Can the Prediction of Complex Transporter (uptake, efflux)/Enzyme-mediated Drug-drug Interactions be Possible Using a Proposed Physiologically-based Pharmacokinetic (PBPK) Model with in Vitro Ki Values?

Yuichi Sugiyama
Sugiyama Laboratory, RIKEN Innovation Center, RIKEN, Research Cluster for Innovation,

DDI-2017 20th International Conference
on Drug-Drug Interactions

June 19-21, 2017
Husky Union Building, University of Washington; Seattle, WA, USA.
Contents

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

2) PBPK model based analysis of OATPs mediated drug-drug interaction (Top down + Bottom-up)

3) Other collaborations
   Time-dependent inhibition
   Substrate-dependent inhibition

4) Necessity of establishing dynamic PISCS
# 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>
</tr>
<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>
</tr>
<tr>
<td><strong>Anti-cancer drug</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<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>Others</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>maravirooc</td>
<td>OATP1B1</td>
<td>CYP3A4</td>
<td>Pgp</td>
</tr>
<tr>
<td>fexofenadine</td>
<td>OATPs</td>
<td>-</td>
<td>Pgp</td>
</tr>
</tbody>
</table>
Impact of the function of each pathway on the overall intrinsic clearance

\[ CL_{\text{int,all}} = PS_{\text{inf}} \times \frac{CL_{\text{bile}} + CL_{\text{met}}}{PS_{\text{eff}} + CL_{\text{bile}} + CL_{\text{met}}} \]

\[ R_{\text{dif}} = \frac{PS_{\text{inf,dif}}}{PS_{\text{inf,act}}} \]

\[ PS_{\text{inf}} = 100, \; PS_{\text{eff}} = 2, \; CL_{\text{bile}} + CL_{\text{met}} = 500 \text{ (Case-1)} \]

\[ R \text{ value} (1 + I/K_i) \]

% of CL_{\text{int,all}} (cont.)

(1/AUC)
Plasma concentrations of atorvastatin and pravastatin were greatly increased by rifampicin, but not by itraconazole.

<table>
<thead>
<tr>
<th></th>
<th>AUC&lt;sub&gt;0-10&lt;/sub&gt; [pg*hr/ml]</th>
<th>ATV</th>
<th>PRV (AUC&lt;sub&gt;0.8&lt;/sub&gt;)</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*
Inhibition of OATPs + mechanism-based inhibition of CYP2C8 by Gem-glu

Atorvastatin, pravastatin, rosubastatin exhibited relatively higher AUCR, while fluvastatin, repaglinide, and glibenclamide do lower AUCR. The latter compounds are lipophilic and have higher Rdif values.

(Yoshida K et al., Annu Rev Pharmacol Toxicol, 53, 581-612 (2013))
How to obtain the in vivo $\beta$ value and $R_{dif}$ values (=$P_{Sinf,dif}/P_{Sinf,act}$) from these clinical studies?

1) $R_{dif}$ values; Effect of single dose of rifampicin;
   Atrovastatin $AUC_R=12$, $R_{dif}$ is known as 0.024 (in vitro; determined in my lab). Then, from $AUC_R$ for other substrates together with the estimates of $fo_{atp}$ (Rifampicin sensitive OATPs) value, in vivo $R_{dif}$ value of OATPs mediated uptake is estimated

   - atorvastatin $AUC_R=12.0$  $R_{dif} = 0.024$
   - simeprevir  $AUC_R = 7.3$  $R_{dif} = 0.07-0.10$
   - bosentan  $AUC_R=3.2$  $R_{dif} = 0.3-0.4$
   - repaglinide  $AUC_R =2.0$  $R_{dif} = 0.7-1.1$

2) $\beta$ values; Effect of iv itraconazole(iv);
   Midazolam  $AUC_R=4.0$, $fm(3A)$ is known as 0.9 (in vitro; determined in my lab) Then, from $AUC_R$ for other substrates together with their $fm$ values, in vivo $\beta$ value of dual substrates of OATP and CYP3A4 is estimated (shown in the next slide)
(Summary) \[ \beta = \frac{CL_{bile} + CL_{met}}{PS_{eff} + CL_{bile} + CL_{met}} \]

# ATV, Bosentan: Close to Case-1 (\( \beta \) high 0.8~1)
# CER, REPG, SIMP: (\( \beta \) intermediate 0.3-0.7)
# DAR (\( \beta \) low < 0.2)

From the clinical DDI studies so far done and also from the I/Ki value of ITZ (CYP3A4 inhibitor), we can estimate the \( \beta \) values of each compound. With high \( \beta \) value of substrate, the inhibition of metabolism and biliary excretion does not affect the blood AUC value of unchanged drug (uptake-limited case)
In vitro–in vivo extrapolation methods for $\beta$ and $R_{\text{dif}}$ values will be established, which is useful for the quantitative prediction of complex DDIs involving influx/efflux transporters and metabolic enzymes.

$\beta$ value; sandwich cultured hepatocytes and HLM

$R_{\text{dif}}$; Initial uptake experiments at a low substrate concentration (<0.5 uM) and at a high concentration (>100 uM) or in the presence of an inhibitor, rifampicin or rifampicin SV (>30 uM).

If we use inhibitors, the in vitro cassette dosing of substrates become possible.
Contents

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

2) PBPK model based analysis of OATPs mediated drug-drug interaction (Top down + Bottom-up)

3) Other collaborations
   Time-dependent inhibition
   Substrate-dependent inhibition

4) Necessity of establishing dynamic PISCS
Quantitative analyses of hepatic OATP-mediated interactions between statins and inhibitors using PBPK modeling with a parameter-optimization method.


Elevation of plasma pitavastatin (PIT) concentration by cyclosporine A (CsA)

Prediction by dynamic model (PBPK model)
Oral administration of CsA (average 131 mg) 1 hr before PIT

Oral administration of PIT (2 mg)

Plasma concentration of PIT (ng/mL)

- PIT + CsA
- PIT (control)

Plasma concentration of PIT (ng/mL)

Time (hr)

AUC × 4.6
Cmax × 6.5

Pitavastatin

OATP1B1 substrate (Hirano et al., 311:139-146(2004))
PBPK model for DDI analyses (PIT, CsA)

Substrate

Inhibitor

PIT: Five liver compartments (for better IVIVE of high clearance drugs) (Watanabe et al., JPET 2009)

PIT: (Kp values; in silico method) Muscle, skin and adipose as rapid equilibrium compartments
Optimization of parameters in PBPK models by fitting ($f_{B\text{CL}_{\text{int},\text{all}}}$, $K_i$, $k_a$, $T_{lag}$)

Initial parameters for PBPK models
- Compartment model ($k_a$, $T_{lag}$)
- Clearance concept ($f_{B\text{CL}_{\text{int},\text{all}}}$)
- in vitro experiments ($R_{\text{diff}}(P_{S\text{diff,inf}}/P_{S\text{act}})$, $f_H$)
- in silico calculation ($K_p$, $\gamma=PS_{\text{diff,inf}}/PS_{\text{diff,eff}}$)
- References (other physiological and pharmacokinetic parameters)

Optimization of parameters including $K_i$ by fitting to both control and DDI condition

Clinical data of DDI

Scheme of the workflow of parameter optimization in the PBPK models to describe hepatic OATP-mediated DDIs.

Step 1
Step 2
Step 3
Step 4
Extended clearance concept

\[ CL_{\text{int,all}} = P_{\text{inf}} \times \frac{CL_{\text{bile}} + CL_{\text{met}}}{PS_{\text{eff}} + CL_{\text{bile}} + CL_{\text{met}}} \]

\( \beta \) value

Where \( P_{\text{inf}} = P_{\text{inf,act}} + P_{\text{inf,dif}} \)

\( P_{\text{eff}} \) assumed to be \( P_{\text{eff,dif}} \)

\[ R_{\text{dif}} = \frac{P_{\text{inf,dif}}}{P_{\text{inf,act}}} \]

\[ K_{p,u} = \frac{P_{\text{inf,act}} + P_{\text{inf,dif}}}{P_{\text{eff,dif}}} \]

\[ \gamma = \frac{P_{\text{inf,dif}}}{P_{\text{eff,dif}}} \]

\[ fbile = \frac{CL_{\text{bile}}}{(CL_{\text{met}} + CL_{\text{bile}})} \]

Parameters for elementary steps

\( P_{\text{inf,act}}, P_{\text{inf,dif}}, P_{\text{eff,dif}}, CL_{\text{bile}}, CL_{\text{met}} \)

Hybrid parameters for describing hepatobiliary elimination steps

\( CL_{\text{int,all}}, \beta, K_{p,u}, R_{\text{dif}}, fbile \)
How to determine $K_{p, uu(true)}$ from in vitro experiment?

$C/M$ ratio(on ice) = $\frac{C_{cell}}{C_{medium}}_{on ice} = \frac{PS_{dif,inf}}{f_T \cdot PS_{dif,eff}}$

Assumption: membrane potential is completely lost under on ice condition (recently demonstrated experimentally)

$PS_{dif,inf} = PS_{dif,eff}$ ($\gamma_{on ice} = \frac{PS_{dif,inf}}{PS_{dif,eff}} = 1$)

$\frac{C/M$ ratio(37°C)}{C/M$ ratio(on ice)} = \frac{PS_{act,inf} + PS_{dif,inf}}{f_T \cdot (PS_{dif,eff} + CL_{int,met})} \cdot f_T = K_{p, uu(true)}$

Comparison of methods for estimating unbound intracellular-to-medium concentration ratios in rat and human hepatocytes using statins
Yoshikado et al.,
Simulated time course of plasma conc. of cyclosporine A and pitavastatin/fluuvastatin

**Cyclosporine A**

\[ \beta = 0.8 \]

**Pitavastatin (EHC model)**

\[ \beta = 0.2 \]

**Fluvastatin (EHC model)**
Impact of the consideration of enterohepatic circulation on the simulated time course of plasma conc. of pitavastatin/fluavastatin

(EHC model gave much better fit to the data)

**EHC**

**Pitavastatin (EHC model)**

**Fluvastatin (EHC model)**

**Non-EHC**

**Plasma**
- control
- +CsA

**Liver**
- control
- +CsA
PBPK models were constructed for pitavastatin, fluvastatin and pravastatin as substrates and cyclosporin A (CsA) and rifampicin (RIF) as inhibitors, where enterohepatic circulations (EHC) of statins were incorporated. Without EHC, good fitting was not obtained for either substrate. (In vitro measured Rdif values, γ values were well incorporated into this modeling)

Similar in vivo inhibition constant (K_i) values of each inhibitor against OATPs were obtained, regardless of the substrates.

CsA: 0.012 uM (pitavastatin) 0.010 uM (fluvastatin)
Rifampicin; 0.23 uM (pitavastatin) 0.19 uM (pravastatin)

Estimated K_i values of CsA were comparable to reported in vitro values with the preincubation of CsA, while those of RIF were 3-5 folds smaller than reported in vitro values.

CsA(+preincubate) ; 0.014–0.080 uM, Trans-inhibition mechanism
Rifampicin; 0.65–1.1 uM,
( Mechanism of lower Ki value in vivo of Rif. compared with in vitro are not known yet.)
Conclusion

* Standardized protocol of top-down analyses of complex DDI (where transporters and enzymes are inhibited) based on PBPK modeling were established. The in vivo Ki values were obtained, leading to the prediction of complex DDI of other substrates.

* We need some in vitro measured parameters such as Rdif value, Kpuu value, γ values (index of the asymmetry of passive diffusion via basolateral membrane) in this model based analyses.

* An important parameter, β which determines rate-determining process of drugs is set to different values (0.2, 0.5, 0.8) in the model analyses, and the outcome of analyses are not so much different as far as the plasma-concentration time profiles are analyzed.

* However, this β values should affect the hepatic concentration time profiles. Therefore, this should be estimated in near future from the in vitro experiments (isolated and sandwich cultured hepatocytes, HLMs)
Contents

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

2) PBPK model based analysis of OATPs mediated drug-drug interaction (Top down + Bottom-up)

3) Other collaborations
   Time-dependent inhibition
   Substrate-dependent inhibition

4) Necessity of establishing dynamic PISCS
Further studies recently done on a collaboration base on the OATP-mediated DDIs

A) Time-dependent inhibition of OATPs by cyclosporine A

Shitara Y et al., (Sanofi) Pharmacol Therap., in press

B) Substrate-dependent inhibition. Collaboration with Eisai


Proposal of a model explaining time dependent inhibition of OATP1B1 by cyclospororin A

Ki,in << Ki,out

Shitara Y. and Sugiyama Y; Pharmacololol & Therapeutics, in press
Contents

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

2) PBPK model based analysis of OATPs mediated drug-drug interaction (Top down + Bottom-up)

3) Other collaborations
   Time-dependent inhibition
   Substrate-dependent inhibition

4) Necessity of establishing dynamic PISCS

   PISCS (Pharmacokinetic Interaction Significance Classification System)
In future,
For substrates/inhibitors of transporters, enzymes,

1) Substrate file of PBPK parameters
   (drug dependent parameters)

2) Inhibitor file of PBPK parameters
   (+ Ki value, parameters for time-dependent inhibition)

Then you can do any kind of prediction by combining
1) and 2)
(as far as substrate dependent inhibition does not take place)

This is the so-called “Dynamic (PBPK based) PISCS”
CYP3A Dependent Pharmacokinetic DDI in Literature

(113 cases reported in 78 articles in 1986-2006)

Increase in Contribution Ratio (CR) of Substrates

Increase in Inhibition Ratio (IR) of Inhibitors

CYP3A4 inhibitors

In many DDI studies, AUC increase was calculated from *in vitro* parameters which correspond to CR and IR. However, if *in vivo* information of AUC changes is rich enough, CR and IR are calculable from AUC changes without *in vitro* information by using the above equation.

By avoiding to use *in vitro* information, the prediction would become free from various errors associated with *in vitro-in vivo* extrapolation.

Contribution Ratio of CYP3A and Inhibition Ratio of CYP3A from 53 Drug Interaction Studies

CONTRIBUTION RATIO OF CYP3A TO ORAL CLEARANCE ($CR_{3A}$)

<table>
<thead>
<tr>
<th>SUBSTRATES</th>
<th>$CR_{3A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>0.2</td>
</tr>
<tr>
<td>Lovastatin</td>
<td>0.4</td>
</tr>
<tr>
<td>Buspirone</td>
<td>0.6</td>
</tr>
<tr>
<td>Nisoldipine</td>
<td>0.8</td>
</tr>
<tr>
<td>Triazolam</td>
<td>1.0</td>
</tr>
<tr>
<td>Midazolam</td>
<td></td>
</tr>
<tr>
<td>Felodipine</td>
<td></td>
</tr>
<tr>
<td>Cyclosporin</td>
<td></td>
</tr>
<tr>
<td>Nifedipine</td>
<td></td>
</tr>
<tr>
<td>Alprazolam</td>
<td></td>
</tr>
<tr>
<td>Atorvastatin</td>
<td></td>
</tr>
<tr>
<td>Telithromycin</td>
<td></td>
</tr>
<tr>
<td>Zolpidem</td>
<td></td>
</tr>
<tr>
<td>Cerivastatin</td>
<td></td>
</tr>
</tbody>
</table>

INHIBITION RATIO OF CYP3A ($IR_{3A}$)

<table>
<thead>
<tr>
<th>INHIBITORS</th>
<th>$IR_{3A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole (200-400mg)</td>
<td>0.2</td>
</tr>
<tr>
<td>Voriconazole (400mg)</td>
<td>0.4</td>
</tr>
<tr>
<td>Itraconazole (100-200mg)</td>
<td>0.6</td>
</tr>
<tr>
<td>Telithromycin (800mg)</td>
<td>0.8</td>
</tr>
<tr>
<td>Clarithromycin (500-1000mg)</td>
<td>1.0</td>
</tr>
<tr>
<td>Saquinavir (3600mg)</td>
<td></td>
</tr>
<tr>
<td>Nefazodone (400mg)</td>
<td></td>
</tr>
<tr>
<td>Erythromycin (1000-2000mg)</td>
<td></td>
</tr>
<tr>
<td>Diltiazem (90-270mg)</td>
<td></td>
</tr>
<tr>
<td>Fluconazole (200mg)</td>
<td></td>
</tr>
<tr>
<td>Verapamil (240mg-480mg)</td>
<td></td>
</tr>
<tr>
<td>Cimetidine (800-1200mg)</td>
<td></td>
</tr>
<tr>
<td>Ranitidine (300-600mg)</td>
<td></td>
</tr>
<tr>
<td>Roxithromycin (300mg)</td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine (100mg-200mg)</td>
<td></td>
</tr>
<tr>
<td>Azithromycin (250-500mg)</td>
<td></td>
</tr>
<tr>
<td>Gatifloxacin (400mg)</td>
<td></td>
</tr>
<tr>
<td>Fluoxetine (20-60mg)</td>
<td></td>
</tr>
</tbody>
</table>

The data collected from 398 studies involving 77 substrates and 64 inhibitors. Potential number of DDIs composed of these drugs are approximately 5,000 in total.
Complex rifampicin DDI effects on glibenclamide pharmacokinetics

Clinical protocol

Day 1 15 22 23 24 25 26 27 28 30
1st 2nd 3rd 4th

Glibenclamide oral dose: 1.25 mg
Rifampicin dose: 600mg

- + Rifampicin single infusion (AUC: 2.25 fold)
- control (Glibenclamide alone)
- + Rifampicin repeated oral dose + single infusion (AUC: 0.78 fold)
- + Rifampicin repeated oral dose (AUC: 0.37 fold)

Clinical Pharmacology & Therapeutics, 85(1) 2009, 78-85
Zheng HX, Huang Y, Frassetto LA, And Benet LZ.

Collaboration with Asaumi (Ono Pharm)

So-called “Dynamic (PBPK based) PISCS
COI

I am a scientific advisory board member of the following companies.

1) SimCYP
2) SEKISUI Medical

I am and have been a consultant of and collaborating with 30 domestic and global pharmaceutical industries
Sugiyama Lab  Main Members

Takashi Yoshikado (wet + dry, IVIVE DDI)
Kota Toshimoto (dry, VCT (Virtual clinical study))
Kim, Soo-Jin (DDI, PGx, IVIVE)
Lee, Kyeong Ryoong (Daewoong) (DDI, IVIVE)
Kim, Sae Hee (Daewoong) (DDI, IVIVE)
Atsuko Tomaru (IVIVE, Bioanalysis with LC/MS/MS)
Aya Kiriake (IVIVE, transporter expression systems)
Kiyoe Morita (IVIVE, isolated hepatocyte (suspension, plated))
In-Soo Yoon (DDI, isolated hepatocytes (suspension, plated))

Secretaries
Sachie Sato
Chinami Yamada
Rie Abe
Aya Miyamoto

RIKEN  Kazuya Maeda, Hiroyuki Kusuhara (Univ of Tokyo)

Azusa Futatsugi (DDI, PGx)
Keiko Ishigame (IVIVE, Bioanalysis)
Hisako Motokawa (IVIVE, Bioanalysis)