Efficient use of chimeric mice with humanized livers for drug development support: a three consecutive experimental case study

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Introduction

Chimeric mice with humanized livers can be used to obtain supportive data for selecting the best candidate compound for new drug development. To utilize this costly animal model efficiently, we tried to verify that consecutive experiments could be conducted with the same animals to generate predictive human ADME.

Method

<Animals, dosing, sample collection>

The humanized TK-NOG chimeric mice were generated using the method of Hasegawa et al[1)]. TK-NOG mice with elevated serum ALT levels by intraperitoneal injections of GCV received transplants of1.0 x 10^6 of human hepatocytes by intrasplenic injection. First of all, a cholesterol lowering drug, torcetrapib, was administered orally to a group of three chimeric mice, and plasma concentration of a sequential metabolite, M4[2)], was analyzed. Following a recovery period of one week, a PPAR-gamma agonist, troglitazone, was dosed orally and the unchanged drug and its metabolites in plasma were measured[3)]. Another three-day washout period was placed and then a polyethylene tube was cannulated into the bile duct of the same animals. Troglitazone was given again orally and its glutathione conjugates[4)] in the bile were analyzed (Fig.1). This study was conducted in accordance with the regulations and guidelines of animal experiments specified by SMD.

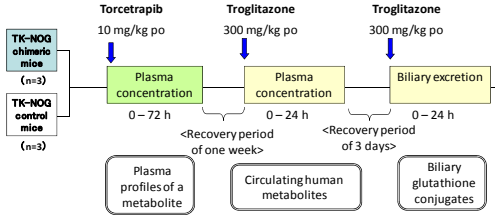

Fig. 1. Three consecutive administration studies using the same chimeric mice with humanized livers.

<Sample analyses>

Torcetrapib and M4 were quantified by LC-MS/MS. The analytical and standard samples were mixed with methanol, centrifuged. The supernatant was mixed with 10 mM ammonium acetate, and was injected into the column. The LC-MS/MS analysis was performed using the LC-20AD HPLC system coupled with an API-5000 tandem mass spectrometer. The column was InertSustain (C18, 40 °C), and a gradient elution was employed (10% to 95% B, 0.4 ml/min) (A: 10 mmol/L ammonium acetate solution, B: acetonitrile). Analytes were detected in the positive ion mode with electrospray ionization using multiple reaction monitoring: torcetrapib, $[M]^+$ m/z 599.1 → 227.0; M4, $[M]^+$ m/z 242.2 → 196.0. The retention times of torcetrapib and M4 were 3.2 and 2.3 minutes, respectively. The limits of quantification for torcetrapib was 1 ng/ml in plasma, and determination coefficient (R) was > 0.9950.

Troglitazone and its conjugates were also quantified by LC-MS/MS. To the analytical and standard samples, 20 µl of IS solution (rosiglitazone) was added. The mixture was centrifuged and the supernatant was mixed with 0.1% formic acid, and injected into the column. The LC-MS/MS analysis was performed using the LC-10Avp HPLC system (InertSustain C18) coupled with an API-4000 system. The analytes were eluted with a linear gradient (0.4 ml/min) (15% B to 90% B [10% formic acid (A), acetonitrile (B)] and were detected in the negative ion mode with electrospray ionization using multiple reaction monitoring: troglitazone, $[M]^-$ m/z 440.1 → 397.0; troglitazone sulfate, $[M]^-$ m/z 520.1→ 440.2; troglitazone glucuronide, $[M]^-$ m/z 616.2→ 440.2; rosiglitazone, $[M]^-$ m/z 356.0 → 313.1. The retention times of troglitazone, troglitazone sulfate and rosiglitazone were 2.1, 1.7 and 1.6 minutes, respectively. The limits of quantification were 10 and 1 ng/ml in plasma, and determination coefficients (R) were > 0.9977 and > 0.9998, for troglitazone and troglitazone sulfate, respectively.

To a 180 µl aliquot of pooled bile, 360 µl of 10 mM ammonium acetate was added. The mixture was filtrated and injected into the HPLC column (YMC-Triart, S-1.9 µm). Analytes were eluted by gradient (5% to 30% B, 0.2 ml/min) (A: 10 mmol/L ammonium acetate solution, B: acetonitrile). Troglitazone and its glutathione conjugates were monitored at their molecular ions listed in Table 1.

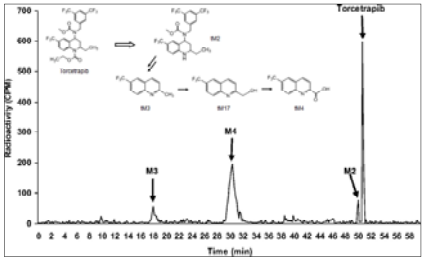

Fig. 2 An HPLC radiochromatogram of human plasma (0-12 h pooled) after single oral administration of [^{14}C]torcetrapib to healthy male volunteers at a dose of 120 mg[2)].

Results

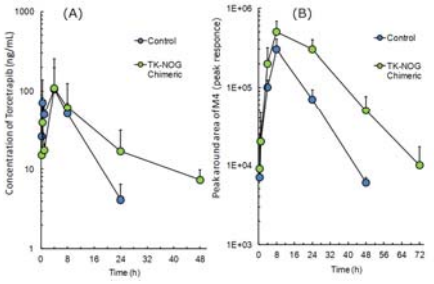

Fig. 3 Plasma concentration of torcetrapib (A) and M4 (B) after oral administration of 10 mg/kg torcetrapib to TK-NOG chimeric mice (-●-) and control NOG mice (-●-). Mean±SD of 3 animals.

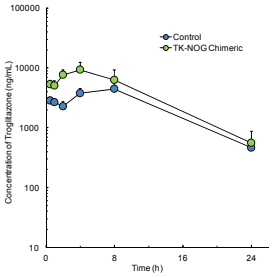

Fig. 4 Plasma concentration of troglitazone after oral administration of 300 mg/kg troglitazone to TK-NOG chimeric mice (-●-) and control NOG mice (-●-). Mean±SD of 3 animals.

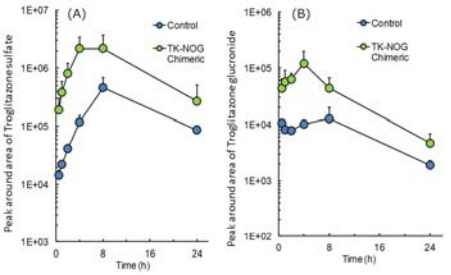

Fig. 5 Plasma concentration of troglitazon sulfate (A) and troglitazon glucuronide (B) after oral administration of 300 mg/kg troglitazone to TK-NOG chimeric mice (-●-) and control NOG mice (-●-). Mean±SD of 3 animals.

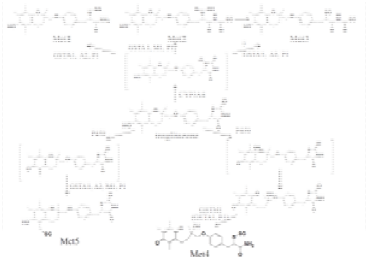

Fig. 6 Proposed metabolic activation pathways of troglitazone followed by GSH conjugation of the reactive products[5)].

Double null alleles of GSTM1 and GSTT1 were considered to be associated with the onset of Tro-DILI[6,7)].

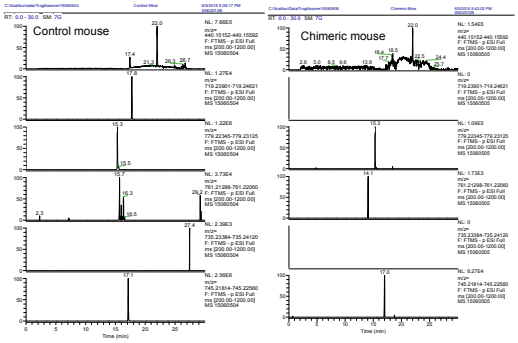

Fig. 7 Selected MS chromatograms of troglitazone GSH conjugates excreted into the bile in control and TK-NOG chimeric mice.

Table 1 Biliary excretion of various troglitazone GSH conjugates in control and TK-NOG chimeric mice.

metabolite		peak area	
	MH^-	Control	TK-NOG chimeric
troglitazone	440	4,560,441	618,822
Met1	719	40,106	ND
Met2	779	5,225,237	421,116
Met3	761	143846	ND
Met4	735	ND	ND
Met5	745	13,374,285	296,482

ND: not detected

Discussion

1. After treatment with torcetrapib, plasma levels of M4 was higher in chimeric mice than those in control mice, reflecting metabolic patterns of these drugs in humans.
2. After treatment with troglitazone, plasma levels of troglitazone sulfate was higher in chimeric mice than those in control mice, reflecting metabolic patterns of these drugs in humans.
3. Several types of glutathione conjugates of troglitazone were reportedly found in rat bile and *in vitro* incubation mixtures with human liver microsomes or hepatpcytes. Possible correlation of null mutation of GSTM1 and GSTT1 to the idiosyncratic hepatic toxicities of this drug was suggested. Therefore, it was of interest to compare species differences in *in vivo* experiments. However, no noticeable types of the glutathione conjugate metabolites were found in the bile of humanized chimeric mice, which suggested that these conjugates might not be excreted in humans.
4. During the consecutive administration period of ten days with repeated blood sampling and, finally, bile collection, no abnormalities of the chimeric mice were observed.

Humanized chimeric mice withstood at least three consecutive experiments as an efficient way of application giving useful information for new drug development.

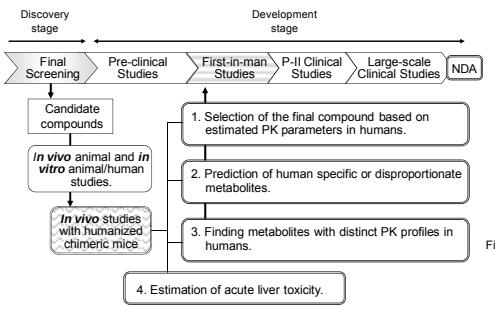

Fig. 8 Potential contributions of chimeric mice with humanized livers in new drug development.NDA, new drug applications[8)]

References

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