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4th Workshop (virtual)
April 19-21, 2021

[https://www.itc-transporter.org/itcw4.html](https://www.itc-transporter.org/itcw4.html) provides links to agenda and registration at **ASCPT**

Pre-Workshop Webinar, March 31st, 2021, 5-6:30 PM EDT ([free registration](#))
Prof Yuichi Sugiyama

Use of Extended Clearance Concept and PBPK Modeling in New Drug Discovery and Development: Predicting Target Tissue Exposure from In Vitro to In Vivo
Contents

1) Introduction;
   Rate-determining process (focusing on the liver)
   (Uptake, efflux, elimination, metabolism) DDI

2) PGx of OATP1B1: PBPK model based analysis

3-1) PBPK model based analysis of OATPs mediated drug-drug interaction
     (Top down + Bottom-up)
     (i) victim drugs-perpetrator drugs
     (ii) endogenous biomarker (CP-I) – rifampicin

3-2) Simple bottom-up predictions dot not always work well.
     (i) prediction of hepatic clearance of highly protein bound drugs
        (albumin-mediated hepatic uptake mechanisms should be considered)
     (ii) Time-dependent inhibition (inhibitors for OATP1B and OCTs)

4) Target-mediated drug disposition (TMDD); To obtain dose-dependent change in molecular target occupancies only from the plasma concentration time-profile
Drug-interaction between Cerivastatin and Gemfibrozil/ CsA

• 52 patients died (US 31).
• Among 31 patients, 12 were given also gemfibrozil.

Cyclosporine A

OATP1B1

OATP1B3

CYP2C8

OATP1B3

CYP2C8, 3A4

Gemfibrozil glucuronide

Gemfibrozil glucuronide

Gemfibrozil glucuronide

Cerivastatin

Dual substrates

Dual inhibitors

Gemfibrozil glucuronide

Gemfibrozil glucuronide

52 patients died (US 31).
Among 31 patients, 12 were given also gemfibrozil.

### Examples of substrates for uptake/efflux transporters and enzymes (1)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Uptake transporter</th>
<th>Metabolic enzymes</th>
<th>Efflux transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-Hyperlipidemic drugs (statins)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>atorvastatin</td>
<td>OATPs</td>
<td>CYP3A4</td>
<td>-</td>
</tr>
<tr>
<td>cerivastatin</td>
<td>OATPs</td>
<td>CYP2C8, 3A4</td>
<td>-</td>
</tr>
<tr>
<td>fluvastatin</td>
<td>OATPs</td>
<td>CYP2C9</td>
<td>-</td>
</tr>
<tr>
<td>pravastatin</td>
<td>OATPs</td>
<td>-</td>
<td>MRP2</td>
</tr>
<tr>
<td>rosuvastatin, pitavastatin</td>
<td>OATPs</td>
<td>-</td>
<td>BCRP</td>
</tr>
<tr>
<td><strong>Anti-hypertension or -cardiovascular disease</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>bosentan</td>
<td>OATPs</td>
<td>CYP3A4, 2C9</td>
<td>-</td>
</tr>
<tr>
<td>torasemide</td>
<td>OATPs</td>
<td>CYP2C9</td>
<td>-</td>
</tr>
<tr>
<td>telmisartan</td>
<td>OATP1B3</td>
<td>UGTs</td>
<td>-</td>
</tr>
<tr>
<td>valsartan</td>
<td>OATPs</td>
<td>-</td>
<td>MRP2</td>
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<tr>
<td><strong>Anti-cancer drug</strong></td>
<td></td>
<td></td>
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<tr>
<td>docetaxel</td>
<td>OATP1B3</td>
<td>CYP3A4</td>
<td>-</td>
</tr>
</tbody>
</table>
Examples of substrates for uptake/efflux transporters and enzymes (2)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Uptake transporter</th>
<th>Metabolic enzymes</th>
<th>Efflux transporter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-diabetes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>repaglinide</td>
<td>OATPs</td>
<td>CYP2C8, 3A4</td>
<td>-</td>
</tr>
<tr>
<td>nateglinide, glibenclamide</td>
<td>OATPs</td>
<td>CYP2C9, 3A4</td>
<td></td>
</tr>
<tr>
<td><strong>Anti-HCV</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>simeprevir, grazoprevir</td>
<td>OATP1B1</td>
<td>CYP3A4</td>
<td>-</td>
</tr>
<tr>
<td>asunaprevir, danoprevir, paritaprevir</td>
<td>OATPs</td>
<td>CYP3A4</td>
<td>Pgp</td>
</tr>
<tr>
<td><strong>Etc.</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montelukast</td>
<td>OATP2B1</td>
<td>CYP2C8, 2C9, 3A4</td>
<td>-</td>
</tr>
<tr>
<td>maraviroc</td>
<td>OATP1B1</td>
<td>CYP3A4</td>
<td>Pgp</td>
</tr>
<tr>
<td>fexofenadine</td>
<td>OATPs</td>
<td>-</td>
<td>Pgp</td>
</tr>
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</table>
Understanding Extended Clearance Concept is very important (I have been training this concept to all the students, post-doc in my lab (UOT, RIKEN)

“Extended Clearance Concept”

• enough to predict the change in AUC and/or Css both in plasma and tissue
  (PGx, DDI (at least for static analyses, and not for dynamic analysis)

“PBPK modeling”

• Appropriate model for describing the drug conc-time course both in plasma and tissue as well as AUC, Css
Overall Hepatic Intrinsic Clearance (\(\text{Cl}_{\text{int,all}}\))

\[ \text{Cl}_{\text{int,all}} = \text{PS}_{\text{inf}} \times \frac{\text{CL}_{\text{int}}}{\text{PS}_{\text{eff}} + \text{CL}_{\text{int}}} \]

1) When \(\text{PS}_{\text{eff}} \ll \text{CL}_{\text{int}}\), (Case-1)

\[ \text{Cl}_{\text{int,all}} = \text{PS}_{\text{inf}} \]

Degree of Sequestration (\(\beta\))

\(\beta = 1\)

Uptake – limited (anionic drugs; statins, sartans)

2) When \(\text{PS}_{\text{eff}} \gg \text{CL}_{\text{int}}\), (Case-2)

\[ \text{Cl}_{\text{int,all}} = \frac{\text{PS}_{\text{inf}}}{\text{PS}_{\text{eff}}} \times \text{CL}_{\text{int}} \]

Degree of Active Uptake (\(\alpha\))

\(\alpha = 1\)

When \(\text{PS}_{\text{inf}} = \text{PS}_{\text{eff}}\) (passive diffusion),

Intrinsic Clearance-limited (lipophilic basic/neutral drugs; quinidine, diazepam)
Impact of the function of each pathway on the overall intrinsic clearance
Plasma concentrations of atorvastatin and pravastatin were greatly increased by rifampicin, but not by itraconazole

### AUC_{0-10} [pg*hr/ml] & ATV & PRV (AUC_{0.8}) & MDZ

<table>
<thead>
<tr>
<th></th>
<th>AUC_{0-10}</th>
<th>ATV</th>
<th>PRV</th>
<th>MDZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont.</td>
<td>38.5 ± 17.5</td>
<td>195 ± 78.7</td>
<td>434 ± 122</td>
<td></td>
</tr>
<tr>
<td>+RIF</td>
<td>439*** ± 134</td>
<td>949*** ± 179</td>
<td>471 ± 168</td>
<td></td>
</tr>
<tr>
<td>+ITZ</td>
<td>36.0 ± 19.2</td>
<td>386 ± 254</td>
<td>755* ± 276</td>
<td></td>
</tr>
</tbody>
</table>

***: P<0.0005
*: P<0.05

---

* Doses of each substrates are 33µg

Maeda K et al., CPT (2011)
Effects of rifampicin and itraconazole on the PK of atorvastatin


CL of atorvastatin was inhibited only by rifampicin.

cf. 2-hydroxyatorvastatin

### Effects of rifampicin and itraconazole on the PK of atorvastatin

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<tr>
<td>AUC&lt;sub&gt;0-10&lt;/sub&gt;</td>
<td>38.5</td>
<td>439***</td>
<td>36.0</td>
</tr>
<tr>
<td>[pg*hr/mL]</td>
<td>±17.5</td>
<td>±134</td>
<td>±19.2</td>
</tr>
</tbody>
</table>

\(AUC_{0-10}\) is significantly greater in the +RIF condition compared to the control and +ITZ conditions. The presence of rifampicin alone is sufficient to inhibit atorvastatin hepatic clearance.

→ atorvastatin hepatic clearance is limited only by hepatic uptake.
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4) Target-mediated drug disposition (TMDD); To obtain dose-dependent change in 
molecular target occupancies only from the plasma concentration time-profile
The impact of OATP1B1 on the PK of drugs
~Plasma conc. of drugs is increased in subjects with OATP1B1*15~

**HMG-CoA reductase inhibitors**
- pravastatin
- simvastatin acid
- pitavastatin
- atorvastatin
- rosuvastatin

**Anti-diabetes**
- repaglinide
- nateglinide
- glybenclamide

**Anti-allergic drug**
- fexofenadine

**Anti-pulmonary hypertension drug**
- Atrasentan
- bosentan

**Anti-cancer drug**
- irinotecan (SN-38)
- docetaxel, paclitaxel

**Chol-absorption inhibitor**
- ezetimibe

**Loop diuretics**
- Torasemide

**Angiotensin receptor antagonists**
- Olmesartan
Relationship between OATP1B1 genetic polymorphism and Pharmacological effect and adverse effect of statins

★Pharmacological effect
Target; HMG CoA-reductase in the liver
No effect or small effect if any

★Adverse Effect
Target; Muscle (via plasma)
Simvastatin-induced myopathy
strong correlation with OATP1B1 polymorphism

Odds ratio of this SNPs for simvastatin-induced myopathy

521C/T vs T/T → 4.5 fold
521C/C vs T/T → 16.9 fold
Summary

All of these pharmacogenetic and DDI studies on OATP1B1 suggested that the hepatic uptake plays an important role in the plasma clearance of therapeutically important drugs (mostly anionic drugs; statins, ARA, ACE inhibitors, anti-HCV drugs, anticancer drugs, etc).

Why did this polymorphism and/or DDI affect only side-effect (myopathy; muscle is a target tissue), and not pharmacological effect (lipid lowering effect; liver is a target organ)?

To answer this question, it is important to estimate the exposure in the plasma (muscle; side effect target) and in the liver (Pharmacological target) (statins, HCV drugs)


PBPK modeling of pravastatin disappearance

Plasma conc-time profile of pravastatin in human

- i.v. 0.134mg/kg (observed)
- o.p. 0.26mg/kg (observed)

Extrapolated parameters based on in vitro (animal, human) and in vivo (animal)
Uptake-limited hepatic elimination of statins

We need PET studies to know the drug exposure change in the liver

Statins
Pravastatin
Pitavastatin
Atorvastatin
Fluvastatin

Systemic exposure

Liver concentration

Liver

Blood

Microsome

IVIVE

CL_{int, all} = \frac{PS_{inf}}{PS_{eff} + CL_{int}}

Metabolism
Biliary excretion (MRP2, BCRP)

0 60 120

Time
Concentration

0 60 120

Time
Concentration

0 60 120

Time
Concentration

We need PET studies to know the drug exposure change in the liver.
Summary:

1) Sensitivity analysis indicated that the change in hepatic uptake ability alters the plasma concentration profile sensitively (toxicity) and may not affect the profile in the liver, target tissue (pharmacological effect). GWAS for simvastatin in fact demonstrated it was the case.

2) Alteration in the biliary excretion ability (MRP2, BCRP) may affect the pharmacological effect (hepatic exposure) much more sensitively than that of the uptake, though there is little change in the plasma exposures.

⇒ We have to confirm it by PET analyses

This prediction has been supported by several studies published by other groups (simvastatin GWAS study, rosvuastatin Jupiter trial)
Drug development with the Use of Microdosing Clinical Trial: Based on the Quantitative Prediction Technology of ADME (NEDO Research Project (2008-2011) collaboration with Y.Watanabe)

- **R(+)-[11C]Verapamil**: P-gp (BBB) and ontogeny in BBB penetration.
- **[11C]Oseltamivir**: OATP1B1,1B3, MRP2.
- **[15R-[11C]TIC-Me**: OATP1B1,1B3, MRP2.
- **[11C]Dehydropravastatin**: OATP1B1, MRP2.
- **[11C]Telmisartan**: OATP1B3.
- **[11C]SC-62807**: OATP1B1,1B3, BCRP.
- **[11C]Metformin** (will be studied very soon): OCT2, MATE 1.
- **[11C]Uric acid**: URAT1, BCRP.
- **[18F]FPEG(2kDa)**: • Unabsorbed marker • Application to PEGylation.

Takashima T et al., J Nucl Med. 2011; 52: 950-7
Takashima T et al., JPET 2010; 335: 314-323
Takashima T et al., Mol Pharm. 2011; 8: 1789-98

PET imaging human studies

[\textsuperscript{11}C]DHP PET studies

[\textsuperscript{11}C]DPV chemical structure

\textbf{Metabolism is minimized}

cf. Pravastatin chemical structure

[11C]DHP dynamic model fitting

[11C]DPV chemical structure

Fitted radioactivity-time profile of DPV on control phase using dynamic model (#2)

cf. Pravastatin chemical structure

Fitted blood radioactivity-time profile of DPV using 2-exponential curve (#2)

Fitted radioactivity-time profile of DPV on +rifampicin phase using dynamic model (#2)

% Dose/mL vs Time (min)

Liver
Bile duct + Gallbladder

% Dose vs Time (min)

Liver
Bile duct + Gallbladder

(17)
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4) Target-mediated drug disposition (TMDD); To obtain dose-dependent change in
molecular target occupancies only from the plasma concentration time-profile
Drug-Drug Interaction between CER and Cyclosporine A/Gemfibrozil

To develop PBPK models to quantitatively describe the transporter- and enzyme-mediated DDIs of CER

\[
\begin{align*}
\text{CLh} & \downarrow \quad \text{Vd} \downarrow \quad T_{1/2} \rightarrow \\
\text{CLh} & \downarrow \quad \text{Vd} \quad \rightarrow \quad T_{1/2} \downarrow
\end{align*}
\]

PBPK analyses of transporter/enzyme mediated complex DDI

Integration of bottom-up approach And top-down approach is important

**Background:**

Cerivastatin (CER)

- **Central**
- **Adipose**
- **Skin**
- **Muscle**

Cyclosporine A (CsA)

- **Central**
- **Adipose**
- **Skin**
- **Muscle**

Gemfibrozil (GEM)

- **Central**
- **Adipose**
- **Skin**
- **Muscle**

**Clinical data of DDI**

- **Step 1:** Determination of the structures of PBPK models according to the PK properties of drugs

**Initial parameters for PBPK models**

- Compartment model ($k_a, T_{eq}$)
- Clearance concept ($V_{cl}\cdot CL_{int}$)
- In vivo experiments (PS$_{int}$/PS$_{int}$, $f_a$)
- In silico calculation ($K_{in}$, PS$_{int}$/PS$_{int}$)
- References (other physiological and pharmacokinetic parameters)

**Optimization of parameters in PBPK models by fitting**

**Step 4** Optimization of parameters including Ki by fitting to both control and DDI condition

**Yoshikado T et al., Clin Pharmacol Ther., (2016)**
Best simulation of CER vs CsA / CER vs GEM

**CER vs CsA**

We may have to consider the inhibition of GI Pgp

In vivo Ki of CsA
Obtained for other drugs

**CER vs GEM**

In vitro Ki value of GEM and its glucuronide (Ki, app and kinact) MBI

Present PBPK models were able to well reproduce the clinical DDIs

Summary

• The concentration-time profiles for CER and GEM/GEM-glu described by PBPK models were well agreed with the clinically observed data.

• The present PBPK models were able to capture the clinical DDIs
  ➢ CER and CsA: using $\frac{1}{2}$ in vivo Ki value for OATP1B1 which is previously reported (substrate-dependent Ki). We may have to take into account the inhibition of intestinal Pgp to better describe this DDI
  ➢ CER and GEM: using reported in vitro $\text{k}_\text{inact}$ value (GEM-glu) and Ki value for OATP1B1 or CYP2C8 when $\text{fm}_{2C8} = 0.85$ value was used

Complex DDIs involving both transporters and metabolic enzymes could be quantitatively predicted by PBPK modeling (Bottom-up approach) based on the in vitro parameters ($K_i, k_{\text{inact}}$)
We now know some cases where simple bottom up prediction (IVIVE) cannot be applied.

1) Albumin-mediated hepatic uptake observed for highly protein bound drugs (OATP1B, OAT2 substrates)

2) Time-dependent inhibition constant (Ki, app value) observed for some OATP1B inhibitors and OCT1 inhibitors
Soo-Jin Kim, Kyeong-Ryoon Lee, Seiji Miyauchi, and Yuichi Sugiyama

Extrapolation of In Vivo Hepatic Uptake Clearance from In Vitro Uptake Clearance by Suspended Human Hepatocytes (IVIVE) for Anionic Drugs with High Binding to Human Albumin: Improvement of IVIVE by Considering the “Albumin-Mediated” Hepatic Uptake Mechanism Based on the Facilitated-Dissociation Model

The interaction of the albumin-ligand complex with the surface of hepatocytes enhances the dissociation of the ligand from albumin.


\[ \lambda = \frac{[Alb]_b}{[Alb]_i} = \frac{B_{\text{max}}}{K_{d,\text{m}} + [Alb]} \]

\( \lambda \): the ratio of bound albumin concentration to total albumin concentration;
\( B_{\text{max}} \) is the binding capacity of albumin to the cell surface.

\[ f_a = \frac{1}{[Alb] \cdot \frac{n}{K_a} + 1} \]

Two pathway
1. Unbound ligand pathway
2. Dissociated ligand pathway from ligand-albumin complex
Improvement of IVIVE of hepatic uptake clearance by taking into account of albumin-mediated hepatic uptake mechanism

IVIVE was improved by taking into account of the albumin-mediated hepatic uptake, though not perfect.

9 compounds
The $CL_{uptake,u}$ (at 5% HSA) predicted by fitting with Taso model was used.
Facilitated-dissociation (FD) model

\[ R = \frac{PS_{\text{inf, u, plasma}}}{PS_{\text{inf, u, buffer}}} = 1 + \left( \frac{1}{f_{u,p}} - 1 \right) \cdot \frac{r \cdot B_{\max}}{K_{d,m} + [\text{Alb}]} \]

The parameter \( r \) represents a ratio of uptake clearance of the unbound ligand dissociated from the ligand-albumin complex at cell surface to the uptake clearance of the unbound ligand dissociated in the plasma away from cell surface. For data fitting, \( r \cdot B_{\max} \) was considered as a hybrid constant as they were individually un-identifiable.
We now know some cases where simple IVIVE cannot be applied.

1) Albumin-mediated hepatic uptake observed for highly protein bound drugs (OATP1B, OAT2 substrates)

2) Time-dependent inhibition constant (Ki,app value) observed for some OATP1B inhibitors and OCT1 inhibitors
Preincubation-dependent and long-lasting inhibition of organic anion transporting polypeptide (OATP) and its impact on drug-drug interactions

Yoshihisa Shitara, Yuichi Sugiyama

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Keywords:
OATP1B1
Drug-drug interactions
Hepatic uptake
Time-dependent inhibition
Long-lasting inhibition
Physiologically based pharmacokinetic model
Modeling & simulation

ABSTRACT

Preincubation with cyclosporin A (CsA), a potent inhibitor of organic anion transporting polypeptide 1B1 (OATP1B1) and OATP1B3, enhanced its inhibitory effects on these transporters in vitro. A similar effect was observed upon preincubation with some other inhibitors. Removing these from the incubation media did not readily reverse the inhibition on OATP1B1 and OATP1B3. This preincubation-dependent long-lasting inhibition appeared to be related to CsA concentration in the cells in addition to that in the incubation media. Thus, we hypothesized that CsA inhibits OATP1B1 and OATP1B3 from inside (trans-inhibition) as well as outside (cis-inhibition) the cells and constructed the cis- and trans-inhibition model. The enhanced inhibitory effect of CsA on OATP1B1 observed after preincubation was quantitatively described using $K_{in}$ and $K_{out}$ as inhibition constants for cis- and trans-inhibitions, respectively. In addition, a long-lasting inhibition was also described by this model. Additional factors taken into consideration when simulating in vivo pharmacometric alterations by CsA are potential inhibition by AM1, a major metabolite of CsA, which has been reported to inhibit OATP1B1 and OATP1B3. Based on the physiologically based pharmacokinetic model incorporating trans- and cis-inhibition of OATP1B1 by CsA, the simulation showed that OATP1B1-mediated drug-drug interaction with CsA was suggested to be time-dependent also in vivo although further clinical studies are required for confirmation.

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Cis- and trans-inhibition of CsA on OATP1B1

\[
\text{CL}_{\text{uptake}}(+I) = \frac{V_{\text{max}}}{K_m} \frac{1 + f_{u,H} \cdot I_H / K_{i,in}}{1 + f_{u,B} \cdot I_{EH} / K_{i,out}} + S
\]

 cis-Inhibition = inhibition from outside

\[
\text{cis}-\text{Inhibition (competitive)}
\]

trans-Inhibition = inhibition from inside

\[
\text{trans}-\text{Inhibition (non-competitive)}
\]

\([\text{CsA}]_{\text{out}}\) (CsA outside cells)

\([\text{CsA}]_{\text{in}}\) (CsA inside cells)

\(K_{i,out}\)

\(K_{i,in}\)

Uptake clearance of OATP1B1 substrates in the liver when co-administered with CsA:

\(V_{\text{max}}\): maximum velocity

\(K_m\): Michaelis constant

\(f_{u,H}\) and \(f_{u,B}\): fraction of unbound CsA in the liver and at the extracellular space of the liver, respectively

\(I_H\) and \(I_{EH}\): inhibitor (CsA) concentrations in the liver and at the extracellular space of the liver, respectively
Simulation analysis of time-dependent enhancement effect of inhibition of OATP1B1 by CsA

IC$_{50}$ = 0.246 µM (preincubation = 0 min)

IC$_{50}$ = 0.0261 µM (preincubation = 60 min)

cis-Inhibition

1/(1 + I$_{out}$/IC$_{50,app}$) = 1/[(1 + I$_{out}$/K$_{i,out}$)x(1 + I$_{u,in,unbound}$/K$_{i,in}$)]

:with respect to time I$_{u,in}$↑, K$_{i,in}$→ ⇒ IC$_{50,app}$↓

Izumi S et al. (2015) Drug Metab Dispos 43, 235
(1) A Systematic In Vitro Investigation of the Inhibitor Preincubation Effect on Multiple Classes of Clinically Relevant Transporters

Péter Tátrai, Patrick Schweigler, Birk Poller, Norbert Domange, Roelof de Wilde, Imad Hanna, Zsuzsanna Gáborik, and Felix Huth

Solvo Biotechnology, Budapest, Hungary; Novartis Institutes for Biomedical Research, Basel, Switzerland and Novartis Institutes for Biomedical Research, East Hanover, New Jersey

Drug Metab Dispos 47:768–778, 2019

OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1, and MATE2-K
(Some inhibitors exhibited more than 200 folds decrease in apparent IC50 value by pre-incubation)

(2) Long-term trans-inhibition of the hepatitis B and D virus receptor NTCP by taurolithocholic acid
Long-term *trans*-inhibition of the hepatitis B and D virus receptor NTCP by tauroliothocholic acid

Kira AAT Lowjaga, Michael Kirstgen, Simon F Müller, Nora Goldmann, Felix Lehmann, Dieter Glebe, Joachim Geyer

*Institute of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Justus Liebig University Giessen, Germany*

What is the best translational approach until the mechanism is fully elucidated?

1) Benefit in using ‘shifted’ IC50 in the PBPK models as conservative approach? (Current recommendation by regulations)

2) More mechanism based PBPK modeling; distribution of inhibitor from extracellular space to intracellular sites ⇒ estimation of Permeability clearance, tissue binding, Ki,in, Ki,out ⇒ estimation of inhibiting effects which are changed with preincubation-time
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     (i) prediction of hepatic clearance of highly protein bound drugs
        (albumin-mediated hepatic uptake mechanisms should be considered)
     (ii) Time-dependent inhibition (inhibitors for OATP1B and OCTs)

4) Target-mediated drug disposition (TMDD); To obtain dose-dependent change in
   molecular target occupancies only from the plasma concentration time-profile
Concerns about the clinical DDI assessment:

**R-value**

R-value = 1 + \( \frac{I_{u, in, max}}{K_i} \) or IC\(_{50}\)

\( I_{u, in, max} = f_u \times I_{max} + (k_a \times \text{Dose} \times F_a \times F_g / Q_h) \)

If \( F_a \times F_g \) values and \( k_a \) values are unknown, use 1 and 0.1 min\(^{-1}\)
If \( f_u \) values are <0.1 or undetermined, assume \( f_u = 0.01 \)

11 substrates and 61 inhibitors (total 106 studies)

<table>
<thead>
<tr>
<th>Method</th>
<th>1: ( I_{max}/K_i \geq 0.1 )</th>
<th>2: ( I_{max}/K_i \geq 0.02 )</th>
<th>EMA ( R \geq 1.04 )</th>
<th>PMDA ( R \geq 1.25 )</th>
<th>FDA ( R \geq 1.25 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN</td>
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<td>TP</td>
<td>40</td>
<td>35</td>
<td>44</td>
<td>37</td>
<td>35</td>
</tr>
</tbody>
</table>

True positive rate

60% 69% 57% 65% 70% 73%

True negative rate

70% 70% 73% 73% 72% 71%

Many false positive prediction


Current criteria may request pharmaceutical industry to conduct false positive DDI study, and overlook DDI risk.
In future, we do not need to perform DDI clinical studies using probe substrates?
Effect of rifampicin on the plasma concentration time profiles of 4 probe drugs and 28 endogenous substrates

Plasma concentrations of the endogenous substrates were determined at designated times in healthy volunteers treated with or without an oral dose of rifampicin (150, 300 and 600 mg).
AUCR with 90% confidence interval assuming log normal distribution of various endogenous substrates with increasing dose of rifampicin. AUC0-24h was used to calculate AUCR.

The basic model structure for OATP1B1 substrates was reported previously (Yoshikado et al., Clin Pharmacol Ther 100:513-523, 2016).

The biosynthesis rate ($v_{syn}$) of CP-I is incorporated.

Asaumi et al., CPT-PSP 7:186-196, 2018
Simultaneously fitted blood concentration–time profiles of CP-I in the absence and presence of RIF after parameter optimization using the PBPK model incorporating the inhibition of OATP1Bs and MRP2.

Strategy to predict DDI for a probe substrate using CP-I as an endogenous biomarker

Step 1

**Phase I trial** of New Chemical Entity (NCE) CP-I levels

Doses of NCE

Conc

Time

Obtain *in vivo* $K_{OATP1B1}^{(CP-I)}$ using CP-I (an endogenous probe)

**Step 2**

*In vitro* transport study to obtain inhibitory potency of NCE using CP-I and a probe drug

*In vivo* $K_{OATP1B1}^{(CP-I)}$

*In vitro* $K_{OATP1B1}^{(Drug)}$

Obtain

*In vivo* $K_{OATP1B1}^{(Drug)}$

*In vitro* $K_{OATP1B1}^{(Drug)}$

**Step 3**

PBPK modeling-based simulations

Quantitative prediction of the impact of NCE on the pharmacokinetics of a probe drug (concentration-time profiles, AUC, $C_{max}$)


Flowchart: Thanks to Dr. Wooin Lee
Prediction of the effect of RIF on blood concentration-time profiles of statins
(Correction of in vivo $K_{i,u}$OATP1B1 based on substrate-dependent difference of in vitro $K_{i,u}$)

Predicted and observed AUC ratios and $C_{\text{max}}$ ratios for statins using our PBPK models

With Taking substrate-dependent $K_{i,u,OATP1B3}$ into consideration


Without taking substrate-dependent $K_{i,u,OATP1B3}$ into consideration

AFE: Average fold errors
Summary

The PBPK modeling approach provides an insightful understanding of the mechanisms governing changes in the plasma conc. of an endogenous biomarker (CP-I) for OATP1Bs and MRP2 (CP-I) and enables complex analyses of the dose-dependent inhibitory effects of RIF on the hepatic OATP1Bs/MRP2-mediated transport of CP-I.

It also lead to the successful prediction of RIF interaction with several probe substrates (statins) for OATP1Bs.

Ki value for MRP2 (as a biliary excretion transporter of CP-I) cannot be determined only from the top-down analyses, and the value obtained by PET imaging analyses of different probe (TIC-Me (PGI2 receptor imaging in the brain)) was used to obtain Ki,MRP2 (0.87 uM).
Perspective

Emphasize the importance of the use of multiple biomarkers (coproporphyrine I, bilirubin glucuronide and glycochenodeoxycholic acid sulfate) to assess the OATP1Bs mediated interaction of new NCE as perpetrators in their phase 1 clinical studies.

Some of recent manuscripts indicate the advantage and validity of the use of coproporphyrine I as a biomarker of OATP1Bs function in vivo. However, it is not so easy to confirm that your NCE does not modulate other transporters and enzymes which will be responsible for the biosynthesis, intestinal absorption and biliary excretion and renal clearance of this biomarker. In fact, rifampicin and cyclosporine which are well known to inhibit OATP1Bs mediated hepatic uptake are also known to affect P-gp/BCRP in the intestine, BSEP and MRP2/BCRP in the liver.

Use of multiple biomarkers will ultimately increase the confidence in our prediction of clinical DDI using biomarkers from pharmaceutical and regulatory perspectives.
1) Introduction;
   Rate-determining process (focusing on the liver)
   (Uptake, efflux, elimination, metabolism) DDI

2) PGx of OATP1B1: PBPK model based analysis

3-1) PBPK model based analysis of OATPs mediated drug-drug interaction
   (Top down + Bottom-up)
   (i) victim drugs-perpetrator drugs
   (ii) endogenous biomarker (CP-I) – rifampicin

3-2) Simple bottom-up predictions do not always work well.
   (i) prediction of hepatic clearance of highly protein bound drugs
      (albumin-mediated hepatic uptake mechanisms should be considered)
   (ii) Time-dependent inhibition (inhibitors for OATP1B and OCTs)

4) Target-mediated drug disposition (TMDD); To obtain dose-dependent change in
   molecular target occupancies only from the plasma concentration time-profile
Target-mediated Drug Disposition (TMDD)

✓ Introduced by Dr. Gerhard Levy (1994)
✓ Type of nonlinear PK
✓ When drugs bind to a target with high affinity and to a significant extent (relative to dose), part of the initial dose is rapidly acquired by the target sites and only then the drug will distribute to other tissues.

Well-recognized with biologics (e.g., monoclonal antibodies), but earliest examples were in fact small-molecule drugs (more cases being reported/identified lately)

Gerhard Levy, PharmD
(1928-2017)

Pharmacologic target-mediated drug disposition
Gerhard Levy, PharmD Annbve, N.Y.
(Clin Pharmacol Ther, 1994)

Courtesy from Wooin Lee
Can the prediction of dose-dependent molecular target occupancy be possible from the phase-1 clinical studies?

Just measuring the blood concentration of drugs over a wide range of dose without measuring the tissue concentration such as PET imaging.

This is the methodology presented in a recent NRDD review (1) and in a recent original article on bosentan (2).


(2) Koyama S, Toshimoto K, Lee W, Aoki Y, and Sugiyama Y. Revisiting nonlinear bosentan pharmacokinetics by PBPK modeling: Target binding, albeit not a major contributor to nonlinearity, can offer prediction of target occupancy. Drug Metab Dispos., in press.

Saturation of OATP1B mediated hepatic uptake mostly accounted for the non-linear PK of bosentan. We did not analyze such a discrepancy between observed value and fitted line at the lowest dose (10mg).

Our previous publication

Recent studies on the analyses of non-linear PK of bosentan

Physiologically based pharmacokinetic modeling of bosentan identifies the saturable hepatic uptake as a major contributor to its nonlinear pharmacokinetics.

(Major mechanism for non-linear PK: Hepatic uptake (OATP1B))

A study on pharmacokinetics of bosentan with systems modeling, part 1: translating systemic plasma concentration to liver exposure in healthy subjects.

(Major mechanism for non-linear PK: Multiple mechanism, OATP1B, CYPs, TMDD)

Volz A-K, Krause A, Haefeli WE, Dingemanse J, and Lehr T:
Target-mediated drug disposition pharmacokinetic–pharmacodynamic model of bosentan and endothelin-1.


Volz A-K, Dingemanse J, Krause A, and Lehr T:
Target-mediated population pharmacokinetic modeling of endothelin receptor antagonists.

(Major mechanism for non-linear PK: Target binding followed by the internalization(TMDD))
Volz A. et al., ClinPharmacokinet. 2017

- Population PK/PD Model considering only TMDD.
- There is no evidence that bosentan can be internalized and eliminated after binding to ET receptor. (Endothelin can be internalized and degraded.)
PBPK Model Structure of Bosentan

Koyama S, Toshimoto K, Lee W, Aoki Y, and Sugiyama Y.
Revisiting nonlinear bosentan pharmacokinetics by PBPK modeling:
Target binding, albeit not a major contributor to nonlinearity, can offer prediction of target occupancy
Drug Metab Dispos., 49:298-304 (2021)

- Combined dissolution/absorption, 5 liver, tissue distribution, and target binding.
- Target binding is directly connected to central compartment.

- Considered saturable hepatic uptake and target binding, and non-saturable metabolism.
- Assuming linear and dissolution-rate limited absorption.
Cluster Gauss-Newton method in comparison to conventional methods

Conventional methods:
- Requires **appropriate initial iterate** for the parameters.
- Obtains only a **single set** of optimised solutions.
- Computationally **expensive**.
  - Need extra model evaluations (e.g., for derivative computation)
  - Need to restart with different initial parameters
- Requires a lot of experience!

Cluster Gauss-Newton method:
- Only requires **setting wide ranges** of initial iterates.
- Obtains multiple **sets** of optimised solutions.
- Computationally **cheap and robust**.
  - No need for extra model evaluation.
  - Can estimate many unknown parameters in a complex model
- Requires less experience.

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https://doi.org/10.1007/s11081-020-09571-2
Estimating the receptor occupancy of bosentan with top-down approach

Koyama, Satoshi, et al. “Revisiting nonlinear bosentan pharmacokinetics by PBPK modeling: Target binding, albeit not a major contributor to nonlinearity, can offer prediction of target occupancy.” Drug Metabolism and Disposition (accepted for publication)
Estimating the receptor occupancy of bosentan with top-down approach

**Initial** Parameter Distribution

**Final** Parameter Distribution

**Initial** model fit

**Final** model fit
Now the data at all the doses are well captured by the model including target binding and saturable hepatic uptake.
Calculated Time-Course of Receptor Occupancy

(A) I.V.  
(B) P.O.

Receptor occupancy was calculated 0.6-0.8 when clinical dose of Bosentan (62.5, 125 mg P.O.) was administered.
Estimating the receptor occupancy of bosentan with top-down approach

Parameters found though topdown approach **including low-dose**

Parameters found though topdown approach **not including low-dose**
Workflow for assessment of drug candidates with possible TMDD. PBPK modeling-based prediction with target considerations can be combined with PK data analysis from a microdose analysis in human subjects.

If a small dose (e.g., microdose) PK data is included in Phase 1 clinical dose escalation study, we may be able to detect the TMDD and predict the doses which exhibit the appropriate target occupancy (therapeutic dose).

Burt T, Young G, Lee W, Kusuhara H, Langer O, Rowland M, Sugiyama Y.

To assess how the inclusion of microdosing (small dosing) data improves the prediction accuracy of overall target occupancy based on blood PK profiles.

We may be able to estimate the therapeutic dose during the phase 1 clinical studies once the starting dose is microdose or relevant small dose.
Disclosure for COI

I am a scientific advisory board member of SimCYP.

I have been serving as a chair and a vice-chair of the global consortia of Pharma Industries:
(i) PET-IVIVE (6 companies) as a chair
(ii) Endogenous biomarker-DDI prediction (8 companies)
Acknowledgement