Is prediction of tissue exposure from in vitro data using PBPK modeling possible? Confirmation by PET imaging to study the clinical disposition of membrane transporter substrates

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Hosted by the International Transporter Consortium (ITC)

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Morning Glow seen from Omni Shopreham Hotel

Contents

1) Quantitative assessment of OATPs-mediated DDI and PGx using PBPK model.

2) In vitro measurement of Kpuu value

3) Use of PET imaging

The impact of OATP1B1 on the PK of drugs

～Plasma conc. of drugs is increased in subjects with OATP1B1*15～（521C/C vs T/T）

HMG-CoA reductase inhibitors

pravastatin
simvastatin acid (3A4)
pitavastatin
atorvastatin (3A4)
rosuvastatin

Anti-diabetes

repaglinide (2C8, 3A4)
nateglinide (2C9, 3A4)
Glibenclamide(2C9, 3A4)

Anti-HCV

simeprevir
asunaprevir
paritaprevir　etc.

Anti-pulmonary hypertension drug

Atrasentan (3A4)
Bosentan (3A4)

Anti-cancer drug

irinotecan（SN-38）

Chol-absorption inhibitor

Ezetimibe (glucuronidation)

Loop diuretics

Torasemide (2C9)

Angiotensin receptor antagonists

Olmesartan、Valsartan

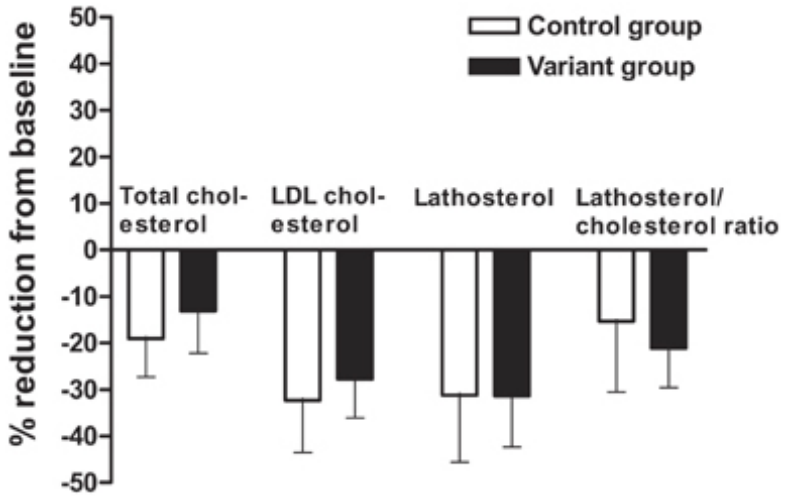
Relationship between OATP1B1 genetic polymorphism and Pharmacological effect and adverse effect of statins

★Pharmacological effect

Target; HMG CoA-reductase in the liver

No effect or small effect if any

(Igel M et al.,Clin Pharmacol Ther, 79, 419-26 (2006))



★Adverse Effect

Target; Muscle (via plasma)

Simvastatin-induced myopathy

strong correlation with OATP1B1 polymorphism

(SEARCH Collaborative Group et al., New Engl J Med, 359, 789-99 (2008))

Odds ratio of this SNPs for simvastatin-induced myopathy GWAS study

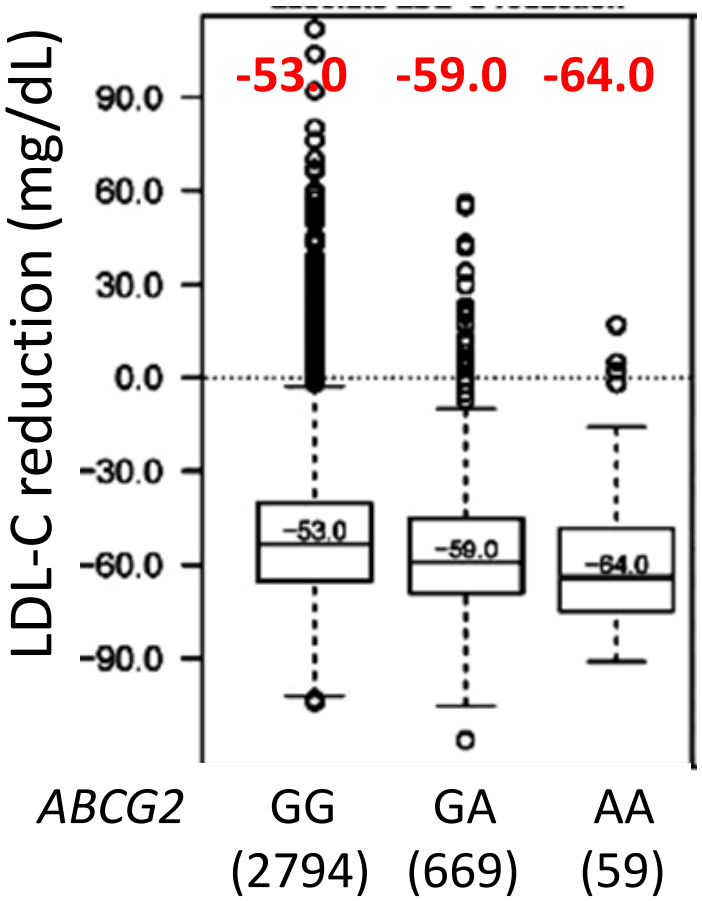
521C/T vs T/T → 4.5 fold

521C/C vs T/T →16.9 fold

Genome wide association study of LDL-cholesterol response to rosuvastatin in JUPITER Trial

Rosuvastatin 20mg/day (n=3523) or placebo (n=3466): Circ Cardiovasc Genet 2012;5:257-264

***ABCG2* (BCRP) is associated with LDL-C reduction** in SNP highly correlated with nonsynonymous 421C>A mutation (Linkage disequilibrium: r^2=0.81) . Two additional reports supported the association of 421C>A mutation with efficacy.



421C>A mutation reduce excretion into intestine and secretion into bile => Increase plasma exposure and hepatic exposure

***SLCO1B1* is (OATP1B1) not associated with LDL-C reduction** in 521T>C mutation, which results in larger plasma exposure by the lower hepatic uptake activity.

Both mutations result in larger plasma exposure of rosuvastatin, while only mutation in *ABCG2* has association with efficacy.

Summary-1

 # 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).

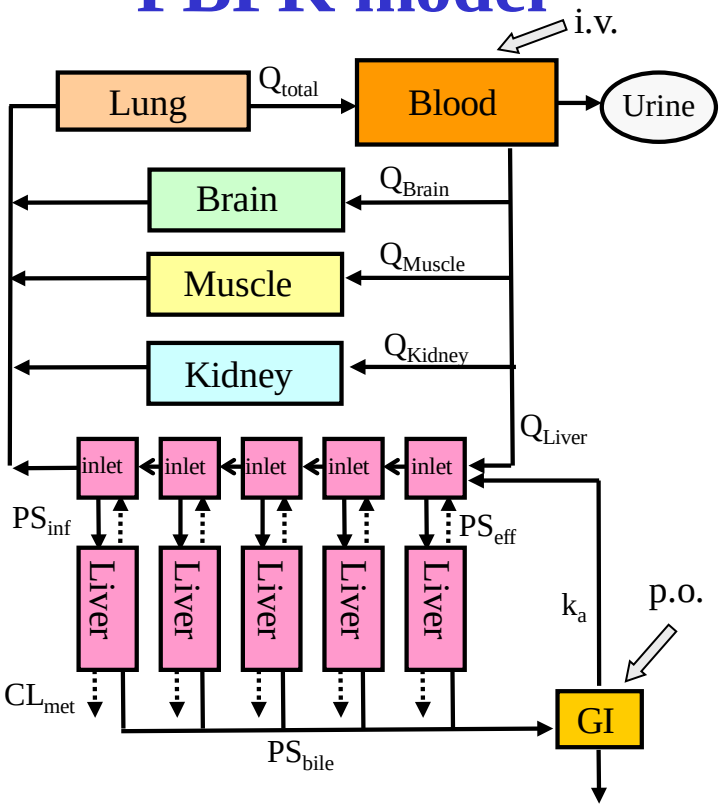
Why did this polymorphism and/or DDI affect **only side-effect (myopathy; muscle is a target tissue),** and **not pharmacological effect (lipid lowering effect; liver is a target organ) ?**

To answer this question, it is important to establish PBPK modeling. (statins, HCV drugs) ↓

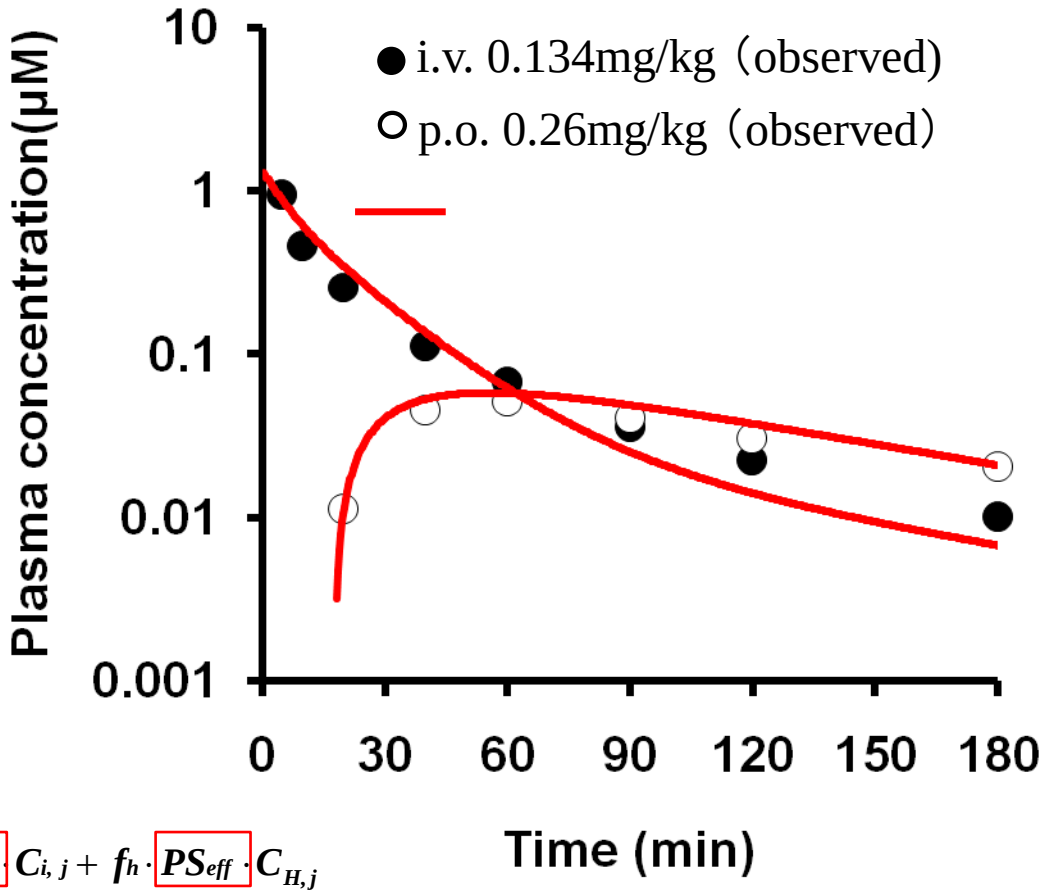
Watanabe T, Kusuhara H, Maeda K, Shitara Y and Sugiyama Y. Physiologically based pharmacokinetic modeling to predict transporter-mediated clearance and distribution of pravastatin in humans. **J Pharmacol Exp Ther** 328:652-662 (2009)

Yoshikado T, Yoshida K, Kotani N, Nakada T, Asaumi R, Toshimoto K, Maeda K, Kusuhara H, Sugiyama Y. Quantitative analyses of hepatic OATP-mediated interactions between statins and inhibitors using PBPK modeling with a parameter-optimization method. **Clin Pharmacol Ther.** 100:513-523(2016)

PBPK model



Plasma conc-time profile of pravastatin in human



Blood $$V_b \cdot \frac{dC_b}{dt} = Q_h(C_i - C_b) - CL_r \cdot C_b$$

Capillary in the liver $$V_{i,j} \cdot \frac{dC_{i,j}}{dt} = Q_h(C_{b,j} - C_{i,j}) - f_b \cdot \boxed{PS_{inf}} \cdot C_{i,j} + f_h \cdot \boxed{PS_{eff}} \cdot C_{H,j}$$

Hepatocytes $$V_{H,j} \cdot \frac{dC_{H,j}}{dt} = f_b \cdot \boxed{PS_{inf}} \cdot C_{i,j} - f_h \cdot (\boxed{PS_{eff}} + \boxed{CL_{met}} + \boxed{PS_{bile}}) \cdot C_{H,j}$$

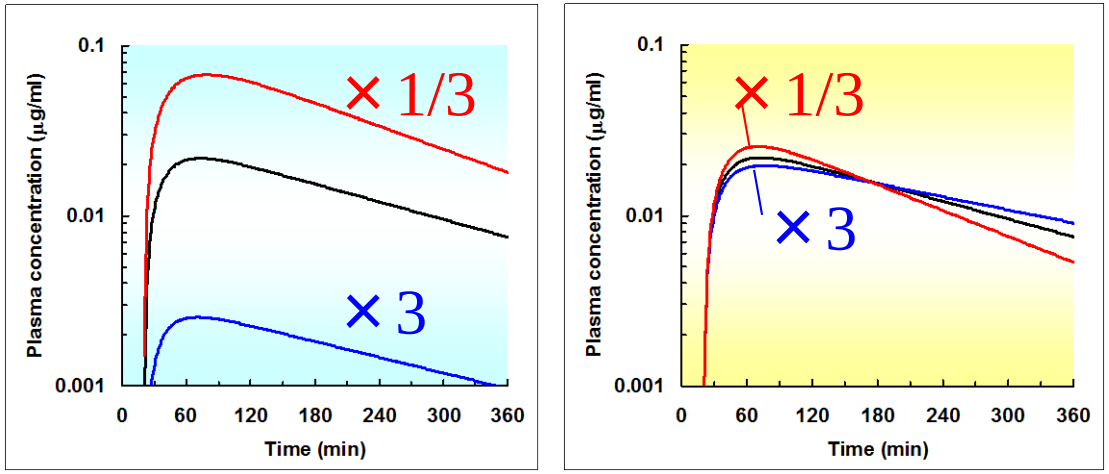
□ Extrapolated parameters based on in vitro(animal, human) and in vivo (animal)

PBPK modeling of pravastatin disappearance

PSinf(OATP1B1)

PSbile (MRP2/BCRP)

Plasma



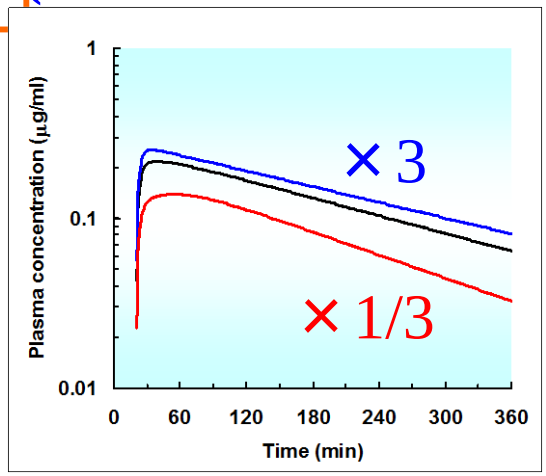
Uptake transporter (OATP1B1, PS_{inf})
(Adverse effect)

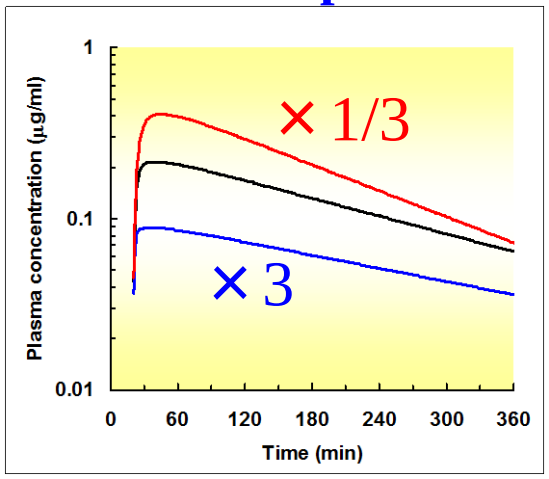
Big effect on plasma and muscle exposure

Small (if any) effect on Liver exposre

Liver

(We need PET studies to confirm this prediction)





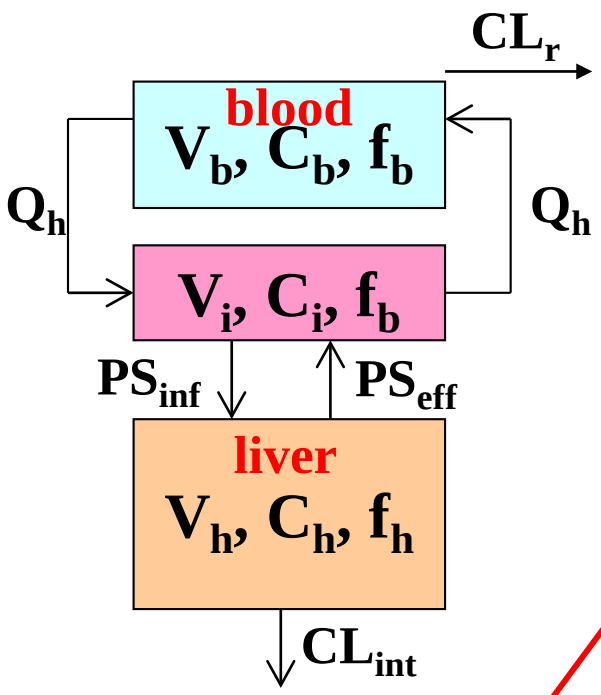
Efflux (biliary) transporter (MRP2, BCRP, OATP1B1, PS_{eff})
(Pharamcological effect)

Small (if any) effect on plasma and muscle exposure

Big effect on Liver exposre

Effect of the transporters(influx, efflux) in the liver on the time-course and exposure of drugs in the plasma and target tissue.

Extended Clearance Concept; Effect of functional alteration of metabolism/efflux process on the plasma and liver concentrations



Liver exposure

$$f_h \cdot AUC_h = \frac{Dose}{CL_{int}}$$

Blood AUC

$$f_b \cdot AUC_b = \frac{Dose}{PS_{inf} \cdot \frac{CL_{int}}{PS_{eff} + CL_{int}}}$$

When $PS_{eff} << CL_{int}$, (uptake limited)

$$f_b \cdot AUC_b = \frac{Dose}{PS_{inf}}$$

When $PS_{eff} >> CL_{int}$ (intrinsic clearance-limited)

$$f_b \cdot AUC_b = \frac{Dose}{PS_{inf} \cdot \frac{CL_{int}}{PS_{eff}}}$$

In either case, the change in PSinf(uptake clearance) does not influence the liver exposure (fh ·AUCh), but influence the blood exposure

“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

Summary-2 :

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 (pharamcological 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 much more sensitively than that of the uptake

⇒This prediction is consistent with a most recent study (Jupiter trial for rosuvastatin) published by other group.

Summary-3:

DDIs where the efflux transporters are inhibited often do not change the plasma exposure (AUC) of victim compounds, though the intracellular exposures should be increased very much. This is mainly because uptake becomes the rate determining process of the blood clearance of drugs. Under such a condition, the change in the abilities of uptake transporters easily change the plasma(and muscle) AUC of drugs, but the change in the abilities of efflux does not change the plasma AUC but changes the liver AUC.

Examples:

Statins; change (↓) in hepatic efflux tranporters (MRP2, BCRP) will enhance the exposure in the liver(↑), but not the exposure in the plasma

Metformin: change (↓) in hepatic efflux tranporters (MATE1) will enhance the exposure in the liver(↑), but not the exposure in the plasma (evidence in mice, and we need PET analysis for human)

Ito S et. al J Pharmacol Exp Ther 340:393-403 (2012).

Ito S, et.al J Pharmacol Exp Ther 333:341-350 (2010).

⇒ **We need PET analysis in human**

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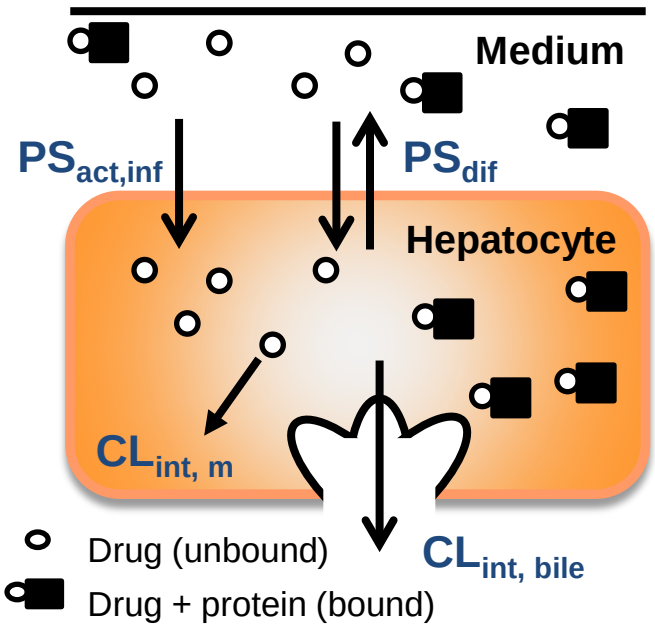
Unbound Concentration Ratio (Liver/Blood), $K_{p,uu}$

Liver/blood unbound concentration ratio（$K_{p,uu}$）is expressed by using not only membrane transport process ($PS_{inf,act}$ and PS_{dif}) but intrinsic clearance (metabolism and bile excretion) as follows：



$$K_{p,uu} = \frac{PS_{act,inf} + PS_{dif,inf}}{CL_{int} + PSdi_{f,\,eff}}$$

$$CL_{int} = CL_{int,m} + CL_{int,bile}$$

Question: **How do we evaluate $K_{p,uu}$?**

→ **Chu X *et al.* (on behalf of ITC consortium), CPT (2013) 94:126-41.**

Published Method for $K_{p,uu}$ (Yabe's Method with Drs. Galetin A and Hosuton B)

Yabe Y et al., DMD (2011) 39: 1808-14.

Step1: Calculate **initial uptake rate** of substrate drugs

Step2: **V_{max}, K_m and PS_{dif}** were **estimated by non-linear regression** from following equation:

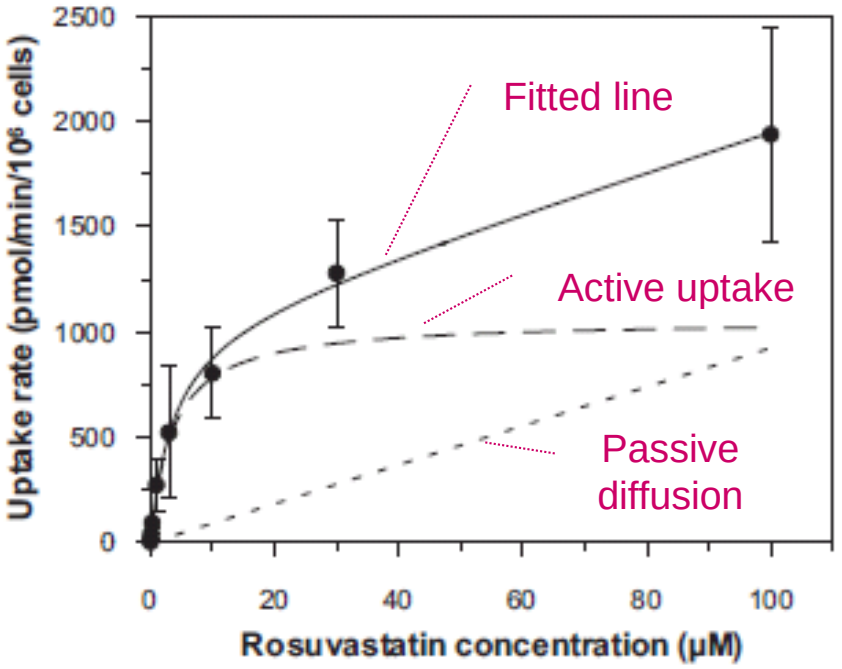
$$v = \frac{V_{\max} \times S}{Km + S} + PS_{dif} \times S$$

Step3: $K_{p,uu,V0}$ and $f_{T,V0}$ values were calculated using the kinetic parameters as follow:

$$K_{p,uu,V0} = \frac{V_{\max} / K_m + PS_{dif}}{PS_{dif}}$$

$$f_{T,V0} = \frac{K_{p,uu,V0}}{K_{p,total}} = \frac{K_{p,uu,V0}}{C_{cell} / C_{medium}(37°C)}$$

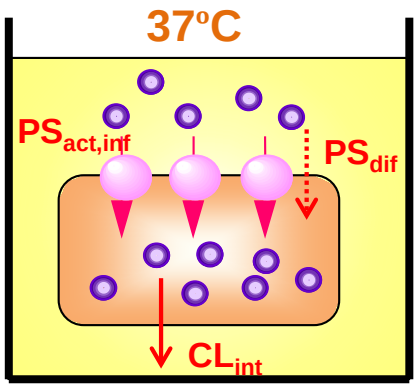
f_T value sometimes exceeds 1. ⇒ probably because of unstable method



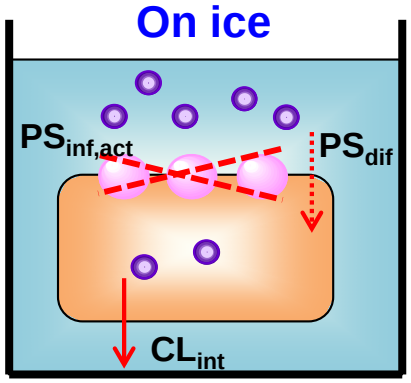
The initial uptake rate has to be determined in this method, and therefore the values are sensitive to the experimental condition and technique. ⇒ need more stable method

Our Method for Determining $K_{p,uu,ss}$

Our Method: Steady-state uptake under 37ºC and On ice



$$C/M\ \ ratio(37^{\circ}C) = \left[\frac{C_{cell}}{C_{medium}}\right]_{37^{\circ}C}$$



$$C/M\ \ ratio(4^{\circ}C) = \left[\frac{C_{cell}}{C_{medium}}\right]_{4^{\circ}C}$$

Step1: Uptake study using hepatocytes was conducted to determine **steady-state uptake** (37ºC/ on ice).

Step2: $K_{p,uu,ss}$ and $f_{T,cell}$ values were calculated using C_{cell}* and C_{medium} as follows:

$$K_{p,uu,ss} = \frac{\left[C_{cell}/C_{medium}\right]_{37^{\circ}C}}{\left[C_{cell}/C_{medium}\right]_{4^{\circ}C}} \qquad f_{T,cell} = \frac{1}{\left[C_{cell}/C_{medium}\right]_{4^{\circ}C}}$$

*, C_{cell} was calculated 3.68 µL (rat) and 2.28 µL (human) as intracellular volume (Nakada *et al.*, *in preparation*).

Theoretical Equation of $K_{p,uu,true}$

- Permeability of Ionized Drugs (Anion)
  - I. The Ratio of Permeabilities of Ionized and Unionized Drugs: λ

$$\lambda = \frac{PS_{dif,inf,ion}}{PS_{dif,inf,union}}$$

  - II. Nernst Equation (Anionic drugs)

$$PS_{dif,eff,ion} = \phi \cdot PS_{dif,inf,ion} = \phi \cdot \lambda \cdot PS_{dif,inf,union}$$

$$\phi = exp\left(\frac{z \cdot F \cdot \Delta\psi}{R \cdot T}\right)$$

R: the gas constant,
T: absolute temperature [K]
z: the valency of the ion,
F: the Faraday constant,
Δψ: the membrane potential of cells [V]

-30-40 mV (at 37C)
close to zero (on the ice)

- Assumption:
  - $PS_{act,eff}$ and CL_{int} are negligible
  - $PS_{dif,inf,union}$ equals to $PS_{dif,eff,union}$

$$K_{p,uu,true} = \frac{C_{cell,unbound}}{C_{medium}} = \frac{\boldsymbol{f_{o,ion} \cdot PS_{act,inf} + (\lambda \cdot f_{o,ion} + f_{o,union}) \cdot PS_{dif,inf,union}}}{\boldsymbol{(\Phi \cdot \lambda \cdot f_{i,ion} + f_{i,union}) \cdot PS_{dif,inf,union}}}$$

Theoretical Consideration of the Difference between $K_{p,uu,ss}$ and $K_{p,uu,V0}$

Theoretical equation of $K_{p,uu}$ ratio:

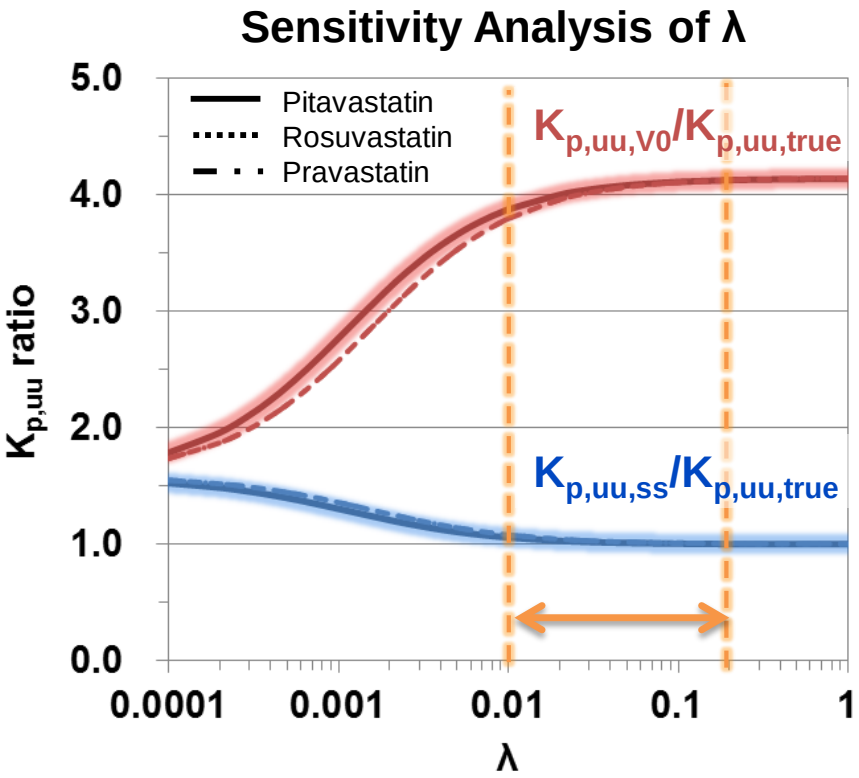
1. **Our method**

$$K_{p,uu,ss} \Big/ K_{p,uu,true} = \frac{\lambda \cdot f_{i,ion} + f_{i,union}}{\lambda \cdot f_{o,ion} + f_{o,uion}}$$

2. **Yabe's method**

$$K_{p,uu,V0} \Big/ K_{p,uu,true} = \frac{\Phi \cdot \lambda \cdot f_{i,ion} + f_{i,union}}{\lambda \cdot f_{o,ion} + f_{o,union}}$$

Δψ = -38 mV (hepatocytes),
Φ ≈ 4.2



When λ is within the range of 0.01–0.2
(experimental result with change in medium pH)),

- ✓ **$K_{p,uu,ss}$ is close to $K_{p,uu,true}$.**
- ✓ **$K_{p,uu,V0}$ is 4-times higher than $K_{p,uu,true}$.**

Comparison of methods for estimating unbound intracellular-to-medium concentration ratios in rat and human hepatocytes using statins

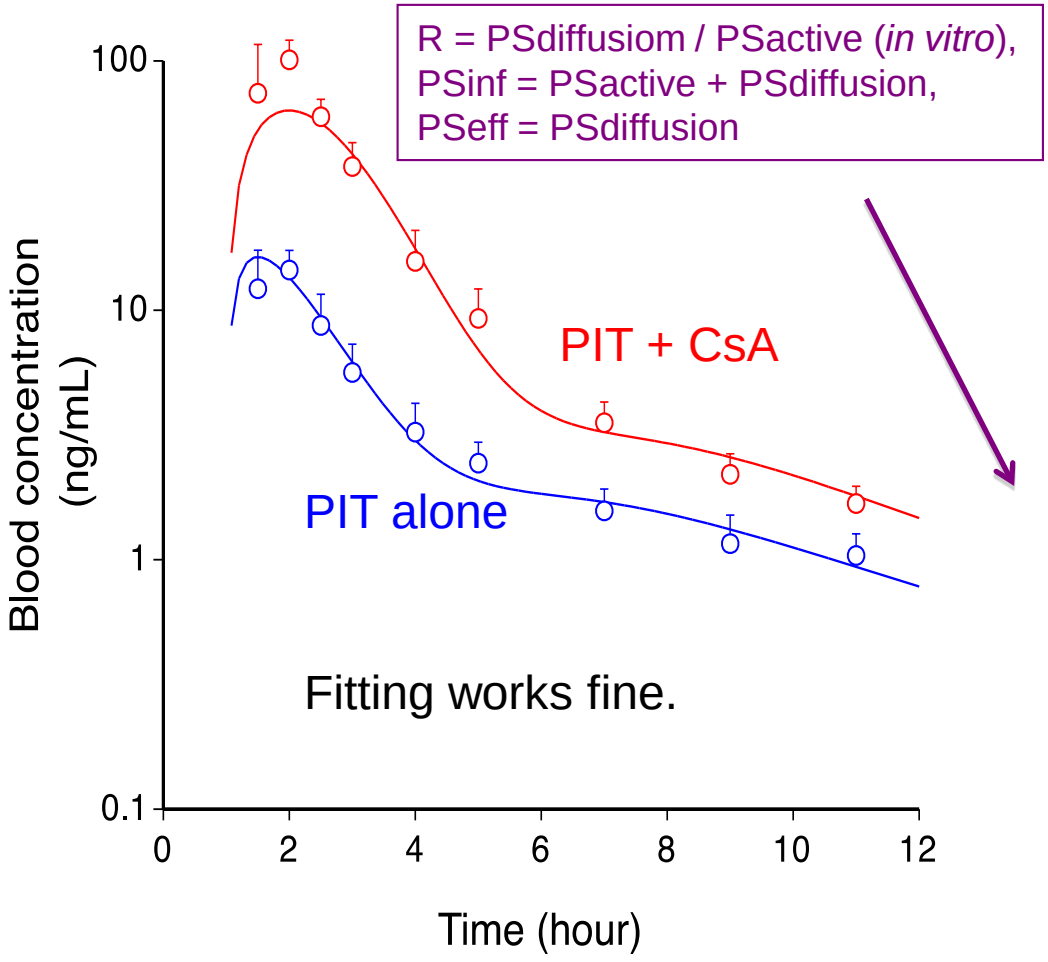
Takashi Yoshikado, Kota Toshimoto, Tomohisa Nakada, Kazuaki Ikejiri, Hiroyuki Kusuhara, Kazuya Maeda, and Yuichi Sugiyama

DMD under revision

$K_{p,uu,ss}$ values of anions are similar to $K_{p,uu,true}$ when the inside-negative membrane potential (-40 mV) at 37C and zero mV on the ice is considered. This suggests that $K_{p,uu,ss}$ method is stable method for estimating the concentration of unbound drugs inside the hepatocytes.

- While the $K_{p,uu,ss}$ for diazepam was ca. 1 (1.2 and 0.41), the values were 13 and 6.9 for pitavastatin, 12 and 6.4 for rosuvastatin, and 2.0 and 1.3 for pravastatin in cells from Lot Hu8075(pooled) and Lot TFF, respectively.
- The obtained $f_{T,cell,ss}$ values were ca. 0.028 and 0.046 for pitavastatin, 0.22 and 0.23 for rosuvastatin, and 0.55 and 0.48 for pravastatin in cells from Lot Hu8075 and Lot TFF, respectively.

(With EHC) Analysis of DDI between PIT vs. CsA considering the inhibition of hepatic uptake process (Ki1)



Parameter	Unit	Fitted
Vb	L	4.92
ka	hr^{-1}	1.02
Lag	hr	0
fbPSinf	L/hr	52.5
R	-	0.312
fhCLint	L/hr	7883
Fbile	-	0.338
Ktransit	hr^{-1}	0.707
Ki1 (total)	μM	0.204
Ki1 (unbound)	μM	**0.0122**

Kpuu = 10 (in vitro)

Reported *in vitro* Ki1 value (unbound): 0.24～0.69 μM.
If preincubated with CsA, *in vitro* Ki1 value: 0.014 μM.

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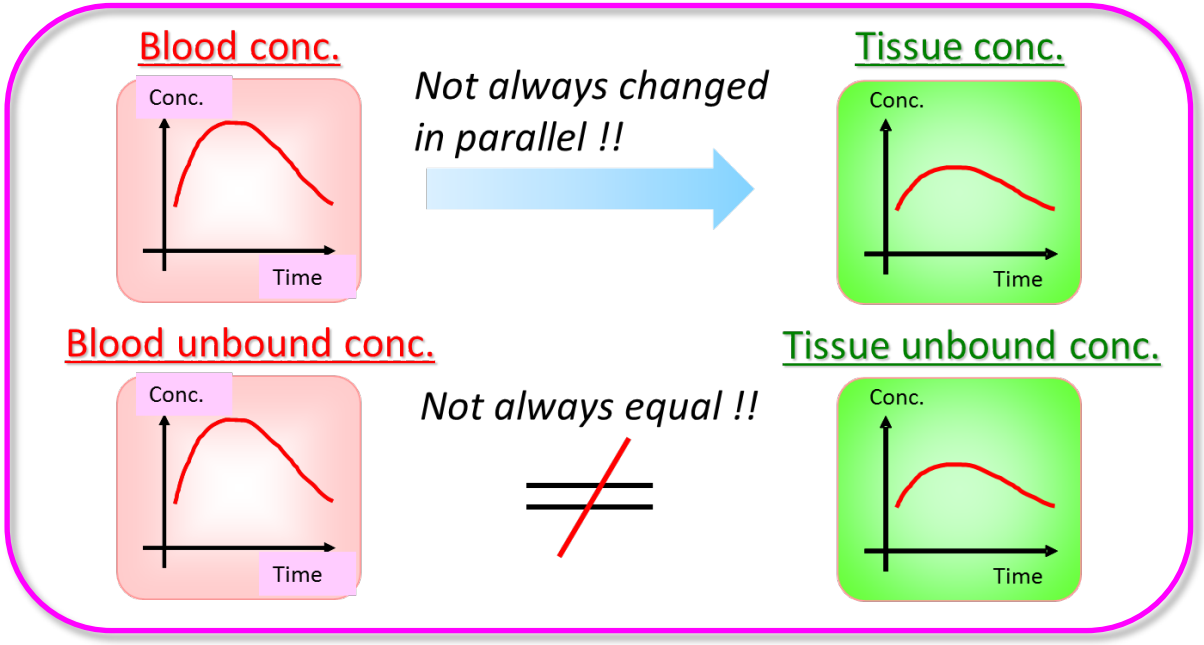
Importance of the estimation of tissue concentration of drugs

Active transport, Metabolism etc.



1) Prediction of pharmacological/toxicological effects of drugs in the target tissue

(e.g. Liver)

HMG-CoA reductase inhibitors (statins) → decrease in plasma cholesterol

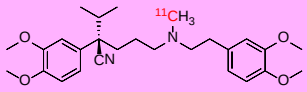
Biguanides → decrease in blood glucose, lactic acidosis

2) Prediction of drug-drug interactions (DDIs) at the target tissue

Inhibitor drugs are **actively accumulated** in the liver → Underestimation of risks of DDIs involving the inhibition of CYPs/efflux transporters

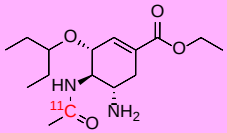
PET probes for the analyses of transporter-mediated PK (in collaboration with Dr.Yasuyoshi Watanabe (RIKEN, Kobe)

R(+)-[^{11}C]Verapamil



P-gp (BBB)

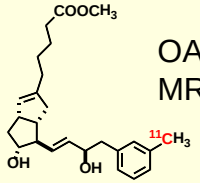
[^{11}C]Oseltamivir



Ontogeny in BBB penetration

Takashima T *et al.*, ***J Nucl Med.*** 2011: 52: 950-7

15*R*-[^{11}C]TIC-Me

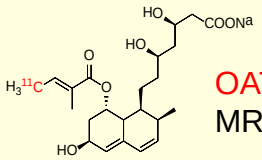


OATP1B1,1B3, MRP2

Takashima T *et al.*, ***JPET*** 2010; 335: 314-323

Takashima T *et al.*, ***J Nucl Med.*** 2012; 53: 1-8

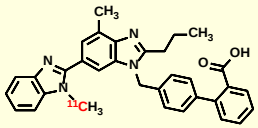
[^{11}C]Dehydropravastatin



OATP1B1

MRP2 ?

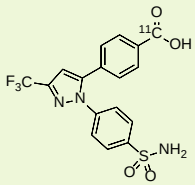
[^{11}C]Telmisartan



OATP1B3

Takashima T et al., ***Mol Pharm.*** 2011; 8: 1789-98

[^{11}C]SC-62807



OATP1B1,1B3, BCRP

Takashima-Hirano M et al., ***Bioorg Med Chem.*** 2011; 19:2997-3004.

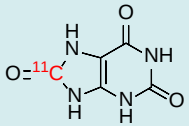
[^{11}C]Metformin

(will be studied very soon)

OCT?

MATE ?

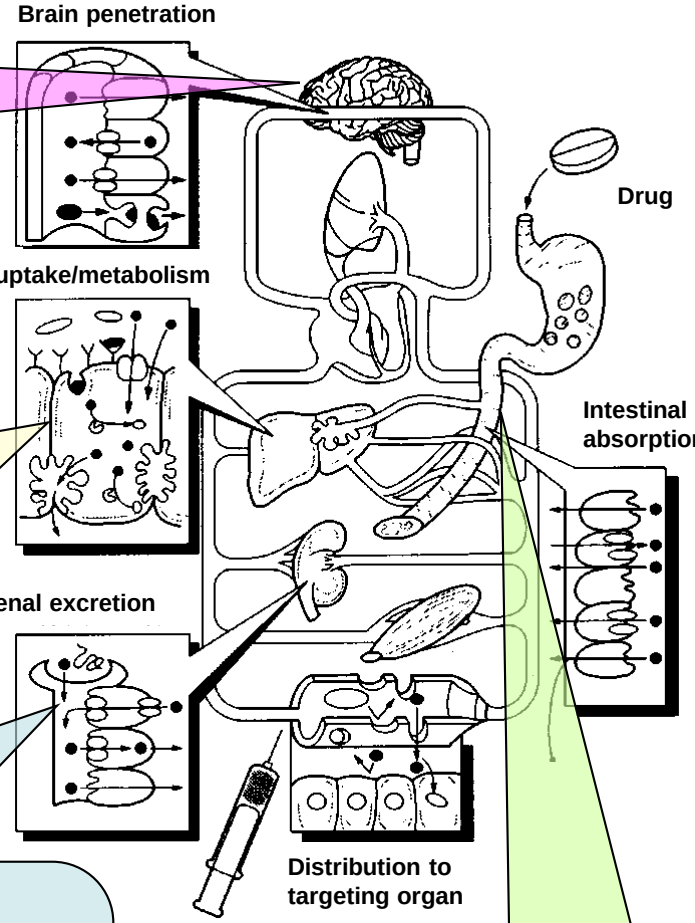
[^{11}C]Uric acid



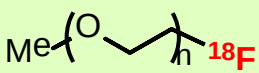
URAT1 ?

BCRP ?

Yashio K *et al.*, ***Bioorg Med Chem Lett.*** *2012 ;22:115-9.*



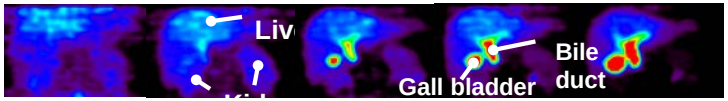
[^{18}F]FPEG(2kDa)



- Unabsorbed marker
- Application to PEGylation

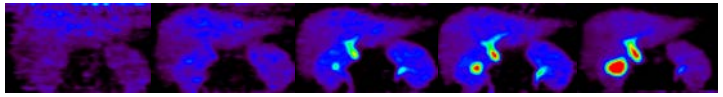
Determination of clearances for tissue uptake (OATP activity) and canalicular efflux (MRP2 activity) in the same subject using 15*R*-[^{11}C]TIC Human studies

Baseline (rifampicin untreated



5 min 10 min 15 min 20 min 30 min

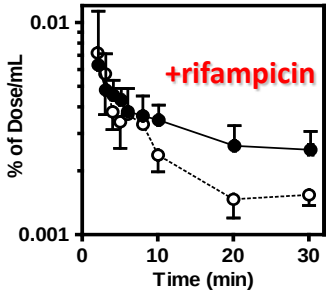
600 mg of rifampicin treated condition



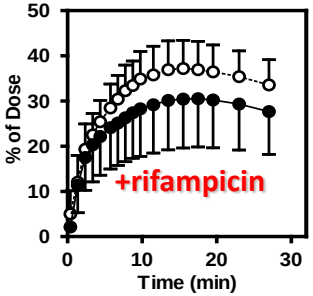
5 min 10 min 15 min 20 min 30 min

%dose/mL: 0.02 0.09

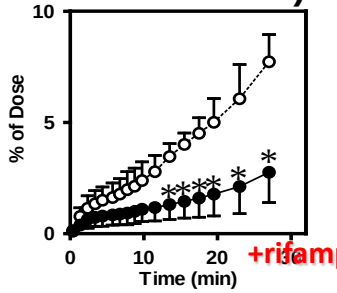
Blood (venous blood)

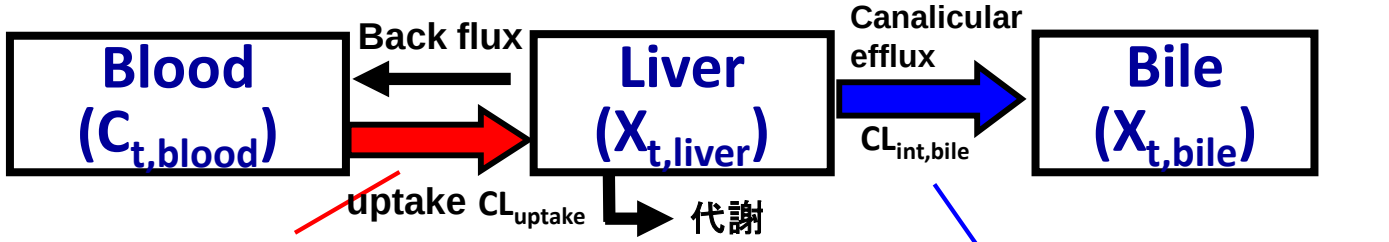


Liver

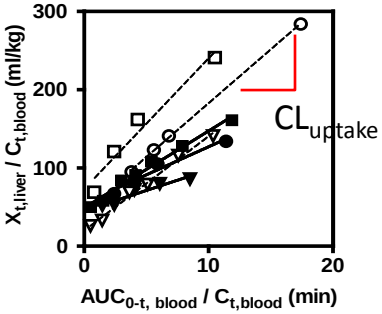


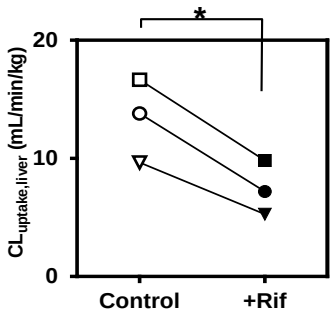
Bile (gall bladder and bile duct)





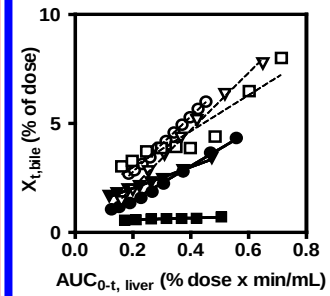
Integration plot

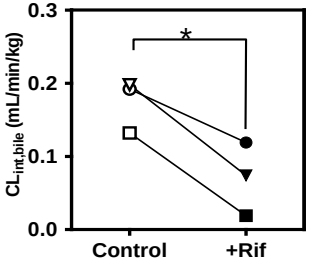




$$\frac{X_{t\_tissue}}{C_{t\_blood}} = CL_{uptake\_tissue} \times \frac{AUC_{0\text{-}t}}{C_{t\_blood}} + V_E$$

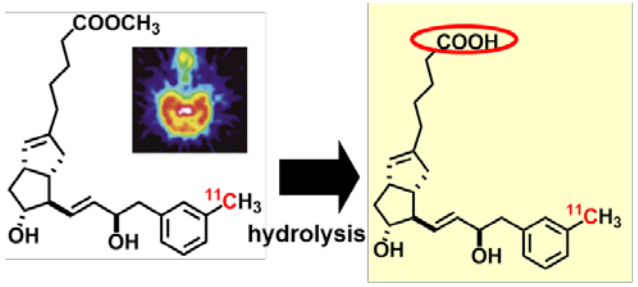
Integration plot





$$X_{t\_bile} = CL_{int,bile} \times AUC_{0\text{-}t,liver}$$

Coadministration of rifampicin with [^{11}C]-15*R*-TIC in humans



([^{11}C]TIC-A)

Hepatic uptake

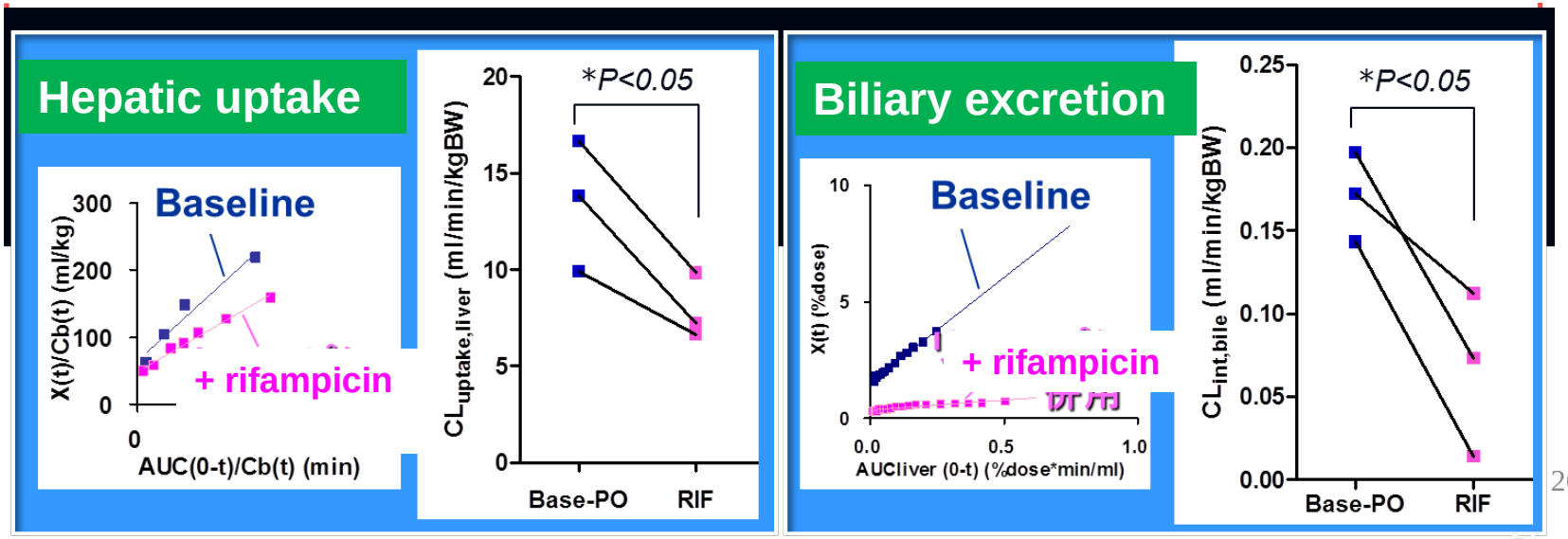
(Takashima T et al., J Nucl Med, 53, 741-8 (2012))

Collaboration with Dr.Yasuyoshi Watanabe in Riken, Kobe

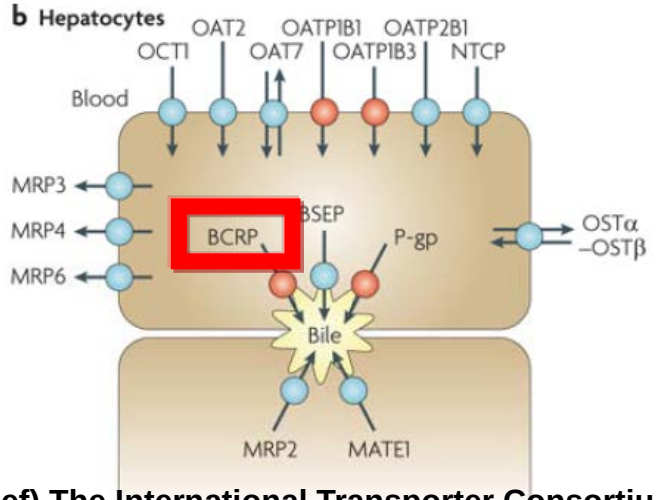
➢ ***PET imaging of radioactivity after administration of [^{11}C]-15R-TIC***

control

+ rifampicin



BCRP/ ABCG2
(Breast cancer resistance protein)

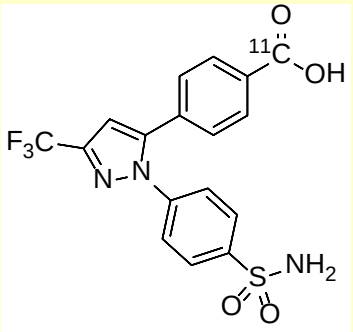


Ref) The International Transporter Consortium (2010) *Nat Rev Drug Discov* **9**:215–236

Functional PET probe for BCRP

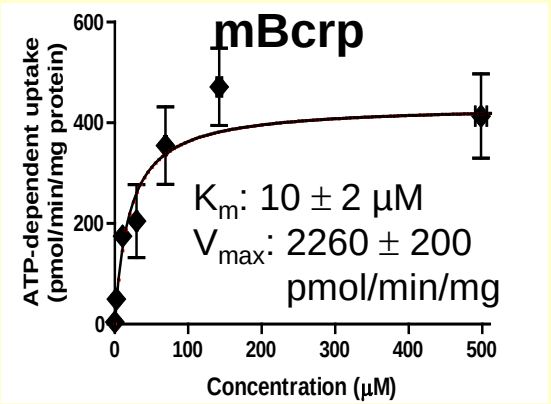
Quantitative evaluation of BCRP-mediated transport in humans: Application to DDI and PGx

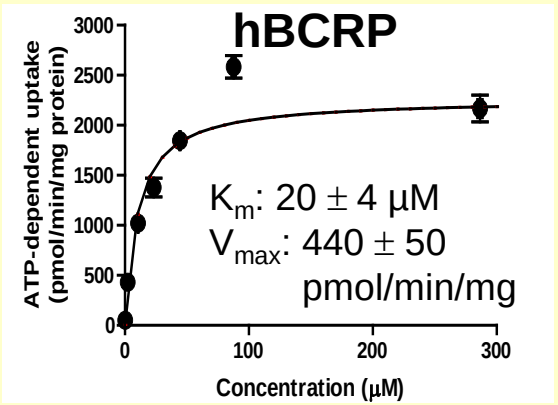
[^{11}C]SC-62807
(metabolite of celecoxib)



Ref) Takashima-Hirano M et al., *Bioorg Med Chem.* **19**: 2997–3004 (2011)

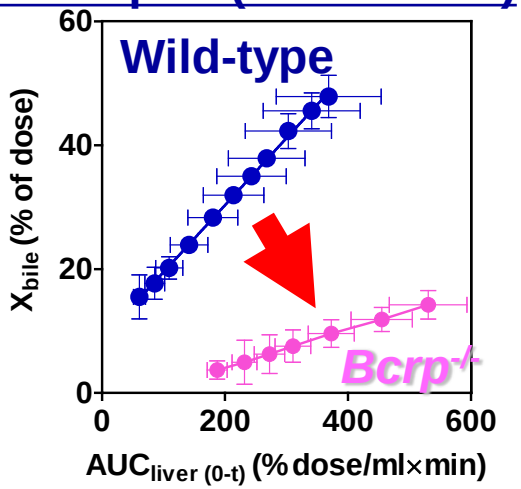
ATP-dependent uptake of SC-62807 by membrane vesicles expressing mBcrp or hBCRP





Comparison of canalicular efflux and brush border efflux of [^{11}C]SC-62807 between wild-type and Bcrp$^{-/-}$ mice

1) Integration plot (liver → bile)

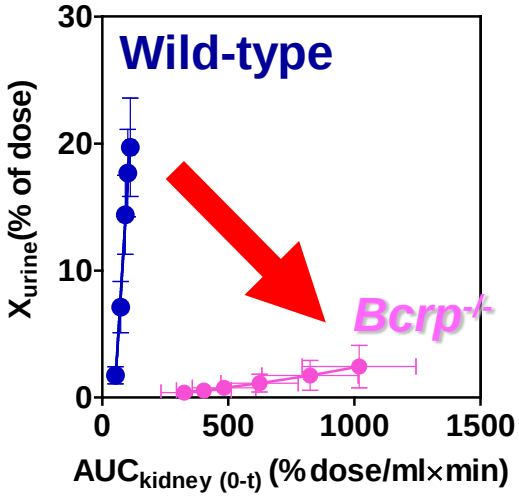


$$X_{bile} = CL_{int,bile,liver} * AUC_{liver} + X_0$$

$CL_{int,bile,liver}$:

- Wild-type 4.5 ± 0.8 ml/min/kg
- *Bcrp$^{-/-}$* 1.2 ± 0.1 ml/min/kg

2) Integration plot (kidney → urine)



$$X_{urine} = CL_{int,urine,kidney} * AUC_{kidney} + X_0$$

$CL_{int,urine,kidney}$:

- Wild-type 13 ± 2 ml/min/kg
- *Bcrp$^{-/-}$* 0.12 ± 0.07 ml/min/kg

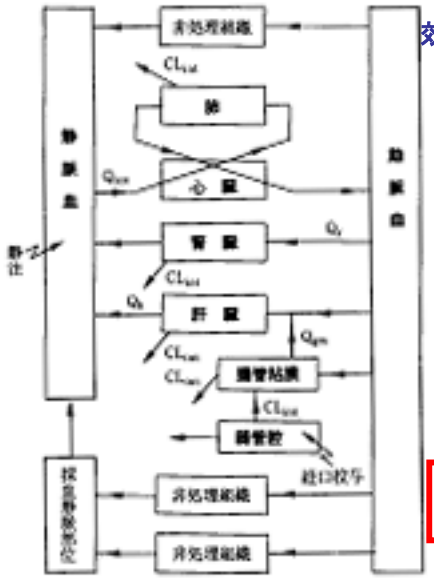
Preclinical studies

Phase I

in vitro screening
ADME parameters（clearance etc）
PD/TD parameters（EC50 etc）

Integration by using mathematical modeliing

PK



効果

PD vs exposure
At the target sites

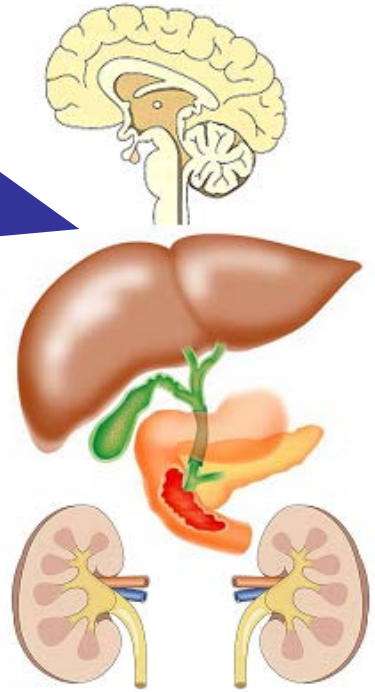
濃度

Prediction of PD

Prediction

Scaling factor
Validation

Analysis of tisse expore uisng PET imaging



Measurement of drug exposure at the target tissues

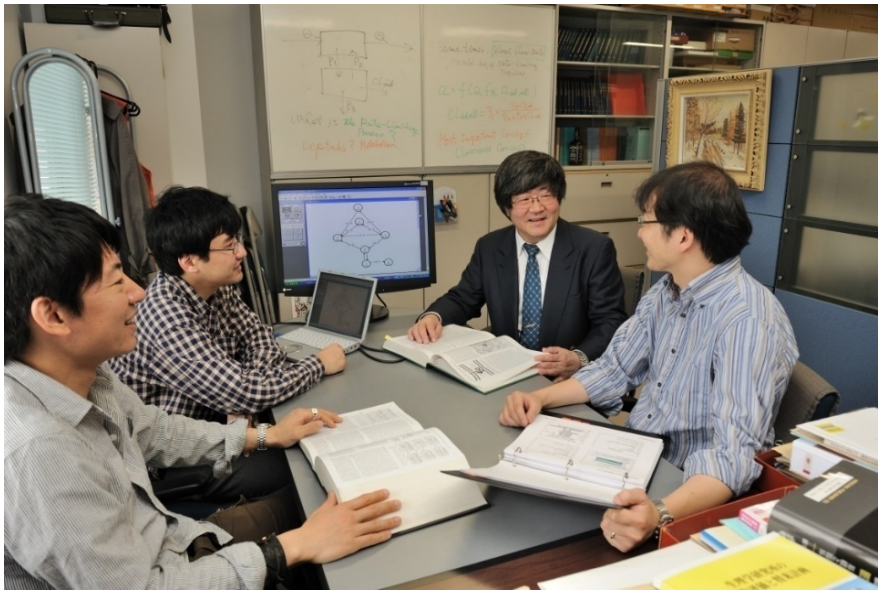
Proposal

1) Comparison of kinetic parameters (PSinf, Clbile) between in vivo (PET imaging) and in vitro (sandwich culture) is necessary for several different PET ligands.

2) Then, we can obtain more reliable scaling factors at least for uptake and biliary excretion process.
（Consortium research is going on by inviting 6 Global Pharma Industries)

3) The PBPK based prediction using thus obtained scaling factors can be compared with that obtained PET imaging data (for example, the increase in drug exposure in the liver by inhibiting the biliary excretion (DDI/PGx))

RIKEN



Kazuya Maeda, Hiroyuki Kusuhara (Univ of Tokyo)

Sugiyama Lab Main Members

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

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

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