CASE STUDY FOR IMPROVEMENTS OF CYP DDI VICTIM RISK GAP BETWEEN PERSPECTIVE AND RETROSPECTIVE PREDICTION USING NEW ADME TOOLS, CHIMERIC MICE

ADDI-2018, Session 6:
Novel Technologies for the Evaluation of Drug-drug interactions

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AGENDA

I Introduction

II Human sandwich-cultured hepatocytes (SCH)

III RI-ADME study using PXB mice

IV DDI study using PXB mice

V Conclusion

CYP DDI VICTIM RISK

A. Determining if the Investigational Drug is a Substrate of Metabolizing Enzymes

1. Conducting In Vitro Studies

The sponsor should routinely evaluate CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 using in vitro phenotyping experiments to determine which enzymes metabolize the investigational drug. However, it is possible that the investigational drug undergoes significant in vivo metabolism that is not mediated by these major CYP enzymes. In this event, the investigational drug is probably a substrate for additional enzymes, and the sponsor should determine what additional enzymes contribute to the metabolism of the investigational drug. These additional enzymes include but are not limited to:

• CYP enzymes including CYP2A6, CYP2J2, CYP4F2, and CYP2E1

• Other Phase I enzymes including monoamine oxidase (MAO), flavin monooxygenase (FMO), xanthine oxidase (XO), and alcohol/aldehyde dehydrogenase

• Phase II enzymes including UDP glucuronosyl transferases (UGTs)

2. Data Analysis and Interpretation

The contribution of a specific metabolizing enzyme to an investigational drug's clearance is considered significant if the enzyme is responsible for > 25% of the drug's elimination based on the in vitro phenotyping studies and human PK data. Under these circumstances, the sponsor should conduct clinical DDI studies using strong index inhibitors and/or inducers of the enzyme.

Refer to the FDA in vitro DDI draft guidance, 2017

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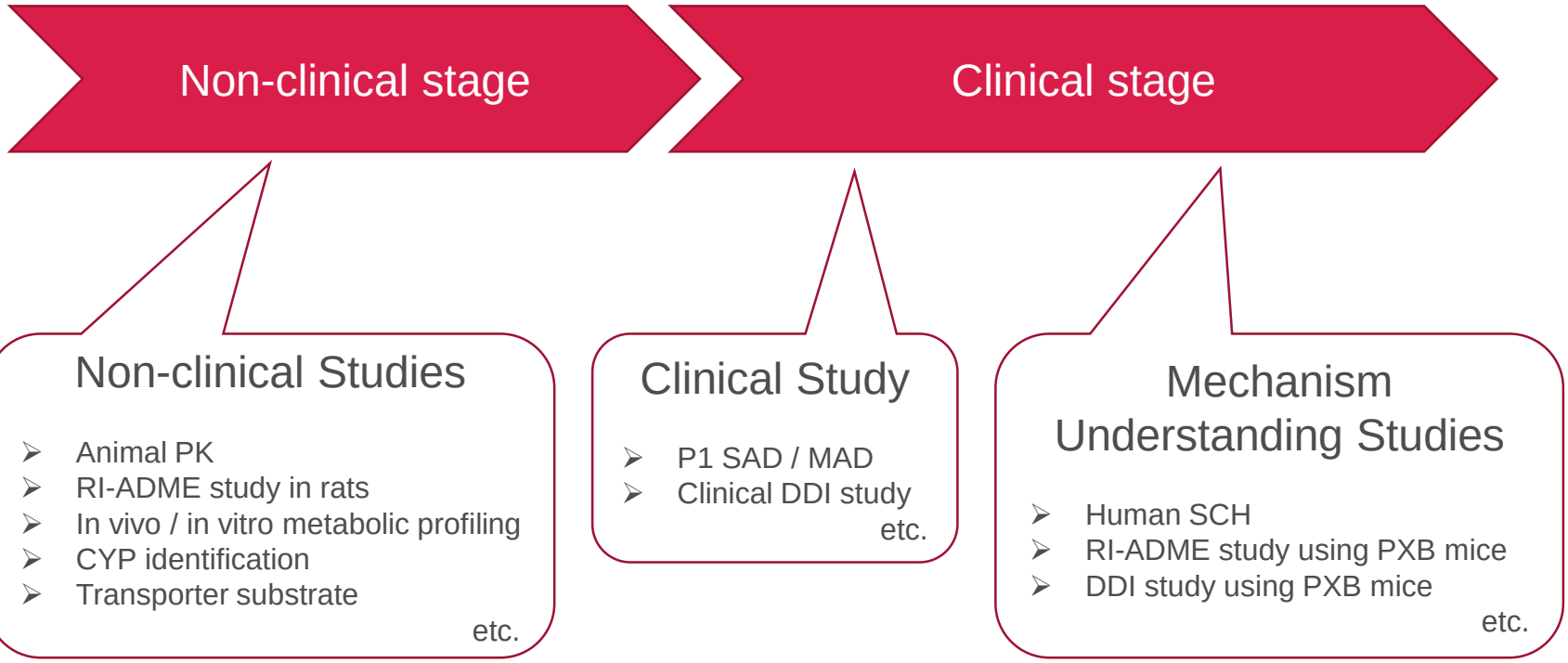
HISTORY OF COMPOUND A



Perspective DDI victim prediction using Simcyp

Retrospective DDI victim prediction using Simcyp

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NONCLINICAL STUDY INDICATING CYP3A VICTIM RISK

Study	Results	Insight
Animal PK	Low urine and bile excretion of compound A in rats	Metabolism is main excretion route in rats (and humans)
RI-ADME in rat In vivo metabolic profiling in rat plasma, urine and bile	Most of compound A and the related compounds were excreted into rat bile (unchanged drug peak is small in the rat bile)	Biliary excretion as metabolites is main excretion route in rats (and humans)
In vitro metabolic profiling	UGT's low contribution to the metabolism (Glu peak is small in human hepatocytes)	CYP metabolism is main route
CYP identification	Compound A is mainly metabolized by CYP3A.	CYP3A victim risk
BCRP substrate P-gp substrate	Compound A is a P-gp and BCRP substrate	Biliary excretion as unchanged drug (not use for Simcyp)

SIMULATION RESULTS USING P1 SAD PK DATA

Background

- Compound A is considered to be a substrate of CYP3A based on in vitro data

Objective

- To simulate the magnitude of DDI that may cause an increase of plasma concentrations of compound A by a co-administered strong CYP3A inhibitor (Itraconazole; ITZ)
- To get the information whether clinical DDI study should be performed as Phase 1 cohort

Criteria and Prediction range

5-fold in AUC_{inf} Ratio, Range of geometric mean from each scenario

Result

- The magnitude of the AUC_{inf} change by ITZ was predicted to be 5.4-30.4 fold.
  -> According to the discussion before the simulation, in vivo DDI cohort should be performed in Phase 1.

Range from the difference of ITZ file

How to estimate Fg	Model	C_{max} ratio	AUC_{inf} ratio
Refer animal data	Animal PK data Fg = 0.636	2.0	5.6-11.3
	with active liver uptake Fg = 0.665	1.9-2.0	5.4-10.8
NOT refer animal data	Qgut model (fu,gut=1) Fg = 0.210	5.4-5.5	15.9-30.4

RESULTS OF CLINICAL DDI STUDY

Fig. Mean Plasma Concentration-time Profiles of Compound A by Treatment: Drug-drug Interaction

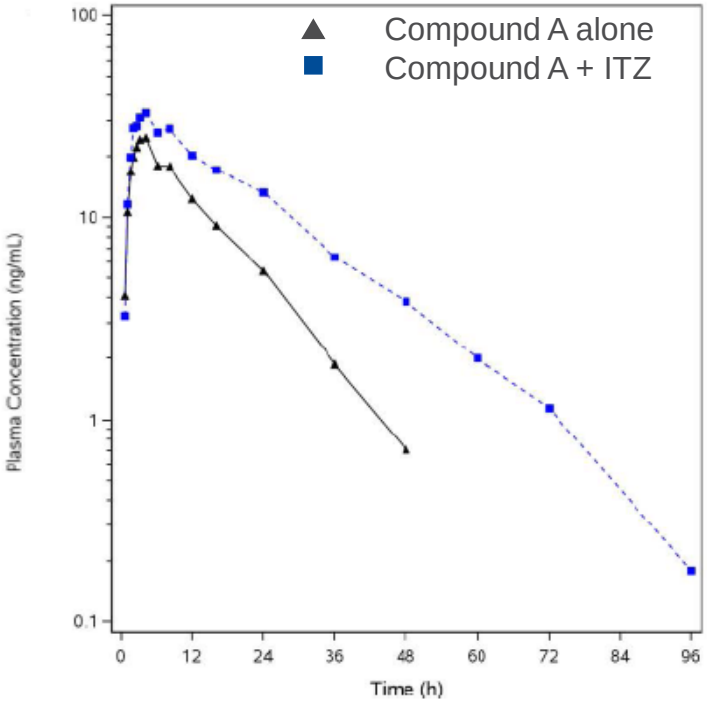

Table. Statistical Assessment of Effect of Itraconazole on the Pharmacokinetics of Compound A: Drug-drug Interaction

Parameters	Compound A + ITZ	Compound A alone	Geometric LS Mean (%)	90% CI of Ratio (%)
AUCinf (h*ng/mL)	732 (n = 8)	370 (n = 8)	197.68	(175.45, - 222.71)
Cmax (ng/mL)	33.2 (n = 8)	24.9 (n = 8)	133.23	(124.84, - 142.18)

CI: confidence interval; LS: least squares.

Cmax Ratio = 1.3

AUCinf Ratio = 2.0

“GAP” between clinical DDI study and simulation result.

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POINTS TO CONSIDER

Underestimation of Fg

- Peff (calculated using physicochemical parameters)
  → Too low permeability ?
- % HepCL (CYP3A4: 96%, others: 4%)
  → The contribution ratio of CYP3A4 was too high ?
- fu,gut (1: assumption)
  → Assumption was too conservative ?

Overestimation of the inhibition of systemic clearance

- % HepCL (CYP3A4: 96%, others: 4%)
  → The contribution ratio of CYP3A4 was too high ?
- Additional elimination routes
  → Renal clearance, biliary clearance or any other routes were existed?
- Rate-limiting step for elimination from the blood
  → Permeability limited instead of metabolizing rate-limited ?

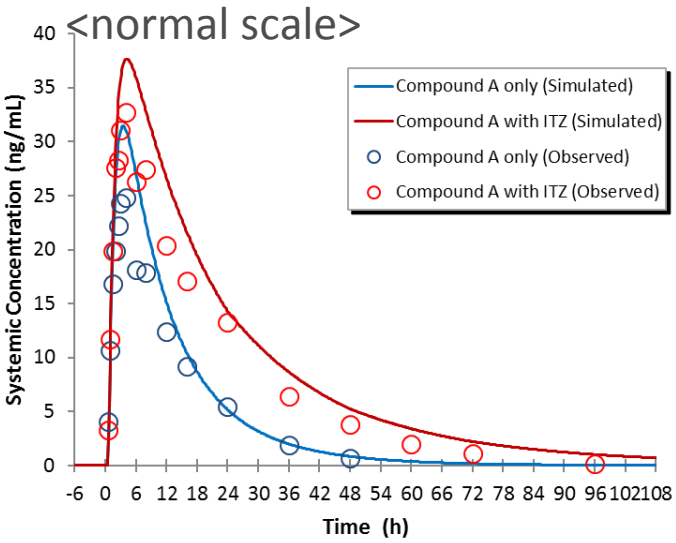
( ): values in the previous simulation, fu,gut: Unbound fraction of drug within the enterocyte

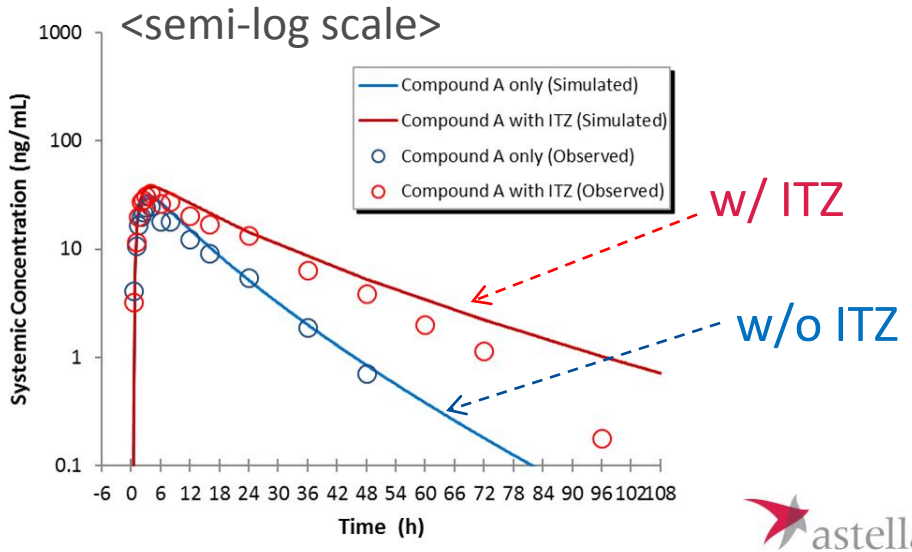
THE EFFECT OF FU,GUT AND %HEPCL SIMULATION RESULTS

fu,gut = fp (0.001177)

%HepCL	Cmax ratio	AUCinf Ratio
96%	1.4	7.3
80%	1.3	3.4
70%	1.2	2.6
60%	1.2	2.1
Clinical result	1.3	2.0

60%





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OBJECTIVE

To understand the GAP between non-clinical and clinical studies about additional elimination route of compound A, especially biliary excretion route, we conducted the following additional ADME studies using the human originated materials.

1. In vitro biliary excretion in human sandwich-cultured hepatocytes (SCH)
2. Mass balance study using chimeric mice with humanized liver (PXB mice)
3. In vivo DDI study using chimeric mice with humanized liver (PXB mice)

ADDITIONAL ADME STUDIES OF COMPOUND A

1. IN VITRO BILIARY EXCRETION IN HUMAN SCH
2. MASS BALANCE STUDY USING PXB MICE
3. IN VIVO DDI STUDY USING PXB MICE

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HEPATIC TRANSPORTER INFORMATION

Study	TP type	Contents
P-gp substrate	Efflux type TP	Compound A is a P-gp substrate.
P-gp inhibition	Efflux type TP	IC50 is 4.26 μM.
BCRP substrate	Efflux type TP	Compound A is a BCRP substrate.
BCRP inhibition	Efflux type TP	IC50 is 1.10 μM.
OATP1B1 inhibition	Uptake type TP	IC50 is 2.13 μM.
OATP1B3 inhibition	Uptake type TP	IC50 is 3.10 μM.

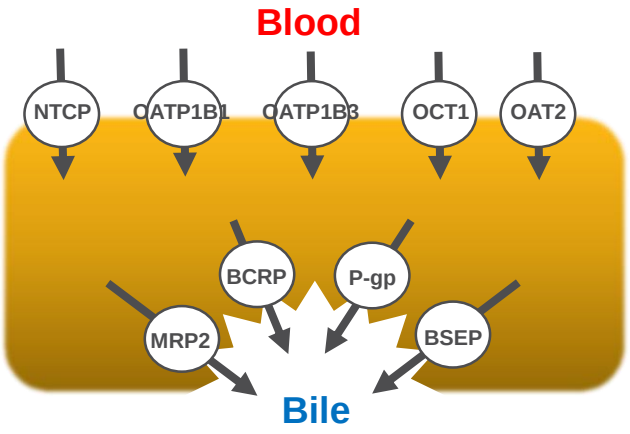

Figure. Selected human hepatic transporters

Uptake transporters in the basolateral (sinusoidal) membrane include the sodium/taurocholate co-transporting peptide (NTCP; SLC10A1); members of the OATP family (OATP1B1 (SLCO1B1), and OATP1B3 (SLCO1B3); organic cation transporter 1 (OCT1); and organic anion transporter 2 (OAT2; SLC22A7).

Apical (canalicular) efflux pumps of the hepatocyte comprise P-gp; bile-salt export pump (BSEP or SPGP; ABCB11); BCRP (ABCG2); and MRP2.

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1. IN VITRO BILIARY EXCRETION IN HUMAN SANDWICH-CULTURED HEPATOCYTES

Objective

- Assessment of biliary excretion of compound A as one of the disposition pathways in human in addition to CYP3A-mediated metabolism

Material

- Human sandwich-cultured hepatocytes

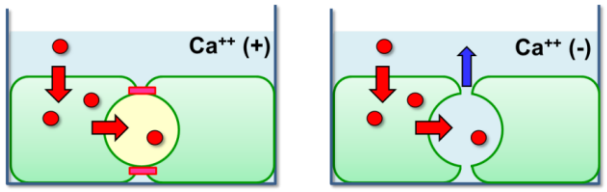
Methods

- Cellular component measurement
- Measureable parameters

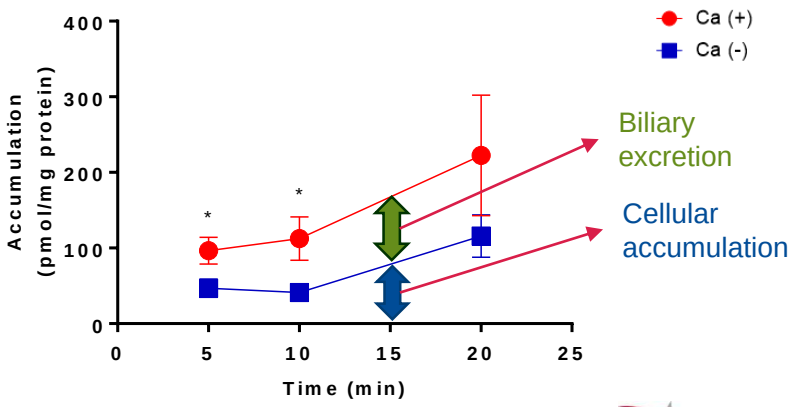
  Biliary excretion and cellular accumulation

- Procedure

  1. Pre-incubation of cells in Ca (+) and Ca (-) conditions
  2. Substrate loading
  3. Lysis and measurement of intracellular compounds



Typical Substrate Results (d8-taurocholic acid)



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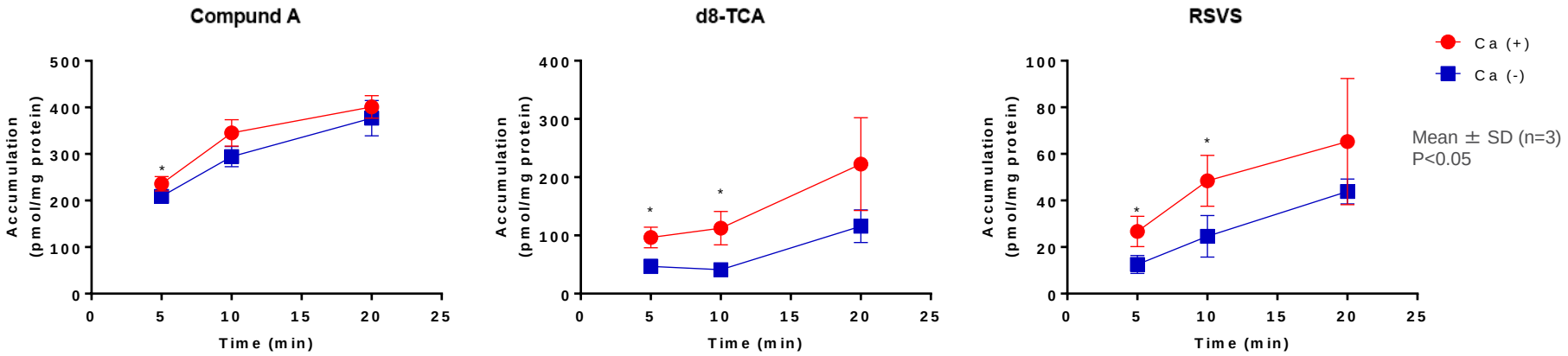
EXP.1 RESULTS

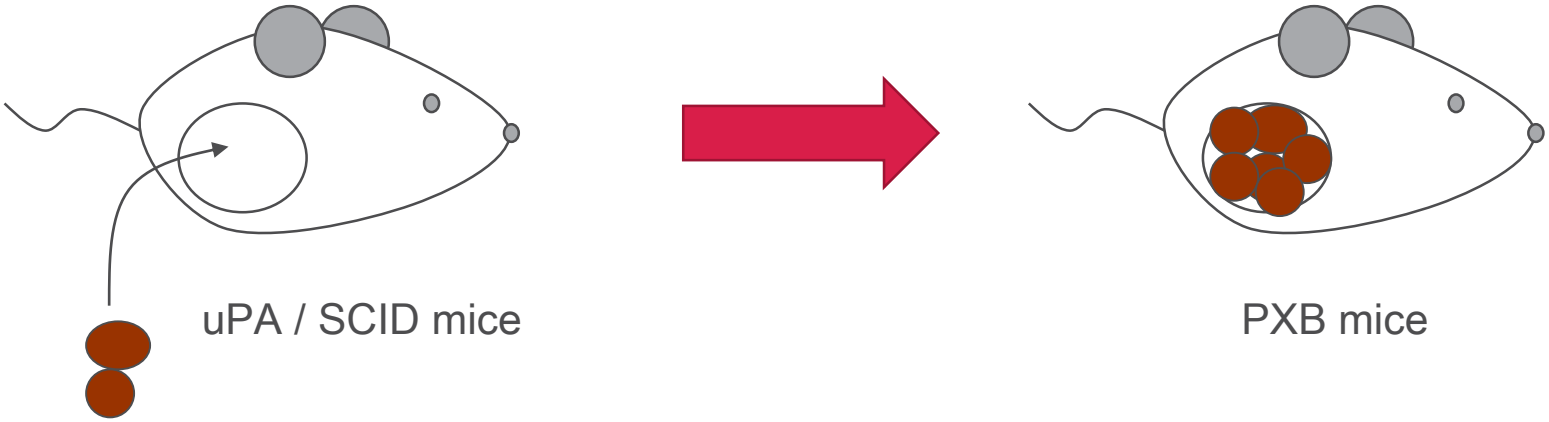
Test compound; Compound A (2.5 μM)
Reference compounds; d8-Taulocholic acid (d8-TCA; 1 μM) and Rosuvastatin (RSVS, 2.5 μM)



Analyte	Accumulation at 10 min, Ca (+) (pmol/mg protein)	BEI* (%)	CLbiliary (in vitro)** (μL/min/mg protein)
Compound A	346±29	5.9 - 14.8	2.2
d8-TCA	113±29	47.9 - 63.4	9.9
RSVS	48±11	32.8 - 52.9	1.1

Data are expressed as the mean values ±SD (n = 3).
* BEI; Biliary excretion index
** CLbiliary was calculated from data of initial time point exhibiting statistically significant differences between Ca(+) and Ca(-) conditions.

PXB MICE (CHIMERIC MICE WITH HUMANIZED LIVER)



uPA: albumin enhancer/promoter urokinase plasminogen activator
SCID: severe combined immunodeficiency transgenic mice

Chimeric mice with humanized liver - Mice engrafted with human hepatocytes

There is abundant data for prediction of DMPK in PXB mice.

Key references are described below.

Katoh M., et al., Drug Metab Dispos 32, 1402-1410 (2004).
Tateno C., et al., Am J Pathol 165, 901-912 (2004).
Katoh M., et al., Drug Metab Dispos 33, 1333-1340 (2005). etc.

2. MASS BALANCE STUDY USING CHIMERIC MOUSE WITH HUMANIZED LIVER

Animal experiments

- Test article: [^{14}C]Compound A (1.86 MBq/mg)
- Animal: PXB mice (chimeric mice with humanized liver) and SCID mice (C.B17/Icr-scidJcl mice) (n=3 each)
- Analytical system: LSC, LC-RAD/MS

<Exp. 2-1>
Dose: 1 mg/kg, iv
Biological sample: Plasma (0.5, 1, 2, 4 and 24 h, pooled for $AUC_{0\text{-}24h}$ calculation), urine (acidified using 10% formic acid) and feces (0-6 and 6-24 h each)
Analysis: Time-course of radioactivity (plasma)
Cumulative excretion of radioactivity (urine and feces)
Metabolite profiling and structure estimation (pooled plasma, urine and feces)
<Exp. 2-2>
(conducted 2 weeks after completion of Exp 1)
Dose: 1 mg/kg, iv
Biological sample: Urine and bile (0-24 h each, acidified using 10% formic acid)
Analysis: Cumulative excretion of radioactivity (bile and urine)
Metabolite profiling and structure estimation (bile)
<Exp. 2-3>
(Immediately conducted after completion of Exp 2)
Dose: 1 mg/kg, iv
Biological sample: Plasma (1 h)
Analysis Metabolite profiling and structure estimation (plasma)

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CUMULATIVE EXCRETION

Cumulative Excretion in Urine and Feces (Exp. 2-1)

Time (h)	Excretion of radioactivity (% of dose)			
	PXB		SCID	
	Urine	Feces	Urine	Feces
0 – 6	3.4±0.5	38.1±6.2	4.1±0.7	54.6±11.6
0 – 24	4.9±0.5	87.9±3.2	5.6±1.0	85.6± 3.0

Cumulative Excretion in Bile and Urine (Exp. 2-2)

Time (h)	Excretion of radioactivity (% of dose)			
	PXB		SCID	
	Bile	Urine	Bile	Urine
0 – 24	67.9±7.6	3.8±1.5	82.3±11.3	2.6±2.2

Data are expressed as the mean values ±SD (n = 3).

Most of the radioactivity was excreted in the feces or bile.
There are no significant difference between PXB and SCID mice.

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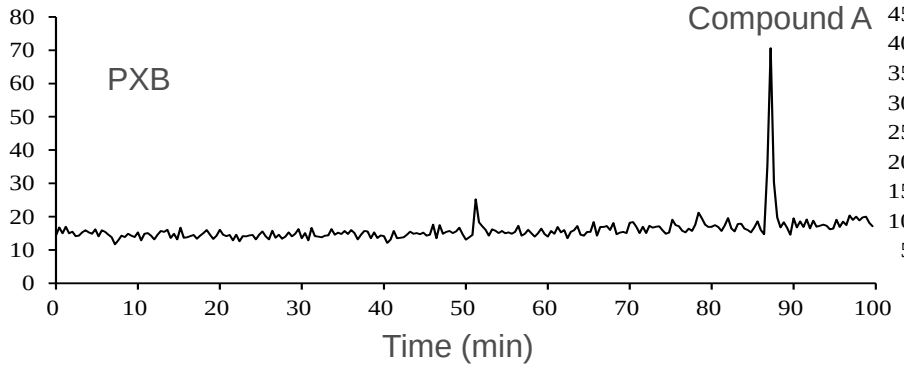
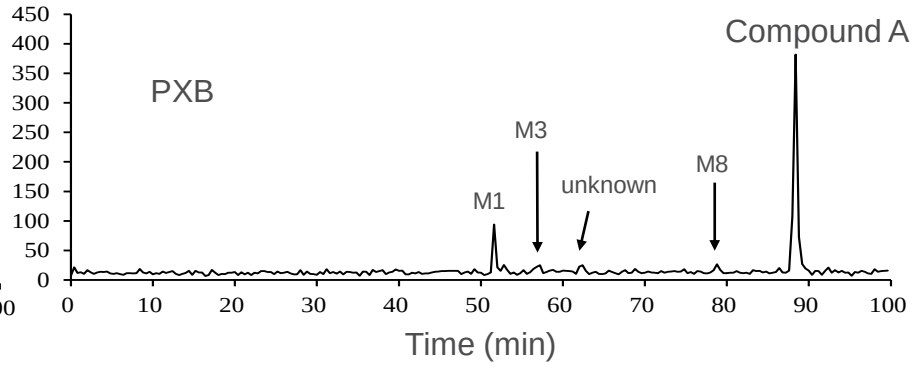
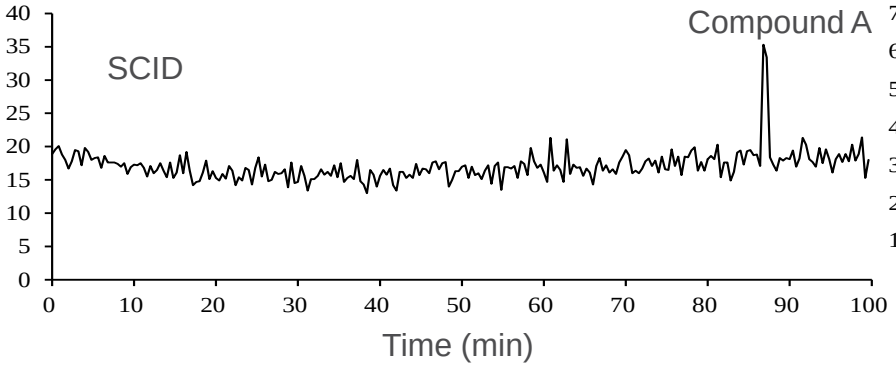
METABOLITE PROFILE IN PLASMA

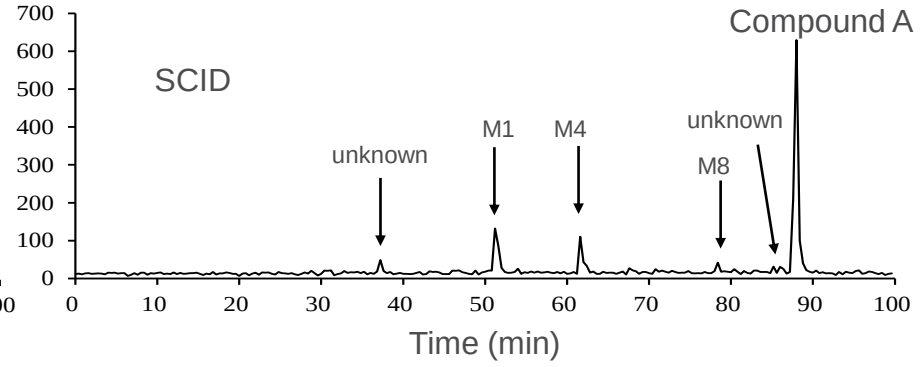
Pooled plasma (AUC_{0-24h}, Exp. 2-1)



Plasma (1 h, Exp. 2-3)

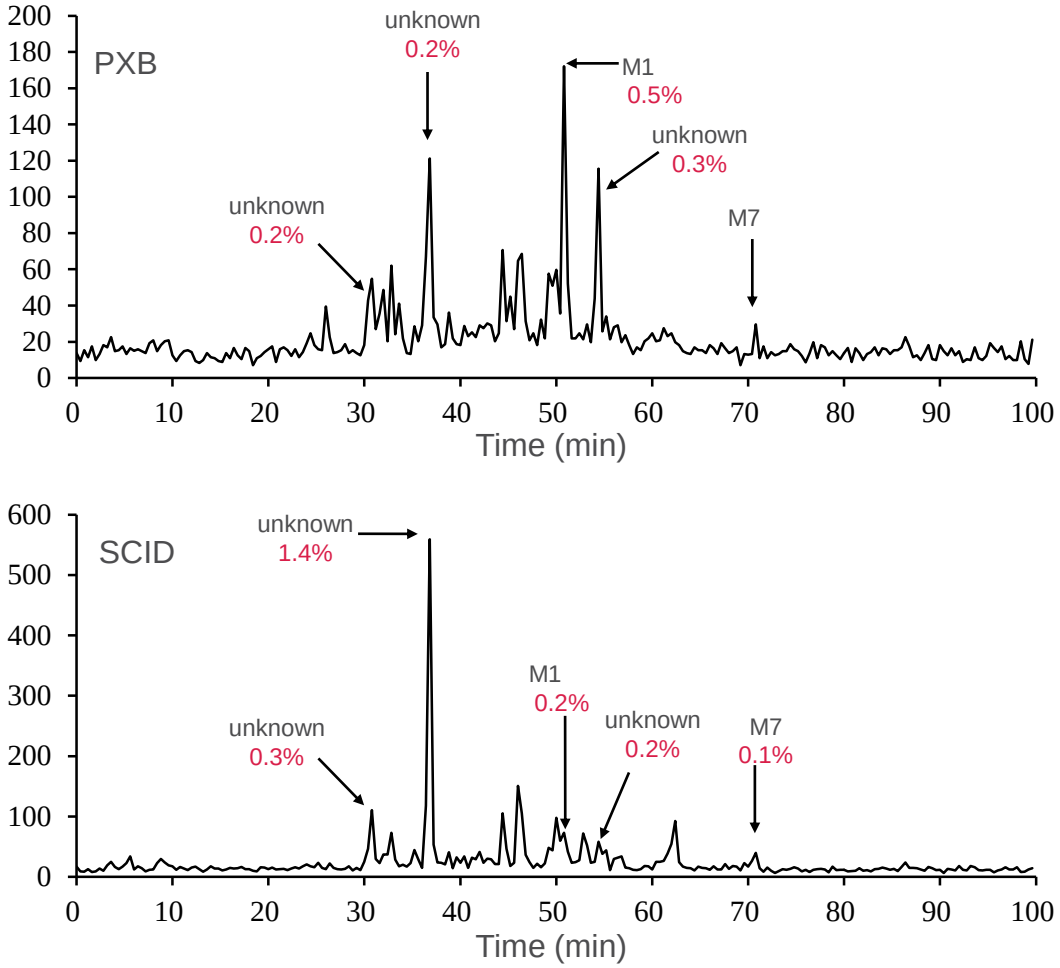






M1 was detected as a major metabolite in the plasma of PXB mice.

METABOLITE PROFILE IN URINE

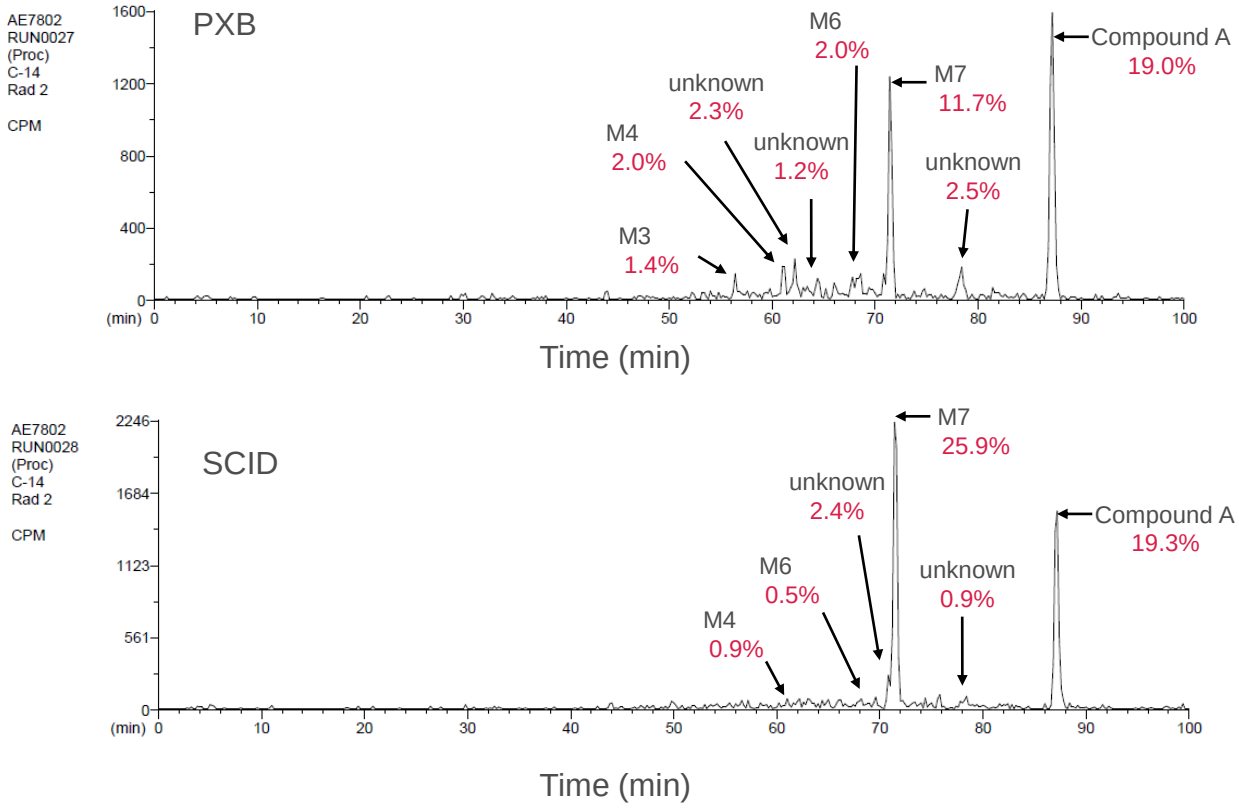


%: % of dose

M1 was also detected as a major metabolite in the urine of PXB mice.

METABOLITE PROFILE IN FECES

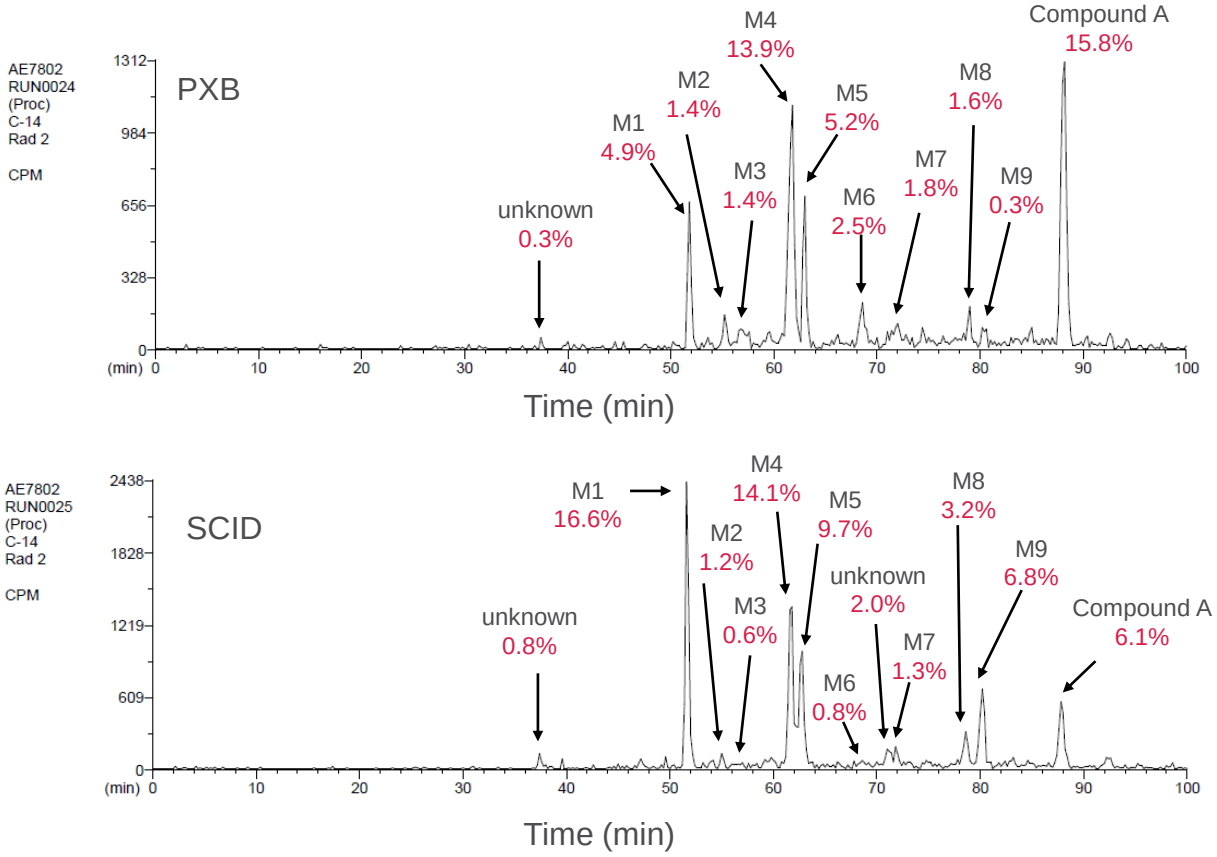
%: % of dose



M7 was detected as major metabolites in the feces of PXB and SCID mice.
Compound A was excreted in feces of PXB and SCID mice.

METABOLITE PROFILE IN BILE

%: % of dose



M1 (−CH3+Gluc), M4 (−CH3+SO3) and M5 (−2H) were detected as major metabolites in the bile of PXB and SCID mice. The biliary excretion of Compound A in PXB mice was observed (15.8% of dose), and it is higher than that in SCID mice. A direct glucuronidation conjugate, M9, was more generated in SCID mice than in PXB mice.

SHORT SUMMARY OF EXP. 2

Most of the radioactivity was excreted in the feces or bile.

M1 ($-CH_3$+Gluc), M4 ($-CH_3$+SO_3) and M5 ($-2H$) were detected as major metabolites in the bile of PXB and SCID mice.

The biliary excretion of Compound A in PXB mice was observed (15.8% of dose), and it is higher than that in SCID mice.

A direct glucuronidation conjugate, M9 (+Gluc), was more generated in SCID mice than in PXB mice.

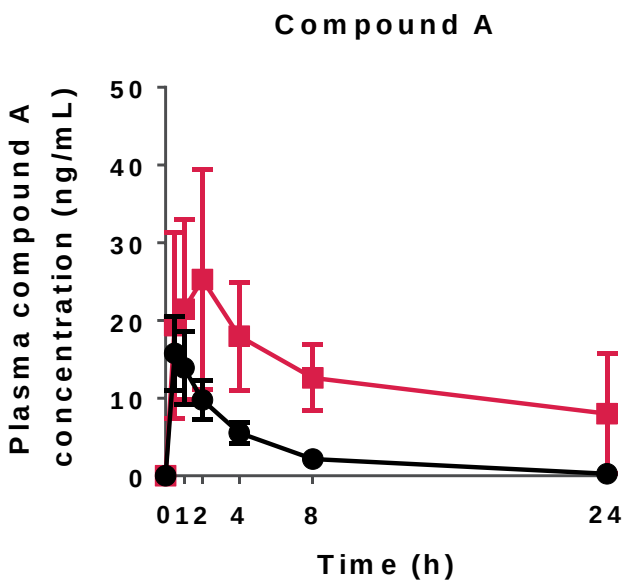
Given from the results, the major excretion pathways of Compound A in humans are supposed to be biliary excretion, oxidation and demethylation of the methoxy group.

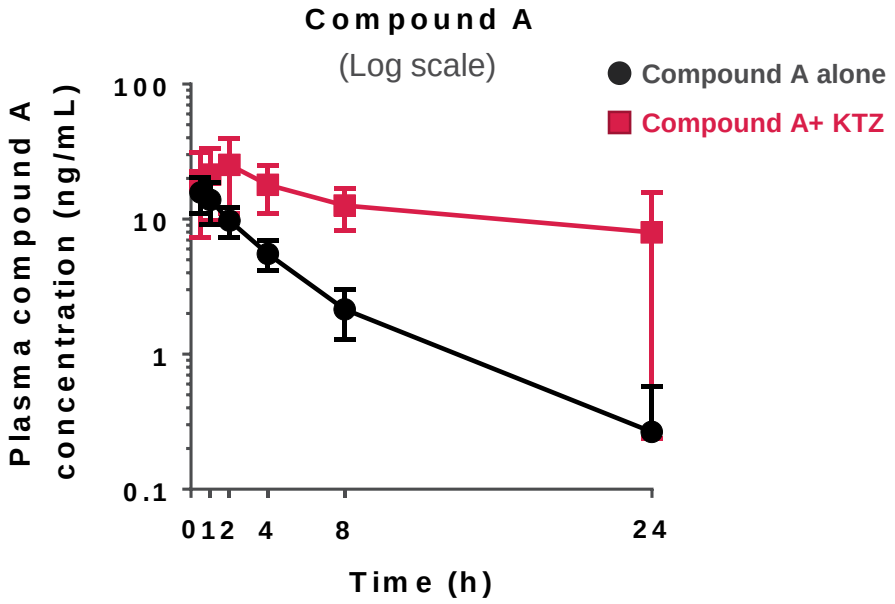
3. IN VIVO DDI STUDY USING CHIMERIC MOUSE WITH HUMANIZED LIVER

Exp. 3-1: DDI Study between Compound A and Ketoconazole

Animal	Chimeric mice with humanized liver (male, n = 4)
Test compound	Compound A
CYP3A inhibitor	Ketoconazole (KTZ)
Administration	Compound A (1 mg/kg), po
	1h pre-dosing of KTZ (0, or 100 mg/kg), po

EXP. 3-1: DDI STUDY BETWEEN COMPOUND A AND KETOCONAZOLE





PK parameters	Compound A alone	Compound A + KTZ	Ratio
C_{max} (ng/mL)	17.1 ± 5.3	29.6 ± 10.4	1.7
AUC_{24} (ng*h/mL)	73.2 ± 21.0	307.6 ± 90.3	4.2
AUC_{inf} (ng*h/mL)	71.9 ± 23.7	496.3 ± 369.7	6.9

Mean±SD (n = 3 - 4)

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COMPARISON BETWEEN CLINICAL OBSERVATION AND CHIMERIC MICE WITH HUMANIZED LIVER

Compound	AUC ratio (with/without strong CYP3A inhibitor)	
	Clinical study [90% CI]	Chimeric mice
Triazolam	40.7 (Ritonavir, 200 mg)	22.8*
	27.1 (Itraconazole, 200 mg)	
	22.4 (Ketoconazole, 400 mg)	
Compound A	2.0 [1.7 - 2.2] (Itraconazole, 200 mg)	6.9
Pitavastatin	0.8 (Itraconazole, 200 mg)	2.1*

AUC ratios of triazolam and pitavastatin in clinical studies were from The Metabolism and Transport Drug Interaction Database (DIDB) (https://www.druginteractioninfo.org/).

*: AUC_t

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UPDATE OF NONCLINICAL STUDY INDICATING CYP3A VICTIM RISK

Study	Results	Insight
Animal PK	Low urine and bile excretion of compound A in rats	Metabolism is main excretion route in rats ~~(and humans)~~
RI-ADME in rat In vivo metabolic profiling in rat plasma, urine and bile	Most of compound A and the related compounds were excreted into rat bile (unchanged drug peak is small in the rat bile)	Biliary excretion as metabolites is main excretion route in rats ~~(and humans)~~
In vitro metabolic profiling	UGT's low contribution to the metabolism (Glu peak is small in human hepatocytes)	CYP metabolism is main route in humans
CYP identification	Compound A is mainly metabolized by CYP3A.	CYP3A victim risk
BCRP substrate P-gp substrate	Compound A is a P-gp and BCRP substrate	Biliary excretion as unchanged drug (not use for Simcyp)
In vitro biliary excretion in human SCH	Compound A showed in vitro biliary excretion with BEI of about 10% in SCH.	Biliary excretion as unchanged drug
Mass balance study using PXB mice	The biliary excretion of Compound A in PXB mice was observed. A direct Glu was more generated in SCID mice than in PXB mice.	Biliary excretion as unchanged drug in PXB mice and difference in Glu conjugation between PXB and SCID mice
In vivo DDI study using PXB mice	The magnitude of the AUCinf change by KTZ was 6.9 fold. The fmCYP3A is suggested to be 86%.	Compound A may be a moderate or sensitive CYP3A substrate

CONCLUSION

The one of the major excretion pathways of Compound A in humans are supposed to be not only oxidation, demethylation but also biliary excretion (i.e., about 15%).

Since there are species difference between rat and human about biliary excretion (and glucuronidation), the GAP may be one of the reason for inducing the false positive about perspective Simcyp analysis before clinical DDI study.

The new ADME technology, e.g., PXB mice and in vitro SCH, will be useful for the assessment of victim DDI risk of a compound, which is excreted by multiple elimination routes including Phase I/II metabolism and biliary excretion in humans.

%HepCL	Cmax ratio	AUCinf Ratio
80%	1.3	3.4
Clinical Study Result	1.3	2.0

fu,gut = fp (0.001177)

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