Humanized Thymidine Kinase–NOG Mice Can Be Used to Identify Drugs That Cause Animal-Specific Hepatotoxicity: A Case Study with Furosemide^S

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ABSTRACT

Interspecies differences have limited the predictive utility of toxicology studies performed using animal species. A drug that could be a safe and effective treatment in humans could cause toxicity in animals, preventing it from being used in humans. We investigated whether the use of thymidine kinase (TK)–NOG mice with humanized livers could prevent this unfortunate outcome (i.e., “rescue” a drug for use in humans). A high dose of furosemide is known to cause severe liver toxicity in mice, but it is a safe and effective treatment in humans. We demonstrate

that administration of a high dose of furosemide (200 mg/kg i.p.) causes extensive hepatotoxicity in control mice but not in humanized TK–NOG mice. This interspecies difference results from a higher rate of production of the toxicity-causing metabolite by mouse liver. Comparison of their survival curves indicated that the humanized mice were more resistant than control mice to the hepatotoxicity caused by high doses of furosemide. In this test case, humanized TK–NOG mouse studies indicate that humans could be safely treated with a high dose of furosemide.

Introduction

Interspecies differences in the drug metabolism and disposition pathways used by humans and animal species have limited the predictive utility of toxicology studies performed in animal species (Peltz, 2013). We previously demonstrated that the human-specific liver toxicity caused by fialuridine (Xu et al., 2014) and bosentan (Xu et al., 2015), which was not predicted by animal toxicology studies, could have been predicted if thymidine kinase (TK)–NOG mice with humanized livers (Peltz, 2013; Xu and Peltz, 2015) were used in toxicology studies; however, there are also drugs that are commonly used in humans that cause animal-specific toxicities. The different drugs that are selected for veterinary and human use result from interspecies differences in susceptibility to their toxicities. For example, cats are exquisitely sensitive to acetaminophen-induced liver toxicity (from a reduced ability to clear the drug via glucuronidation) (Court and Greenblatt, 2000); dogs and rodents are highly susceptible to the nephrotoxicity of non-steroidal anti-inflammatory agents (Khan et al., 1998). If these drugs were being developed today, toxicology studies in conventional animal species could have prevented their use in humans. The inability to use a drug that would have provided a safe and effective therapy for humans, resulting from a false-

positive result in an animal study, is a costly and unfortunate outcome.

Furosemide (4-chloro-*N*-furfuryl-5-sulfamoyl-anthranilic acid) is a potent diuretic that has been widely used for more than 40 years (Ponto and Schoenwald, 1990a,b). Although adverse events are due primarily to fluid and electrolyte disturbances, furosemide was selected as a test case because it causes species-specific hepatotoxicity. A single high dose of furosemide (200 mg/kg or greater) causes extensive liver necrosis in mice (Mitchell et al., 1974; Walker and McElligott, 1981) but not in rats (Williams et al., 2007) or hamsters (Mitchell et al., 1976). Furosemide-induced hepatotoxicity in mice is proportional to the extent of hepatic proteins bound by a cytochrome P450-generated epoxide metabolite of furosemide (Mitchell et al., 1976; Williams et al., 2007). The toxicity is increased when biliary furosemide excretion is blocked by inhibitors or is saturated after treatment with a high dose of the drug (Spitznagle et al., 1977), and it is not associated with glutathione depletion (Mitchell et al., 1974; Wong et al., 2000). Because the average human daily furosemide dose is usually less than 6 mg/kg, the high-dose hepatotoxicity in mice may not appear to be a significant concern; however, the S-shaped dose-response curve to loop diuretics is shifted to the right in patients with acute heart failure, which renders them less responsive to these agents. Administration of a high dose of furosemide (ranging from 250 mg to 1 g per day i.v.) has been shown to be an effective treatment of heart failure in these acute settings (Tuttolomondo et al., 2011). There are no reports of hepatotoxicity in humans after high-dose furosemide

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ABBREVIATIONS: ALP, alkaline phosphatase; ALT, alanine aminotransferase; TK, thymidine kinase; TK–NOG, a NOG mouse expressing a thymidine kinase transgene.

treatment. Allometric conversion indicates that a 1-g dose in a 65-kg human corresponds to a 200-mg/kg dose in a mouse (<http://www.fda.gov/downloads/Drugs/Guidances/UCM078932.pdf>). If high-dose furosemide was now being developed for treatment of acute heart failure, the hepatotoxicity appearing in mice could have prevented it from being used in humans. Therefore, we investigated whether studies using humanized TK-NOG mice would have correctly predicted that high-dose furosemide would not cause hepatotoxicity in humans (i.e., “rescue” this drug for use in humans).

Materials and Methods

Study Design. This study was designed to compare the response of control and humanized TK-NOG mice to treatment with a high dose (200 mg/kg) of furosemide.

Preparation and Characterization of Chimeric TK-NOG Mice. All animal experiments were performed according to protocols that were approved by the Stanford Institutional Animal Care and Use Committee, and the results are reported according to the Animal Research Reporting of In Vivo Experiments guidelines (Kilkenny et al., 2010). TK-NOG mice were obtained from and housed at In Vivo Sciences International (Sunnyvale, CA). TK-NOG mice with humanized livers were prepared by ganciclovir-conditioning and human hepatocyte transplantation using a previously described protocol (Hu et al., 2013). All mice used in this study were male. Human liver cells were transplanted when the mice were 8 weeks old, and cryopreserved human hepatocytes were obtained from Celsis In Vitro Inc. (Baltimore, MD). The chimeric mice, the hepatocyte donors, and the level of human serum albumin in the humanized mice 8 weeks after transplantation are shown in Supplemental Table 1. Only chimeric mice having a human plasma albumin level greater than 7.5 mg/ml were used in this study. The plasma human albumin level, which was previously shown to correlate with the extent of liver humanization, was measured by enzyme-linked immunoassay (Hasegawa et al., 2011).

Fig. 1. Treatment with a high dose of furosemide increases the plasma ALT level in control (but not in humanized) TK-NOG mice. Humanized ($n = 4$) or control male TK-NOG mice ($n = 5$) were treated with furosemide (200 mg/kg i.p.) or vehicle. Plasma ALT or ALP levels were measured before and 24 hours after dosing. In control TK-NOG mice, the pre-treatment plasma ALT level (62.5 ± 2.5 U/L) was increased to 1771 ± 467 U/L ($P = 0.01$) at 24 hours after furosemide dosing. In contrast, there was no change in the plasma ALP levels in humanized TK-NOG mice ($P = 0.7$) 24 hours after furosemide treatment. The plasma ALP levels were not altered in control ($P = 0.5$) or humanized mice ($P = 0.6$) after furosemide treatment. Each bar represents the average $\pm$ S.D. of measurements made in humanized or control mice.

Toxicology Study. Furosemide (Sigma-Aldrich, St. Louis, MO) was dissolved in a normal saline solution with 10% dimethylsulfoxide. Eight weeks after hepatocyte transplantation, control and humanized TK-NOG mice were treated with furosemide (200, 400, or 600 mg/kg i.p.) or vehicle (10% dimethylsulfoxide in normal saline). Blood was obtained from the mice before and 24 hours after dosing, and plasma was collected by centrifugation of the blood at 1910g for 10 minutes at 4°C. Plasma liver enzyme levels [alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] were measured using a Heska DryChem 7000 analyzer (HESKA, Loveland, CO) according to the manufacturer's instructions. The P values were determined using a two-sample, two-sided t test, which tests the significance of the observed differences between drug-treated and the corresponding vehicle-treated humanized mice. Liver tissue was obtained from control and humanized TK-NOG mice 24 hours after treatment with vehicle or furosemide. The liver tissue was fixed in 10% formalin, and sections of formalin-fixed paraffin-embedded liver tissue were stained with hematoxylin and eosin. The tissue sections were evaluated by a pathologist who was blinded to the type of mouse that was the source of the liver tissue. Mouse survival was monitored for 5 days after drug treatment. The statistical significance of the difference in the survival curves after treatment was compared using the “survival” package in R (version 3.1, www.r-project.com) for the log-rank test.

Analysis of Drug Disposition. Control and humanized mice were dosed with furosemide (200 mg/kg i.p.) and placed in individual metabolic cages (Hatteras Instruments, Inc. North Carolina) for 24 hours. During this period, feces and urine were collected for analysis. Liver and bile were obtained 24 hours after furosemide dosing. The plasma, urine, and bile samples were extracted with 3 volumes of cold

Fig. 2. Hematoxylin and eosin-stained sections of liver prepared from control or humanized male TK-NOG mice 24 hours after treatment with either furosemide (200 mg/kg i.p.) or vehicle. Each panel shows a central vein and the surrounding hepatocytes. In the vehicle-treated control mice (upper left), there is no evidence of necrosis in the hepatocytes surrounding the central vein, but there is acute hepatocyte necrosis in the furosemide-treated control mice (upper right). In contrast, no evidence of necrosis is seen in the human or mouse hepatocytes around the central vein in vehicle- (lower left) or furosemide-treated (lower right) humanized mice. In the humanized livers, human hepatocytes have a clear cytoplasm, whereas mouse hepatocytes have a pink cytoplasm (arrow). Original magnification, 300 $\times$.

TABLE 1

Amounts of furosemide in the liver, bile, and feces obtained from control and humanized male TK-NOG mice ($n = 4$ mice per group)

Measurements were done over a 24-hour period after administration of furosemide (200 mg/kg i.p.). Each bar represents the average $\pm$ S.E.M. percentage of the total furosemide dose present in each of the collected materials. Urine was the predominant route of furosemide elimination in humanized and control mice, and humanized and control mice eliminated similar amounts of the drug via biliary (feces and bile) and renal clearance pathways.

	Liver	Feces	Urine	Bile
Control	0.3 ± 0.05	0.5 ± 0.04	30.2 ± 7.91	0.5 ± 0.13
Humanized	0.2 ± 0.05	1.6 ± 0.08	26.3 ± 2.43	0.24 ± 0.07
<i>P</i> value	0.11	0.21	0.79	0.14

acetonitrile. D₅-furosemide (Toronto Research Chemicals, Toronto, ON, Canada) was added as internal standard to the extracts. After incubation at -20°C for 30 minutes, the mixtures were centrifuged at 15,000g for 10 minutes; the supernatants were transferred and dried in speed-vac. The dried pellets were resuspended in an equal volume of 5% acetonitrile and 0.1% formic acid and then analyzed on an Agilent QTOF 6520 (Agilent Technologies, Santa Clara, CA) coupled with Agilent infinity 1290 ultra-high-performance liquid chromatography.

A negative electrospray source was used in full-scan mode to monitor furosemide and its metabolites. Accurate mass and isotope pattern of chloride were used to ensure that the correct ions were identified. An Agilent Eclipse Plus C18 RRHD 1.8 μm column (2.1×100 mm; Agilent Technologies) was used with 5% acetonitrile and 0.1% formic acid as the A solvent and 100% acetonitrile and 0.1% formic acid as B solvent. Quantitative analysis of furosemide and its metabolites was performed using a calibration curve that was generated using control mouse plasma that was spiked with 9 to 5000 ng/ml of furosemide and treated as described already herein. The relative amounts of furosemide and its metabolites in each sample were calculated using the assumption that all compounds had the same mass spectrometry response factor. Agilent MassHunter Quantitative Analysis software (Agilent Technologies) was used to analyze the data. The major metabolites were identified according to Williams et al. (2007). The mass-to-charge ratio (m/z) for furosemide and its metabolites was calculated based on their molecular formula. The mass accuracy of the instrument was less than 20 ppm by reference ions. Differences in the amounts of furosemide and metabolites in control or humanized mouse samples were analyzed using a two-sample two-sided t test (on log-transformed data) or a Mann-Whitney test). The latter test was applied to compare the amount of the γ -ketocarboxylic acid metabolite in liver and urine samples because this test was a more appropriate

Fig. 3. (A) A diagram of the pathways used for metabolism of furosemide. Furosemide can be converted to an *N*-dealkylated structure, conjugated with glucuronic acid, or converted to an epoxide that causes hepatotoxicity by reacting with cellular proteins (Williams et al., 2007). The epoxide can be conjugated with glutathione or converted into a γ -ketocarboxylic acid, which is a marker for the formation of the epoxide. (B) The relative amounts of the γ -ketocarboxylic acid metabolite of furosemide in bile and liver samples collected 24 hours after four control or four chimeric male TK-NOG mice were treated with furosemide (200 mg/kg i.p.) are shown. For the bile and urine samples, each bar represents the average $\pm$ S.D. of four independent measurements. For the liver samples, which had much lower amounts of this metabolite, each symbol represents the result obtained from one mouse. The ion suppression caused by the large amount of bile acids present in bile prevented us from determining the γ -ketocarboxylic acid concentration in bile (present in very low abundance) relative to the standard curve. Therefore, the amount of γ -ketocarboxylic acid metabolite in bile is shown as its relative abundance. The amount of the γ -ketocarboxylic acid metabolite in urine ($P = 0.028$), bile ($P = 0.04$), and liver ($P = 0.028$) was significantly increased in samples obtained from control mice relative to humanized mice.

statistical test than the *t* test. In the liver and urine samples, the dynamic range of the amount of this metabolite was very large, and the data distribution was highly non-Gaussian even after a proper transformation of the data. For the bile, samples from only three control mice and three humanized mice could be obtained for analysis (because of the difficulty in obtaining a sufficient amount of bile). Because of the small sample size, the Mann-Whitney test was underpowered: even though the value in one group was always higher than that in the other group, the *P* value was 0.1. Therefore, we applied the *t* test on the log-transformed signal.

Results

To determine whether furosemide-induced hepatotoxicity was a mouse-specific phenomenon, five male control and four highly humanized male TK-NOG mice were treated with a single dose of furosemide (200 mg/kg i.p.), and the plasma ALT and ALP levels were measured before and 24 hours after drug treatment. The pretreatment plasma ALT levels in control TK-NOG mice were within normal limits (62.5 ± 2.5 U/l) but were significantly increased ($P = 0.01$) 24 hours after furosemide dosing (1771 ± 467 U/l). In contrast, there was no change in ALT levels in humanized TK-NOG mice after they received furosemide ($P = 0.7$) (Fig. 1). Of note, the baseline ALT levels in humanized mice can be mildly elevated because ganciclovir conditioning, which causes damage to mouse hepatocytes, is used to prepare the TK-NOG mice for human transplantation. Their ALT levels decline with time after the human cells are transplanted, and the ALT values were well within normal limits at baseline. Consistent with a direct toxic effect on mouse hepatocytes, furosemide treatment did not alter the plasma ALP levels in control ($P = 0.5$) or humanized ($P = 0.6$) TK-NOG mice (Fig. 1).

Liver tissue obtained from control and humanized mice 24 hours after treatment with vehicle or furosemide was examined. There was acute hepatocyte necrosis in the area surrounding the central veins in liver tissue obtained from furosemide-treated control mice, but there was no evidence of this (or any other type of toxicity) in liver tissue obtained from furosemide-treated humanized mice. There was also no evidence of hepatocyte necrosis in livers obtained from any vehicle-treated mice (Fig. 2). Given the selective susceptibility of mouse hepatocytes, we carefully examined areas in the humanized liver that had both mouse and human hepatocytes near the central vein. Interestingly, there was no evidence of necrosis in the mouse hepatocytes in the livers of furosemide-treated humanized mice. Of note, increased vacuolization is commonly seen in the cells within the humanized areas of the chimeric liver.

Interspecies Difference in Furosemide Metabolism.

We previously demonstrated that humanized TK-NOG mice could be used to characterize interspecies differences in drug metabolism (Nishimura et al., 2013) and disposition (Xu et al., 2015). Therefore, we compared the furosemide clearance pathways in control and humanized male TK-NOG mice by measuring the amount of furosemide and its metabolites in urine and feces collected over the 24-hour period after administration of furosemide (200 mg/kg i.p.). Liver and bile samples were also obtained 24 hours after furosemide dosing. Renal elimination was the predominant disposition pathway in both control and humanized mice, and the percentage of furosemide eliminated via biliary clearance (i.e., in feces and bile)

and renal clearance was the same in control and humanized mice (Table 1).

Furosemide metabolites have been characterized (Fig. 3A), and furosemide toxicity in mice has been attributed to the cytochrome P450-mediated generation of an activated epoxide metabolite, which damages hepatocytes through binding to cellular proteins (Williams et al., 2007). The epoxide is a transient intermediate that is rapidly converted to a γ -ketocarboxylic acid, which is a marker for epoxide formation. Of note, there was a 47-fold (Mann-Whitney test, $P = 0.028$; Fig. 3B) and 8-fold increase (two-sample *t* test, $P = 0.04$; Fig. 3B) in the amounts of the γ -ketocarboxylic acid metabolite in the liver and bile of control mice, respectively, relative to humanized mice. Moreover, there was a 3-fold increase in the total amount of the γ -ketocarboxylic acid metabolite in the urine (collected over a 24-hour period) of control mice relative to humanized mice (Mann-Whitney test, $P = 0.03$; Fig. 3B). Although no difference was seen in the amount of the glucuronide conjugated metabolite, the dealkylated metabolite was increased in liver ($P = 0.03$) and urine ($P = 0.02$; Table 2) obtained from control mice relative to humanized mice. The very small amount of the glutathione-conjugated metabolite in these samples is consistent with the fact that furosemide-induced liver toxicity is not associated with glutathione depletion (Mitchell et al., 1974; Wong et al., 2000). Thus, the increased amount of the γ -ketocarboxylic acid metabolite in liver urine and bile obtained from control mice indicates that they produce more of the epoxide metabolite that causes hepatotoxicity.

Dose-Response and Survival Curves. We also examined the liver toxic effect of increasing doses of furosemide by treating control ($n = 4$ per group) and humanized male TK-NOG ($n = 4$ per group) mice with 400 or 600 mg/kg furosemide administered intraperitoneally. The plasma ALT levels of the mice were measured before and 24 hours after drug treatment. The plasma ALT levels measured in control mice were significantly increased ($P = 0.0002$) from 60 ± 6.1 U/l (pretreatment) to 1635 ± 758.7 U/l at 24 hours after treatment

TABLE 2

Disposition pattern for the three major metabolites of furosemide

Furosemide is metabolized into three different metabolites in mice (Williams et al., 2007): 1) a dealkylated metabolite (dealkylated-FS); 2) a glucuronide (FS-glucuronide); and 3) a ketocarboxylic acid metabolite, which can also be conjugated with glutathione (glutathione-FS). Their amounts in urine and feces obtained from control and humanized male TK-NOG mice ($n = 4$ mice per group) were measured over a 24-hour period after administration of furosemide (200 mg/kg i.p.). The liver samples were obtained 24 hours after furosemide dosing. Each data point represents the average of the relative abundance of the indicated metabolite in each of the collected materials obtained from control or humanized mice. *P* values for the difference between the values obtained for control and humanized TK-NOG mice are also shown.

		Liver	Feces	Urine
Dealkylated-FS	Control	7.02	1.82	54.40
	Humanized	2.49	4.60	34.13
	Ratio	2.8	0.4	1.6
	<i>P</i> value	0.032	0.171	0.024
Ketocarboxylic acid	Control	1.50	0.02	100.82
	Humanized	0.03	0.57	38.34
	Ratio	47.3	0.04	2.6
	<i>P</i> value	0.028	0.324	0.028
Glucuronide-FS total	Control	9.26	0.03	206.11
	Humanized	2.34	13.07	249.17
	Ratio	4.0	0.003	0.8
	<i>P</i> value	0.291	0.328	0.681
FS-glutathione	All below the level of detection			

with the 400-mg/kg dose of furosemide. This furosemide dose caused a smaller increase ($P = 0.006$) in plasma ALT levels in the humanized mice from 108 ± 10.4 U/l (pretreatment) to 402 ± 69.6 U/l at 24 hours after treatment (Fig. 4A). All the control mice were dead within 24 hours after treatment with the 600-mg/kg doses of furosemide, which precluded measurement of their ALT levels. In contrast, all the humanized mice survived for 24 hours after dosing, but their plasma ALT levels were significantly increased ($P = 0.004$) from 116 ± 10.1 U/l (pretreatment) to 1856 ± 493.2 U/l at 24 hours after dosing (Fig. 4B). The survival rates for the control and humanized TK-NOG mice after treatment with the 200-, 400-, or 600-mg/kg doses of furosemide were measured over a 5-day period (Fig. 5). All the humanized mice and 75% of the control mice survived for 5 days after treatment with a 200-mg/kg dose furosemide. All the humanized mice survived for the 5-day period after treatment with a 400-mg/kg dose furosemide. Survival of the humanized mice indicates that there was no evidence that any type of delayed toxicity developed in the humanized mice that were treated with the 200- or 400-mg/kg doses of furosemide. All the control mice that were treated with 600- or 400-mg/kg doses of furosemide died within 24 or 48 hours, respectively. In contrast, all the humanized mice survived for 48 hours after treatment with the 600-mg/kg dose of furosemide but were dead by 96 hours. The survival curves after treatment with the 400- ($P = 0.01$) and 600-mg/kg ($P = 0.008$) doses of furosemide were significantly prolonged in the humanized mice relative to control mice. Moreover, there was evidence of a dose-dependent effect furosemide on the survival of humanized mice; their survival after treatment with the 600-mg/kg dose of furosemide was reduced relative to that after the 400-mg/kg dose ($P = 0.01$).

Fig. 4. Control ($n = 4$ per group) or humanized male TK-NOG ($n = 4$ per group) mice were treated with 400 mg/kg (A) or 600 mg/kg (B) furosemide i.p., and their plasma ALT levels were measured before and 24 hours after drug treatment. The plasma ALT levels in control mice were significantly increased ($P = 0.0002$) from 60 ± 6.1 U/l (pretreatment) to 1635 ± 758.7 U/l at 24 hours after treatment with a 400-mg/kg dose of furosemide. This dose also caused a significant ($P = 0.006$) but much smaller increase in plasma ALT levels in the humanized mice from 108 ± 10.4 U/l (pretreatment) to 402 ± 69.6 U/l at 24 hours after treatment. (B) All control mice were dead within 24 hours after treatment with the 600-mg/kg doses of furosemide, which precluded measurement of their ALT levels. In contrast, all humanized mice survived for 24 hours after dosing, but their plasma ALT levels were significantly increased ($P = 0.004$) from 116 ± 10.1 U/l (pretreatment) to 1856 ± 493.2 U/l at 24 hours after dosing. Each bar represents the average $\pm$ S.D. of measurements made in humanized or control mice.

Fig. 5. Survival rates for control ($n = 4$ per group) or humanized ($n = 4$ per group) male TK-NOG mice after treatment with 200-, 400-, or 600-mg/kg i.p. furosemide were measured over a 5-day period. All the humanized mice and 75% of the control mice survived for 5 days after treatment with 200-mg/kg furosemide. All the control mice that were treated with 600- or 400-mg/kg furosemide died within 24 or 48 hours, respectively. All humanized mice survived for 48 hours after treatment with the 600-mg/kg dose of furosemide but were dead by 96 hours. Survival curves after treatment with the 400- ($P = 0.01$) and 600-mg/kg ($P = 0.008$) doses of furosemide were significantly prolonged in the humanized mice relative to control mice. Moreover, survival of the humanized mice after treatment with the 600-mg/kg dose of furosemide was reduced relative to the 400-mg/kg dose ($P = 0.01$).

Discussion

This study demonstrates how TK-NOG mice with humanized livers could be used to efficiently assess the safety a drug that caused hepatotoxicity in mice. In this test case, a simple comparison of the responses of control and humanized mice demonstrated that it is highly likely that humans could be safely treated with a high dose of furosemide. Comparison of the dose-response and the survival curves of humanized and control mice confirmed that control mice had markedly increased sensitivity to the hepatotoxicity caused by treatment with high-dose furosemide. Because of interspecies differences in drug metabolism and drug disposition pathways, some of the drugs that cause toxicities in rodents or in other animal species will be safe for human use. Given the safety concerns associated with the drug development process of today, however, toxicity observed in an animal could easily prevent a safe and effective drug from being used in humans. Rodent-specific drug toxicities are usually addressed by performing toxicity studies in multiple other animal species and then hoping that the results obtained in the other species will somehow be a better indicator of whether a drug is safe for human use. We believe that testing these drugs in humanized mice provides a far superior method for assessing their safety for human use.

This study also demonstrates how humanized TK-NOG mice can be used to characterize the mechanisms underlying a species-specific drug-induced liver toxicity. Control mouse liver produces a markedly increased amount of the metabolite that causes the liver toxicity than the humanized liver. This explains why humans do not develop hepatotoxicity after treatment with high-dose furosemide. The mechanistic understanding

provides important information that supports the indication that this drug will not cause hepatotoxicity in humans. Our prior toxicology studies using humanized mice examined two drugs [fialuridine (Xu et al., 2014) and bosentan (Xu et al., 2015)] that caused liver toxicity in humans, which was not predicted by conventional animal toxicology testing. In those instances, the use of humanized mice could have provided important additional information that would improve the safety of drugs that will be administered to humans. In this study, we demonstrate how their use could prevent rodent-specific toxicity from blocking the use of a safe and effective drug in humans.

Authorship Contributions

Participated in research design: Peltz, Xu.

Conducted experiments: Xu, Wu, Takeda.

Performed data analysis: Xu, Wu, Michie, Zheng.

Wrote or contributed to the writing of the manuscript: Peltz, Michie, Wu, Xu.

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