td>
</tr>
</tbody>
</table>
**Screening Approach**

- **Hypothesis:** All ABCG2 Substrates will Accumulate in Milk
- **Identify which drugs are at greatest risk for accumulation**

<table>
<thead>
<tr>
<th>ABCG2 Substrates</th>
<th>Name</th>
<th>log D</th>
<th>M/S</th>
<th>Name</th>
<th>log D</th>
<th>M/S</th>
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<tbody>
<tr>
<td><strong>M/S Known</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>MS/Unknown</strong></td>
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<tr>
<td>Acyclovir</td>
<td>-1.76</td>
<td>3.0</td>
<td></td>
<td>Bisantrene</td>
<td>-1.13</td>
<td>?</td>
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<tr>
<td>Cimetidine</td>
<td>0.11</td>
<td>5.8</td>
<td></td>
<td>Daunorubicin</td>
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<td>?</td>
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<td>Diflomotecan</td>
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<tr>
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<td>?</td>
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<td>Etoposide</td>
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<td>Flavopiridol</td>
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<td>Lamivudine</td>
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<td>2.2</td>
<td></td>
<td>Genistein</td>
<td>2.61</td>
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<tr>
<td>Levofoxacin</td>
<td>-1.93</td>
<td>1.05</td>
<td></td>
<td>Irinotecan</td>
<td>2.76</td>
<td>?</td>
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<tr>
<td>Methotrexate</td>
<td>-1.00</td>
<td>0.04</td>
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<td>Mitoxantrone</td>
<td>0.28</td>
<td>?</td>
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<td>Nitrofurantoin</td>
<td>-1.17</td>
<td>6.2</td>
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<td>Prazosin</td>
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<tr>
<td>Pantoprazole</td>
<td>1.54</td>
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<td></td>
<td>Quinazoline</td>
<td>0.89</td>
<td>?</td>
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<td>Zidovudine</td>
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<td>1.44</td>
<td></td>
<td>Rosuvastatin</td>
<td>-2.63</td>
<td>?</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Teniposide</td>
<td>1.71</td>
<td>?</td>
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<tr>
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<td></td>
<td></td>
<td>Topotecan</td>
<td>0.98</td>
<td>?</td>
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</table>
Role of ABCG2/SLCO4C1 in Lactating Mammary Epithelia

- What is the pharmacological/toxicological implications of ABCG2 in lactating mammary epithelial cells?
  - Most scenarios (GI, placenta, etc.) ABCG2 appears protective of sensitive tissues
    - Is ABCG2 simply protecting milk production?
    - Is newborn exposure to potential toxins simply an unintended consequence?

- What is the biological function of ABCG2/OATP-H in lactating mammary epithelial cells?
  - Lactogenic regulation may imply a natural function
    - What is the natural substrate(s)?
    - From blood or MEC metabolic product
Summary

- Diffusion is the major mechanism of drug transfer into milk favoring higher M/S for unbound, lipophilic cations
- Active transport of drugs into milk does occur for a number of drugs
- Transporters in mammary epithelial cells
  - Clear role for ABCG2 in transfer of drugs / toxins into milk
  - Need to identify role of SLCO4C1 in drug transfer into milk
  - Build predictive tool for drug screening
    - Cell Culture (double transfected cell line)
    - Mathematical Models
- What is the physiologic role / substrates for ABCG2 and SLCO4C1?
  - Paired up regulation strongly suggests an important biological role!
Acknowledgements

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  – Jeff Moscow
  – Peggy Neville

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SLCO4C1, Substrates

• Again…not widely studied
• Typical OATP substrates
  – Digoxin
  – Ouabain
  – T3
• Sitagliptin
  – Dipeptidyl-peptidase 4 inhibitor for type 2 diabetes

[Chu, 2007 #7223]

[Mikkaichi, 2004 #7225]
Systems Biology: MDR and ABCG2

- **ABC2G**
  - Down-regulated post weaning
- **MDR**
  - Up-regulated post weaning
Systems Biology: SLCO4C1 and ABCG2

- Maternal
  - ABCG2 and SLCO4C1 are up-regulated in LMEC

- Neonate
  - ABCG2 is down-regulated in intestine & up-regulated in liver
  - SLCO4C1 is low (relative to kidney), however it is initially higher in liver and intestine, but fall (to near zero in liver)

- Need to measure protein
- Hematopoietic function in liver?
Pantoprazole in vivo

• Proton Pump Inhibitor
• Drug is marketed as stereoisomer…..
  – Isomers: Plus (R) and Minus (S) around sulfoxide center
• Pharmacokinetics
  – Some interconversion of plus to minus (rats, Masubuchi, 1998)
  – Conflicting evidence for differences in stereo isomers rat PK (Xie, 2005 and Masubuchi, 1998)
  – Humans; cleared by CYP2C19 and CYP3A4
  – Pharmacogenomics (CYP2C19)
    • EMs limited stereo isomer effect
    • PMs greater stereo isomer effect
• What about Abcg2?
  • No reports of stereoisomer interaction
  • Schinkel group work with racemic mixture

---

<table>
<thead>
<tr>
<th>S-Mephenytoin (C19)</th>
<th>EM</th>
<th>PM</th>
</tr>
</thead>
<tbody>
<tr>
<td>t½ (h)</td>
<td>0.94</td>
<td>7.84</td>
</tr>
<tr>
<td>CL/F (ml/min)</td>
<td>150</td>
<td>13</td>
</tr>
<tr>
<td>t½ (h)</td>
<td>1.09</td>
<td>2.26</td>
</tr>
<tr>
<td>CL/F (ml/min)</td>
<td>120</td>
<td>45.3</td>
</tr>
</tbody>
</table>

Tanaka, CPT (2001)
Pantoprazole – Abgc2

- mAbcg2 related flux
- $K_M = 100\mu$M
- Inhibition of imatinib flux

[Breedveld, 2004 #6665]

[Breedveld, 2005 #6658]
Pantoprazole in vivo

- **Principal Goal**: In vivo study performed in rats
  - Identical protocol to NF infusion with GF918 administration
  - Sample serum and measure milk concentration at SS
  - Assess protein binding…..implications for M/S

- **Secondary Goal**: Are there differences in stereoisomers with respect to interaction with Abcg2?

- Assay on a chiral column….works for HPLC, but sensitivity was a insufficient.  LC-MS was problematic

- Phil Mayer (UK Alum) is a Wyeth….provided pure isomers

- MDCKII (and Sf9) with both human and rat ABCG2
Cimetidine Flux

- Directionality of CM flux
- Inhibition of CM B to A flux by pantoprazole isomers (100uM)
- Both isomers knocked out CM flux
  - Inconclusive similar affinity or [I] too large!
- Need to study flux of pantoprazole itself
Pantoprazole Isomers (10 uM)

- Rat Abcg2 cloned into MDCKII
- Examine isomer flux using HPLC
Pantoprazole Isomers (25 & 50 uM)

Or Are they???
# Pantoprazole Isomers

\[
PS_{\text{Apparent}} = \frac{dX_{\text{Receiver}}}{dt} \cdot \frac{C^0_{\text{Donor}}}{2} + PS_{PC}
\]

### Table: PS (uL/hr*cm²)

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Empty</th>
<th>PS(_{PC})</th>
<th>PS(_{\text{Apparent}})</th>
<th>10uM</th>
<th>25uM</th>
<th>50uM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plus</td>
<td>B to A</td>
<td>0.97</td>
<td>112.31</td>
<td>1.70</td>
<td>102.72</td>
<td>0.71</td>
</tr>
<tr>
<td>Plus</td>
<td>A to B</td>
<td>0.96</td>
<td>105.15</td>
<td>2.01</td>
<td>114.68</td>
<td>0.67</td>
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<tr>
<td>Minus</td>
<td>B to A</td>
<td>1.21</td>
<td>120.00</td>
<td>1.51</td>
<td>100.32</td>
<td>0.76</td>
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<tr>
<td>Minus</td>
<td>A to B</td>
<td>1.40</td>
<td>113.39</td>
<td>1.79</td>
<td>106.65</td>
<td>0.81</td>
</tr>
</tbody>
</table>

### rAbcg2

\[
PS_{\text{Apparent}(A,B \rightarrow A)} \Rightarrow [PS_D + PS_{PC}]
\]

\[
PS_{\text{Apparent}(B,A \rightarrow B)} \Rightarrow [PS_{PC}]
\]

### Summary

<table>
<thead>
<tr>
<th></th>
<th>Plus</th>
<th>Minus</th>
<th>Overall</th>
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<tbody>
<tr>
<td>PS(_{PC})</td>
<td>1.27</td>
<td>1.19</td>
<td>1.23</td>
</tr>
<tr>
<td>PS(_D)</td>
<td>229</td>
<td>227</td>
<td>228</td>
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</table>
Pantoprazole & Abcg2

• Two isomers appear to differ with respect to interaction with Abcg2
• Is this true with human ABCG2?
• Is it clinically relevant?

• Do isomers differ in affinity ($K_m$) or capacity ($T_{Max}$)?
  – Initial guess would be affinity since binding to transporter would be logical
  – Use isomers as inhibitors of MTX in Sf9 cells ($IC_{50}$ a reflection of their $K_m$

• Can simulations tell us anything about relative parameters?