Ketoconazole inhibition of midazolam metabolism to 1'OH-midazolam in the chimeric mouse with humanized liver and the SCID mouse

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INTRODUCTION

In human, CYP3A represents one of the most important subfamilies of the CYP superfamily and is the most abundantly expressed CYP in human liver. It is involved in the biotransformation of ~50% of metabolized drugs, and as a result, drug-drug interactions (DDI) associated with modulation of CYP3A-mediated metabolism are of clinical importance. The PXB-Mouse®, a chimeric mouse with a humanized liver, can be used as a predictor of human hepatic elimination and of the potential for DDI. Midazolam (MDZ) and ketoconazole (KCZ) are widely used as a probe and inhibitor, respectively, for evaluating the metabolism of CYP3A4 clinically and *in vitro*. The aim of this study was to evaluate the *in vivo* pharmacokinetics (PK) of MDZ, administered as a single *p.o.* dose, and its primary metabolite, 1'OH-MDZ, alone and when co-administered with KCZ in the PXB-Mouse® and SCID mouse as a control.

METHODS

In-life: PXB-mice (PhoenixBio Co, Ltd), with a replacement index of >70%, and SCID mice (Charles River Labs Japan) were used. MDZ was administered as a 12.5 mg/kg single *p.o.* dose alone and co-administered with KCZ (100 mg/kg and 250 mg/kg). Serial blood samples (25 µL) were collected under isoflurane anesthesia via the retro-orbital plexus/sinus, plasma isolated & stored at -80°C until analysis.

Sample analysis: Concentrations of KCZ, MDZ and 1'OH-MDZ in plasma samples (5 µL) were quantified by a qualified 3-in-1 LC-MS/MS method (Sciex 4000 QTrap, Agilent LC & CTC PAL autosampler), following the addition of a 3-in-1 internal standard solution (deuterated analogs) in 50/50 methanol/acetonitrile to precipitate proteins. A ZORBAX Eclipse XDB-C18, 2.1X30 mm, 3.5 µm column was used with gradient elution. Mobile phase A: 0.1% formic acid in water, B: 0.1% formic acid in methanol.

Data analysis: PK parameters were estimated for each mouse using non-compartmental analysis.

[1] Samuelson, K. *et. al*. Xenobiotica (2012) 42:1128.

RESULTS

Figure. 1. Mean (± SD, n=3) plasma concentration *versus* time curve of MDZ and it's formed metabolite, 1'OH-MDZ, following dosing to PXB and SCID mice.

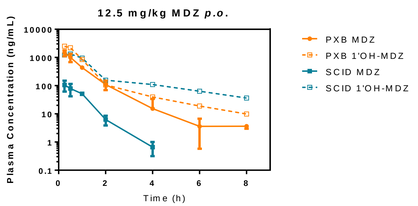

Table 1. Mean (± SD, n=3) PK parameters for MDZ and 1'OH-MDZ, determined for PXB and SCID mice following a single 12.5 mg/kg *p.o.* MDZ dose.

MDZ

Parameter	PXB-Mouse®	SCID Mouse
t_{max} (h)	0.250 ± 0.000	0.250 ± 0.000
C_{max} (ng/mL)	1460 ± 335	106 ± 44.0
Apparent $t_{1/2}$ (h)	n/a[a]	0.497 ± 0.0799
$AUC_{0\text{-}tlast}$ (h*ng/mL)	1170 ± 122	93.7 ± 23.1
$AUC_{0\text{-}inf}$ (h*ng/mL)	n/a	94.2 ± 22.8
$MRT_{0\text{-}inf}$ (h)	n/a	0.847 ± 0.0957

[a] na denotes not applicable.

1'OH-MDZ

Parameter	PXB-Mouse®	SCID Mouse
t_{max} (h)	0.333 ± 0.144	0.667 ± 0.289
C_{max} (ng/mL)	2590 ± 176	1410 ± 659
Apparent $t_{1/2}$ (h)	1.99 ± 0.154	2.60 ± 0.263
$AUC_{0\text{-}tlast}$ (h*ng/mL)	2170 ± 414	2000 ± 599
$AUC_{0\text{-}inf}$ (h*ng/mL)	2200 ± 402	2130 ± 587
$MRT_{0\text{-}inf}$ (h)	1.13 ± 0.278	2.44 ± 0.404
1-OH-MDZ/MDZ AUC ratio	1.85	22.6

RESULTS

Figure 2. Mean (± SD, n=4) plasma concentration *versus* time profiles of KCZ, MDZ and 1'OH-MDZ, in PXB and SCID mice following a single 12.5 mg/kg *p.o.* MDZ dose alone or co-administered with 100 mg/kg or 250 mg/kg KCZ.

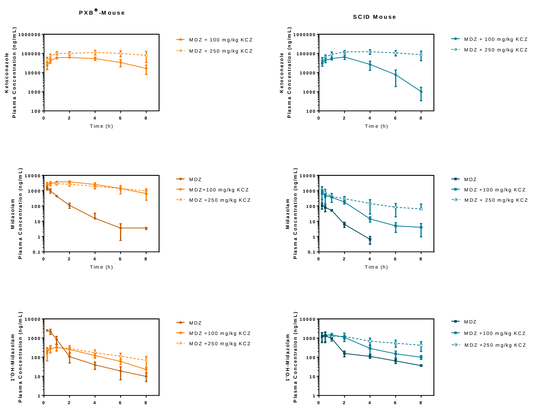

Table 2. Mean (± SD, n=4) PK parameters for KCZ, determined for PXB and SCID mice, following a single co-administered 12.5 mg/kg *p.o.* dose of MDZ and 100 mg/kg or 250 mg/kg KCZ.

Parameter	PXB-Mouse®		SCID Mouse	
	100 mg/kg KCZ	250 mg/kg KCZ	100 mg/kg KCZ	250 mg/kg KCZ
t_{max} (h)	1.50 ± 0.577	2.75 ± 1.50	1.75 ± 0.500	3.00 ± 1.15
C_{max} (ng/mL)	64500 ± 7050	115000 ± 28100	67900 ± 15100	129000 ± 27100
Apparent $t_{1/2}$ (h)	1.90 ± 0.489	3.69 (n=2)	0.831 ± 0.129	12.6 ± 12.4
$AUC_{0\text{-}tlast}$ (h*ng/mL)	347000 ± 60300	767000 ± 217000	222000 ± 67700	823000 ± 201000
$AUC_{0\text{-}inf}$ (h*ng/mL)	397000 ± 84100	834000 (n=2)	223000 ± 68300	2730000 ± 2110000
$MRT_{0\text{-}inf}$ (h)	4.32 ± 0.896	6.42 (n=2)	2.48 ± 0.323	19.4 ± 17.2
$AUC_{0\text{-}inf}$/Dose (h*kg*ng/mL/mg)	3970	3340	2230	10900

RESULTS

Table 3. Mean (± SD, n=4) PK parameters for MDZ and 1'OH-MDZ for PXB and SCID mice, determined following a single co-administered 12.5 mg/kg *p.o.* dose of MDZ and 100 mg/kg or 250 mg/kg KCZ.

MDZ

Parameter	PXB-Mouse®		SCID Mouse	
	100 mg/kg KCZ	250 mg/kg KCZ	100 mg/kg KCZ	250 mg/kg KCZ
t_{max} (h)	1.50 ± 0.577	0.688 ± 0.375	0.375 ± 0.144	0.688 ± 0.875
C_{max} (ng/mL)	4050 ± 472	3130 ± 496	754 ± 283	1010 ± 832
Apparent $t_{1/2}$ (h)	1.95 ± 0.613	4.86 ± 2.21	0.959 ± 0.129	3.67 ± 3.58
$AUC_{0\text{-}tlast}$ (h*ng/mL)	18200 ± 4550	15200 ± 3570	847 ± 39.2	1720 ± 686
$AUC_{0\text{-}inf}$ (h*ng/mL)	20300 ± 6120	22600 ± 7260	853 ± 41.5	2230 ± 1100
$MRT_{0\text{-}inf}$ (h)	3.79 ± 0.886	7.14 ± 3.00	1.34 ± 0.221	4.82 ± 3.84

1'OH-MDZ

Parameter	PXB-Mouse®		SCID Mouse	
	100 mg/kg KCZ	250 mg/kg KCZ	100 mg/kg KCZ	250 mg/kg KCZ
t_{max} (h)	0.875 ± 0.250	1.38 ± 0.750	0.625 ± 0.433	1.31 ± 1.80
C_{max} (ng/mL)	341 ± 115	339 ± 79.1	1600 ± 293	1390 ± 608
Apparent $t_{1/2}$ (h)	1.80 ± 0.449	3.14 ± 0.920	2.94 ± 1.19	7.46 ± 6.94
$AUC_{0\text{-}tlast}$ (h*ng/mL)	1130 ± 246	1440 ± 549	4260 ± 405	6300 ± 2070
$AUC_{0\text{-}inf}$ (h*ng/mL)	1200 ± 231	1770 ± 800	4650 ± 392	11700 ± 6590
$MRT_{0\text{-}inf}$ (h)	3.11 ± 0.691	4.72 ± 0.841	3.10 ± 0.174	10.6 ± 8.95
1'-OH-MDZ/MDZ AUC ratio	0.0591	0.0783	5.45	5.25

CONCLUSIONS

- A lower metabolic rate of human compared to mouse hepatocytes was confirmed by higher plasma exposure and lower 1'OH-MDZ/MDZ AUC ratio observed in the PXB *versus* SCID mouse (Fig. 1, Table 1). The latter ratio is more comparable to humans (0.5-0.8)[1].
- Plasma exposure of KCZ was dose-proportional in the PXB but not in SCID mouse (Table 2).
- KCZ increased exposure of MDZ and decreased the 1'OH-MDZ/MDZ AUC ratio in both PXB and SCID mouse (Fig. 2, Table 3).
- The PXB-Mouse® is a suitable model for the preclinical evaluation of human metabolism and DDI potential for compounds that are metabolized by CYP3A4.