The Importance of Concept of Fraction of Drug Transported (ft) in Understanding and Predicting Drug Disposition and DDI

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Tissue/Membrane Localization of Drug Transporters

- Liver
  - MATE1
  - MRPs 1, 3, 4, 5, 6
  - BCRP
  - BSEP
  - OCT1, NTCP, OATP 1B3, OATP 1B1

- Intestine
  - MRPs 1, 3, 5
  - BCRP
  - MRP2
  - OCT1, ENT1
  - OATP, PEPT, CNT

- Kidney
  - MRPs 1, 3, 5, 6
  - MRP2
  - BCRP
  - OCTN2, OAT3

- Blood
  - OCTN1, OAT3
  - OATP 1A2, OATP 2B1
  - P-gp, BCRP

- Brain Interstitial Space
  - OCTN1, OAT3
  - OATP 1A2, OATP 2B1
  - P-gp, BCRP

- Cerebrospinal Fluid
  - OCTN1, OAT3
  - OATP 1A2, OATP 2B1
  - P-gp, BCRP

- Placenta
  - OCT3
  - OATP 2B1

- Intestinal Lumen

- Fetal blood

- Urine

Unadkat JD, Enzyme-and Transporter-Based Drug-Drug Interactions: 2010.
When are Transporters Relevant to ADME?

- When a significant **fraction** of the drug **dose** is absorbed, distributed into an eliminating or non-eliminating tissue, or cleared from the body via transporters (i.e. $f_t$ is large)

- Modulation of the transporter(s) by DDI or SNPs will
  - **NOT** affect exposure of the tissue to the drug (i.e. AUC) if the drug is cleared **primarily** through that organ/tissue. However the Cmax and Cmin in the tissue will be affected.
  - **NOT** significantly affect the systemic CL of the drug
  - If the **fraction of the dose** distributing into a tissue via a transporter is small
    - **But**, will have a **profound** impact on the tissue concentration and therefore potentially the toxicity and efficacy of the drug – disconnect between the plasma and tissue conc.

- $f_t$ will determine the impact of transporter DDI or SNPs on systemic or tissue conc. of drugs
Some Principles

- When a new molecular entity is found to be a substrate of a transporter, consider the following:
  - Is the transporter(s) present in the tissue of interest?
  - If so, what is its contribution relative to $C_{L_{\text{diffusion}}}$ and $C_{L_{\text{other}}}$. 
  - In vitro transport $\neq$ in vivo relevance because transfected cell lines (or $X. \ oocytes$) often exaggerate $ft$ due to high expression of the transporter.
  - A substrate can be a potent inhibitor of a transporter without being a substrate.
  - Even if the affinity of the substrate for the transporter is low and the expression of the transporter in the tissue of interest is low, that transporter could still be important in determining the clearance and/or tissue conc. of the drug if $ft$ via that transporter is large.
  - $ft$ is king!
Illustration of these Principles Via a Magic School Bus Tour Through the Body

Endres et al., Eur J. Pharm. Sci., 2005
Intestinal Transport

Liver
- MATE1
- MRP 1,3,4,5,6
- BCRP
- BSEP
- OCT1
- NTCP
- OATP 1B3, 1B1
- OATP 2B1

Brain Interstitial Space
- OCTN1
- OAT3
- OCTN2
- OATP 4A1
- OATP 1A2
- OATP 2B1

Cerebrospinal Fluid
- OCTN1
- OAT3
- OCTN2
- MRP 1,2,4,5
- OATP 3A1
- OATP 2B1

Blood
- P-gp
- BCRP

Intestine
- BCRP
- MRP 1,3,5
- ENT1
- OATP 1A2
- PEPT 1,2
- CNT 1,2

Placenta
- MRP2
- BCRP
- P-gp
- OCTN1
- OATP 4C1
- OCT2
- OATP 1A2
- OATP 2B1

Fetal blood
- MRP2
- BCRP
- P-gp

Kidney
- BCRP
- MRP 1,3,5,6

Urine
- P-gp

Absorption: Transport can be Rate-limiting Step(s) in Bioavailability of Drugs

- fraction absorbed (fa) via transporters >> fraction absorbed (fa) via passive diffusion (BDDCS class 3 & 4)

- dual rate-limiting steps for class 3 & 4 drugs – apical and basal membrane

Benet, J Pharm Sci, 2012
Concentrative Nucleoside Transporter 2 Limits the Intestinal Absorption of Ribavirin ($ft=\sim0.8$)

Moss et al., Mol. Pharm.  2012

![Diagram showing vectorial transport of Na+ and Ribavirin through CNT2 and ENT1](image-url)
**Equilibrative Nucleoside Transporter 1 (ENT1) Limits the Egress of Ribavirin from the Intestine to the Blood**

Moss et al., Mol. Pharm. 2012
Green Tea-Nadolol DDI – OATP1A2?

Nadolol is transported by OATP1A2 but not by OATP2B1
But, OATP1A2 mRNA or protein was not detected in human intestinal tissue! This DDI must be due to some other mechanism(s)

For IVIVE and mechanism of DDI, important to ask if the transporter is PRESENT in the tissue of interest
Summary

- When a drug is found, in vitro, to be a substrate of a transporter, ask:
  - Is the transporter present in the tissue of interest?
  - What is the contribution of this transporter relative to diffusion or other transporters, i.e. what is $ft$ for each transporter?
  - In specialized cells (e.g. enterocytes), transporters can pose dual rate-limiting steps (apical and basal) in bioavailability or clearance of drugs
  - Inhibition of efflux transporters (e.g. at the basal membrane) can profoundly increase the local tissue conc. and therefore potential toxicity of the drug
Tissue/Membrane Localization of Drug Transporters

Unadkat JD, Enzyme-and Transporter-Based Drug-Drug Interactions: 2010.
Parallel Routes of Elimination

- Metabolic (*CL*<sub>int</sub>)
- Canalicuclar Efflux (*CL*<sub>c<sub>ef</sub></sub>)

Bi-Directional Hepatic Distribution

- Sinusoidal Influx (*CL*<sub>s<sub>in</sub></sub>)
- Sinusoidal Efflux (*CL*<sub>s<sub>ef</sub></sub>)
- Diffusion
Hepatic Well-Stirred Model

\[ CL = \frac{Q_L \times f_u CL_{int}}{Q_L + f_u CL_{int}} \]


Key Assumptions Ignore Transporters or Permeability Limitations:

- The transfer from perfusate to the liver is instantaneous and not limited by permeability
- Unbound drug concentration in the plasma and the liver are the same

NEDMDG
Modification of the Well Stirred Model to Include Transporters

Endres et al., Mol Pharm. 2009 6:1756-65
Modified Clearance Concepts

\[ CL = \frac{f_u CL_{in} CL_{Other} (CL_{ef} + CL_{int}) + Q_L (CL_{Other} (CL_{ef} + CL_{int} + CL_{ef}) + f_u CL_{in} (CL_{ef} + CL_{int}))}{(f_u CL_{in} (CL_{ef} + CL_{int}) + Q_L (CL_{ef} + CL_{int} + CL_{ef}))} \]

When \( CL_{other} = 0 \)

\[ f_u Q_L CL_{in} (CL_{ef} + CL_{int}) \]

\[ \frac{f_u Q_L CL_{in} (CL_{ef} + CL_{int})}{(f_u CL_{in} + Q_L)} \]

and when \( CL_{ef} \approx 0 \) or \( << (CL_{ef} + CL_{int}) \)

\[ f_u Q_L \]

and when \( CL_{int} / CL_{ef} = \infty \) (no permeability limitation)

\[ Q_L + f_u (CL_{ef} + CL_{int}) \]

and when \( CL_{ef} = 0 \) (only metabolic elimination)

\[ \frac{f_u Q_L CL_{int}}{Q_L + f_u CL_{int}} \]

the equation reduces to the well-stirred model

NEDMDG

Endres et al., Mol Pharm. 2009 6:1756-65
Dependence of Systemic Clearance on Sinusoidal Permeability of the Drug

When $\text{CL}^s_{\text{in}}$ is low or $<<$ $\text{CL}^s_{\text{ef}}$, hepatic distribution becomes permeability rate limited - changes in either $\text{CL}_{\text{int}}$ or $\text{CL}^c_{\text{ef}}$ have decreasing impact on $\text{Cl}_{\text{sys}}$. A metabolic drug interaction may be predicted from microsomal data but NONE is observed in vivo.

Assumptions: $\text{CL}_{\text{other}} = 0$
$Q_L = 1$ (arbitrary vol/time units);
$F_p/F_L = 1$

Endres et al., Mol Pharm. 2009 6:1756-65
When sinusoidal distributional CL is 100-fold $Q_L (=1)$, then $C_{L_{sys}}$ is limited by $Q_L$. When $C_{L_{in}}$ is $<< CL_{ef} + CL_{int}$, CL will be determined by $C_{L_{in}}$ and will be insensitive to inhibition (DDI) of $CL_{ef}/CL_{int}$. However, such inhibition will have a dramatic effect on hepatic conc.

When the NET sinusoidal CL is low (e.g. when the drug is highly permeable) $CL_{ef}/CL_{int}$ determines CL.

Assumptions: $CL_{other} = 0$

$Q_L = 1$ (arbitrary vol/time units); $F_p/F_L = 1$
When $CL_{\text{in}}^s$ is $<< CL_{\text{ef}}^c + Cl_{\text{int}}$ and $Clother=0$

As $CL_{\text{in}}^s \uparrow$, systemic CL $\uparrow$, AUC reservoir $\downarrow$

BUT

AUC liver $\leftrightarrow$

As $Cl_{\text{int}} \uparrow$, systemic CL $\leftrightarrow$, AUC reservoir $\leftrightarrow$, BUT AUC liver $\downarrow$

Even when $CL_{\text{in}}^s$ is not $<< CL_{\text{ef}}^c + Cl_{\text{int}}$ $Cl_{\text{int}}=0.3$, $Cl_{\text{ef}}^c =0$
Changes in ALL clearance pathways \textbf{WILL} affect systemic AND liver AUC

When $\text{Cl}^s_{\text{ef}}$ is not $<< \text{Cl}^c_{\text{ef}} + \text{Cl}_{\text{int}}$ and $\text{Cl}_{\text{other}} > 0$

\[
\text{CL}_{\text{sys}} = \frac{f_u Q_L \text{CL}^s_{\text{in}}}{{(f_u \text{CL}^s_{\text{in}}(\text{CL}^c_{\text{ef}} + \text{CL}_{\text{int}}) + Q_L (\text{CL}^c_{\text{ef}} + \text{CL}_{\text{int}} + \text{CL}^s_{\text{ef}}))}} + \text{CL}_{\text{other}}
\]

$\text{CL}_{\text{other}} = 0.4; \text{CL}_{\text{int}} = 0.3, \text{Cl}^c_{\text{ef}} = 0$
Hepatic OATPs limit Systemic CL of Atorvastatin: Rifmapin DDI

Lau et al., 81, 2007
$^{11}$C-Rosuvastatin – Rifampin DDI in the Rat

$^{11}$C-Rosuvastatin blood conc. –time profile

He et al., Mol Pharm. 2014
Hepatic Uptake and Biliary Excretion of $^{11}$C-Rosuvastatin in the Rat

He et al., Mol Pharm. 2014

He et al., Mol Pharm. 2014
$^{11}$C-Rosuvastatin – Rifampin DDI in the Rat

$^{11}$C-Rosuvastatin blood conc. – time profile

AUC$_{0-15m}$ (AUC$_{RIF}$/AUC$_{control}$) $\uparrow$ 230%

$^{11}$C-Rosuvastatin hepatic conc. – time profile

AUC$_{0-15m}$ $\uparrow$ 5%

Blood\nHepatocyte\nBile

ROS\nBCRP/ MRP2\nOATPs

NEDMDG

He et al., Mol Pharm. 2014
Summary

- Hepatic transporter(s):
  - What is the contribution of the transporter relative to diffusion or other transporters, i.e. what is ft for each transporter?
  - If the uptake transport is a concentrative, it may be the rate-limiting step. Modulation of this transport (e.g. DDI, SNPs) may profoundly affect the systemic conc. of the drug. But, the impact on hepatic conc. is likely to be much smaller because:
    - \( \frac{dX}{dt}_{\text{hepatic uptake}} = CL_{\text{uptake remainder}} \times C_{p,u} \) and \( C_{p,u} \) is ↑
    - If the drug is mostly cleared by hepatic CL, it will eventually be eliminated by passage through the liver
  - Inhibition of efflux transporters (e.g. MRP2) can profoundly increase the hepatic conc. and therefore potential toxicity/efficacy of the drug
Tissue/Membrane Localization of Drug Transporters

Unadkat JD, Enzyme-and Transporter-Based Drug-Drug Interactions: 2010.
**P-gp Transport of $^{11}$C-verapamil at the Human BBB and Inhibition by Cyclosporine A (CsA)**

<table>
<thead>
<tr>
<th>$^{15}$O-water</th>
<th>$^{11}$C-verapamil</th>
<th>$^{15}$O-water</th>
<th>$^{11}$C-verapamil</th>
<th>$^{11}$C-CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 min</td>
<td>~45 min</td>
<td>15 min</td>
<td>~45 min</td>
<td>12 min</td>
</tr>
</tbody>
</table>

CsA infusion (2.5 mg/kg/hr) for 60 min

Continue CsA infusion for ~45 min

Eyal et al., *Clin Pharmacol Ther.* 2010
Plasma and Brain $^{11}$C-verapamil radioactivity concentration-time profiles in the absence and presence of CsA

Effect of CsA different than that in rodents: 87% vs. 1000%

What is the ft of verapamil? Can the effect of maximal inhibition of P-gp be discerned from our data?

Eyal et al., *Clin Pharmacol Ther.* 2010

Cb, cerebellum
FC, frontal cortex
OC, occipital cortex
Pu, putamen
PI, pituitary
T, thalamus
V, lateral ventricle
Regional 0-10 min brain distribution of [¹¹C]-radioactivity

Before cyclosporine
During cyclosporine

Eyal et al., *Clin Pharmacol Ther*. 2010
ft of $[^{11}\text{C}]$-Verapamil radioactivity is $\sim 0.8$ in humans and macaques

Based on these data, the MAXIMUM increase (on complete inhibition of P-gp) in verapamil distribution into the human brain is predicted to be $\sim 4$-5 fold.

confirmed by studies in nonhuman primates, the macaque

Eyal et al., Clin Pharmacol Ther. 2010

Ke et al., J Nucl. Med. 2013
### ft and P-gp DDI at the Mouse BBB

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Brain to Blood Ratio</th>
<th>ft (P-gp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nelfinavir</td>
<td>40</td>
<td>Very High (~ 0.975)</td>
</tr>
<tr>
<td>Verapamil</td>
<td>9.5</td>
<td>High (~ 0.9)</td>
</tr>
<tr>
<td>Cetirizine (Zyrtec®)</td>
<td>2.3</td>
<td>Intermediate (~ 0.6)</td>
</tr>
</tbody>
</table>
Magnitude of P-gp based DDI at the Human BBB Depends on $f_{t(P-gp)}$ of the P-gp substrate

Hsiao et al., Mol. Pharm. 2014
ft and apparent P-gp/BCRP synergism

All values are for unbound drug and are arbitrary

ft = 0.95 due to efflux by P-gp and BCRP

ft_{P-gp} = 0.475, ft_{BCRP} = 0.475
fts_{dif} = 0.05

• $\frac{CSS_b}{CSS_p} = \frac{CL_{dif}}{CL_{efflux}} = 0.05$
• when $CL_{Pgp}=0$, $\frac{CSS_b}{CSS_p} = 0.095$
• when $CL_{BCRP}=0$, $\frac{CSS_b}{CSS_p} = 0.095$
  ~ 2-fold ↑

when $CL_{Pgp}+CL_{BCRP}=0$, $\frac{CSS_b}{CSS_p} = 1$ (~20-fold ↑)
Summary

- BBB transporter(s):
  - For high $f_t$ drugs (>0.9, via P-gp, BCRP or both), there is potential for inadvertent DDI at the human BBB to be clinically significant if these drugs have a narrow therapeutic window.
  - Dual inhibition of both P-gp and BCRP could result in a profound increase in brain conc. – but it’s not synergism, it’s just $f_t$!
  - To estimate the maximum liability for P-gp-based drug interactions that a NME will produce (perpetrator), I propose that rodent studies be conducted with nelfinavir as the substrate and the NME as the inhibitor.
  - To estimate the maximum liability for P-gp based drug interactions that a NME will be subjected to (i.e. victim), I propose that the $f_{t(P-gp)}$ of the NME be determined in rodents. Then, the maximum DDI be computed.
Overall Summary: When are Transporters Relevant to ADME?

- When a significant fraction of the drug dose is absorbed, distributed into an eliminating or non-eliminating tissue, or cleared from the body via transporters (i.e. $f_t$ is large)

- If the fraction of the dose distributing into a tissue via a transporter is small, modulation (e.g. DDI) of this transporter by DDI or SNPs
  - will NOT significantly affect the systemic CL of the drug
  
  BUT,
  - if the drug is cleared primarily through that organ/tissue, the AUC in the tissue will not be affected, but the Cmax will be
  - if the drug is NOT cleared primarily through that organ, it will have a profound impact on the tissue concentration and therefore potentially the toxicity and efficacy of the drug – disconnect between the plasma and tissue conc.

- $f_t$ will determine the impact of transporter DDI or SNPs on systemic or tissue conc. of drugs

- Transporters can serve dual rate-limiting step in the absorption or clearance of drugs
How Does One Determine $f_t$?

- Use primary cells and selective inhibitors
- But primary cells are not available for many organs (e.g. BBB or intestine)
- Alternative approach is to use quantitative proteomics and transfected cell lines

University of Washington Research Affiliate Program on Transporters

- Goal is to quantify the expression of transporters (and interindividual variability) in various human tissues using LC/MS/MS
- Funded by a consortium: Merck & Co, Genentech, Biogen Idec (and Astra Zeneca)
Relative Transporter Abundance Pie Chart

Human liver

- OCT1: 27%
- NTCP: 13%
- Ntcp: 13%
- MRP2: 9%
- MRP3: 3%
- MATE1: 3%
- BSEP: 9%
- OATPs: 29%
- OATP1B3: 7%
- OATP2B1: 10%
- OATP1B1: 12%

Interspecies Variability in Expression of Hepatobiliary Transporters across Human, Dog, Monkey, and Rat as Determined by Quantitative Proteomics

Li Wang, Bhagwat Prasad, Laurent Salphati, Xiaoyan Chu, Anshul Gupta, Cornelis E.C.A. Hop, Raymond Evers, and Jashvant D. Unadkat

Li et al. DMD 2015
Transporter Expression in Human Intestines

Relative contribution

small intestine
- PEPT1 50%
- ABCB1 8%
- ABCC2 10%
- ABCC3 7%
- ABCG2 4%
- ASBT 6%
- OCT1 8%
- OCT3 1%
- OATP2B1 6%

colon
- PEPT1 5%
- ABCB1 5%
- ABCC2 25%
- OATP2B1 12%
- OCT3 2%
- OCT1 12%
- ABCG2 3%
- ABCC3 36%

OATP1A2 could not be detected in the small intestine

N=6, 5 males, 1 female;
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