Organic anion transporting polypeptides (OATPs)-mediated drug-drug interaction (DDI) study between rosuvastatin and cyclosporin A in chimeric mice with humanized liver (PXB-mouse®)

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Background:

Nowadays, the evaluation of potential risk of drug-drug interactions (DDIs) is of high importance in the drug development, in order to improve safety and reduce the attrition rate of new drug candidates. FDA, EMA and MHLW/PMDA have published the guidance/guideline and they recommend some in vitro assays to help us define pharmacokinetic drug interactions between an investigational new drug and others. These assays are very useful whether a compound has a potential for DDIs with other concomitants, however it is very difficult to quantify the in vivo potential from just in vitro data because commonly a drug has multiple clearance pathway and the rate-limiting step for the clearance is usually different for every drug. A chimeric mice with humanized liver is a mouse model with liver repopulated with human hepatocytes. They have human transporters and metabolic enzymes in the liver, and the metabolism and biliary excretion of drugs are similar to those in human. Therefore, many DDIs studies such as hepatic enzyme (e.g. cytochrome P450) inhibition and induction have been reported in the mouse model.
In the present study, we used the mouse model (PXB-mouse®, PhoenixBio, Co., Ltd.) and conducted the DDI study about hepatic uptake transporters, which is organic anion transporting polypeptides (OATPs), to assess the utility of the mouse model for evaluation of the in vivo DDI potential of drug candidates with concomitant drugs.

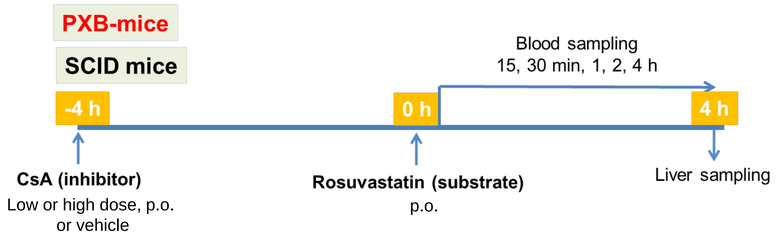
OATPs, Rosuvastatin, Cyclosporin A:

OATPs (OATP1B1 and 1B3) are major uptake transporters and expressed in sinusoidal (basolateral) membrane of hepatocytes. A lot of OATPs-mediated DDIs have been reported in clinical between its substrates, such as statins (e.g. rosvastatin), and inhibitors (e.g. cyclosporin, CsA). A 7.1-fold increase in the plasma concentration of rosuvastatin with CsA was reported in heart transplant patients. Rosuvastatin is a substrate of human OATP1B1/1B3 and these ortholog, rodent Oatp1b2. Many reports have shown that the transport activitiy of Oatp1b2 is higher than that of OATP1B1/1B3. The systemic clearance of rosuvastatin is mostly mediated by hepatic uptake transporter (OATPs) and biliary efflux transporters (BCRP and MRP2). Therefore, the systemic clearance of rosuvastatin in rodent is higher than that in human and then the systemic exposure of rosuvastatin in rodent is lower than that in human.

Methods:

1. Animal Experiment

In PXB-mice and SCID mice (3-4 animals per group), rosuvastatin is administered orally at 4 h after the pretreatment of CsA or vehicle (control).



2. Determination of blood and liver concentration

Blood and liver concentration of rosuvastatin and its metabolites, lactone form and desmethyl form, and cyclosporin A (CsA) were determined by liquid chromatography tandem mass spectroetry (QTRAP5500, SCIEX).

PXB-mouse®:

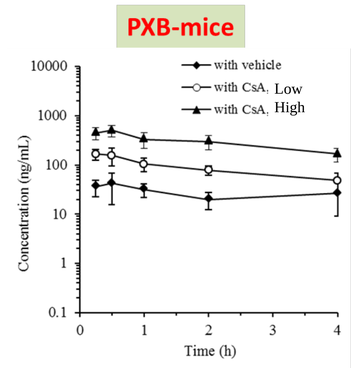
Is world's most widely used human liver chimeric mouse model. Urokinase-type plasminogen activator/severe combined immunodeficiency (cDNA-uPA/SCID) mice with humanized liver, which is repopulated with 80% or more human hepatocytes. In the present study, repopulation rates are 88 - 95% in the liver of the animals.

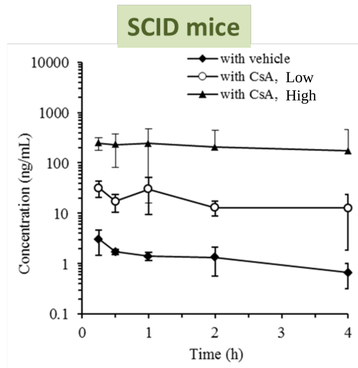
Ethics statement:

This study was reviewed and approved by Animal Care and Use Committee of Toray Industries, Inc. and the Laboratory Animal Ethics Committee of PhoenixBio Co., Ltd. All animals were handled in strict accordance with good animal practice under the supervision of veterinarians. Every effort was made to alleviate animal discomfort and pain by appropriate and routine use of anesthetic and/or analgesic agents.

Results:

1. Blood concentrations of rosuvastatin with/without CsA

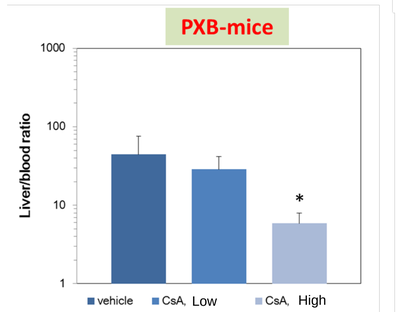


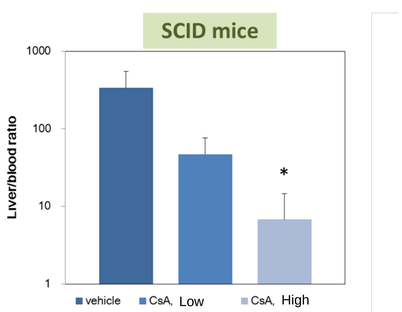


PK Parameters	With CsA, High dose	With CsA, Low dose	With vehicle
C_{5min} (ng/mL)	506	196	46.7
AUC_{0-4h} (ng·h/mL)	1162	344	105
AUC ratio	11	3.3	-

PK Parameters	With CsA, High dose	With CsA, Low dose	With vehicle
C_{5min} (ng/mL)	329	40.5	3.31
AUC_{0-4h} (ng·h/mL)	812	69.2	5.14
AUC ratio	160	13	-

2. Liver concentrations of rosuvastatin with/without CsA





	Liver (ng/g)	Plasma (ng/mL)	$K_{p,liver}$
With vehicle	829	26.4	44.4
With CsA, Low dose	1218	48.4	28.8
With CsA, High dose	989	166	5.89

	Liver (ng/g)	Plasma (ng/mL)	$K_{p,liver}$
With vehicle	270	0.669	336
With CsA, Low dose	403	12.6	46.9
With CsA, High dose	456	220	6.79

Results:

3. Blood concentrations of CsA

PXB-mice	Dose	C_{max} (μmol/L)	AUC (μmol·h/L)
CsA	Low	0.131	0.292
	High	0.349	1.02

SCID mice	Dose	C_{max} (μmol/L)	AUC (μmol·h/L)
CsA	Low	0.403	1.20
	High	1.58	5.25

4. Liver concentrations of CsA

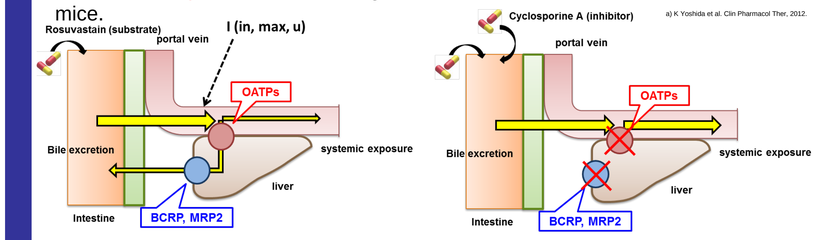
	Dose	C_{4h} (μmol/kg tissue)	
		PXB-mice	SCID mice
CsA	Low	1.07	4.96
	High	5.89	30.0

5. Blood concentrations of rosuvastatin metabolites

Lactone form was rarely detected(< 3 ng/mL)in both PXB- and SCID mice. Desmethyl form was 3-4%(human specific metabolite) of rosuvastatin in PXB-mice, while it was not detected in SCID mice.

I (in, max, u), IC_{50}:

Reported IC_{50} of CsA to OATP1B1, OATP1B3, MRP2, and BCRP were **0.15**, **0.68**, **9.3** and **1.5** μmol/L[a]. Theoretically inhibition of hepatic uptake transporters occurs based on "I (in, max, u)" of inhibitors, and inhibition of hepatic efflux transporters occurs based on liver concentrations of inhibitors. In clinical, a 7.1-fold increase in the plasma concentration of rosuvastatin with CsA occurred when I (in, max, u) of CsA was **1.3** μmol/L[a]. In the present study, I (in, max, u) of CsA are **0.36 to 0.39** and **1.8 to 1.9** μmol/L at low and high dose of CsA in both PXB-mice and SCID mice.



a) K Yoshida et al. Clin Pharmacol Ther, 2012.

I (in, max, u) : the estimation of maximum protein-unbound concentration at the inlet to the liver
I (in, max, u) = fu,b × (Cmax,b + FaFg × ka × Dose / Qh)
fu, b: unbound fraction in blood, FaFg: the fraction absorbed after oral administration, ka: the first order absorption rate constant
Qh: liver blood flow rate

Summary:

- In SCID mice, the blood concentrations of rosuvastatin are lower than those in PXB-mice.
- In PXB- and SCID mice, the AUCs of rosuvastatin were increased when pretreated with CsA in dose-dependent manner.
- In SCID mice, CsA concentrations in blood and liver are higher than those in PXB-mice, and a high dose of CsA significantly increased blood rosuvastatin concentraions (160-fold to vehicle) because of high liver and systemic exposures of CsA compared to PXB mice.
- In PXB- and SCID mice, liver-to-blood concentration ratios of rosuvastatin were decreased by the pretreatment with CsA in dose-dependent manner.

Conclusion:

- **Pharmacokinetics profiles of both rosuvastatin and CsA are different between PXB-mice and SCID mice. The cause is probably based on the difference between human and mouse hepatocytes.**
- **In PXB- and SCID mice, systemic exposure of rosuvastatin is increased by the inhibitions of hepatic uptake transporters with CsA.**
- **In SCID mice, the systemic rosuvastatin concentration is significantly increased by the inhibition of hepatic efflux transporters with CsA at high dose .**
- **PXB-mouse® is a useful experimental animal model to predict in vivo hepatic DDIs in clinical.**