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Species differences in drug glucuronidation: Humanized *UDP-glucuronosyltransferase 1* mice and their application for predicting drug glucuronidation and drug-induced toxicity in humans

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Abstract

More than 20% of clinically used drugs are glucuronidated by a microsomal enzyme UDP-glucuronosyltransferase (UGT). Inhibition or induction of UGT can result in an increase or decrease in blood drug concentration. To avoid drug-drug interactions and adverse drug reactions in individuals, therefore, it is important to understand whether UGTs are involved in metabolism of drugs and drug candidates. While most of glucuronides are inactive metabolites, acyl-glucuronides that are formed from compounds with a carboxylic acid group can be highly toxic. Animals such as mice and rats are widely used to predict drug metabolism and drug-induced toxicity in humans. However, there are marked species differences in the expression and function of drug-metabolizing enzymes including UGTs. To overcome the species differences, mice in which certain drug-metabolizing enzymes are humanized have been recently developed. Humanized *UGT1* (*hUGT1*) mice were created in 2010 by crossing *Ugt1*-null mice with human *UGT1* transgenic mice in a C57BL/6 background. *hUGT1* mice can be promising tools to predict human drug glucuronidation and acyl-glucuronide-associated toxicity. In this review article, studies of drug metabolism and toxicity in the *hUGT1* mice are summarized. We further discuss research and strategic directions to advance the understanding of drug glucuronidation in humans.

Keywords

UDP-glucuronosyltransferase; Glucuronidation; Species difference; Humanized mice; Drug metabolism; Toxicity

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1. Introduction

Most drugs are subject to metabolism by xenobiotic-metabolizing enzymes in the body. While enzymes that catalyze oxidation, reduction, and hydrolysis of drugs are categorized as phase I drug-metabolizing enzymes, enzymes that catalyze conjugation reactions such as glucuronidation, sulfation, and glutathionation are categorized as phase II drug-metabolizing enzymes. UDP-glucuronosyltransferases (UGTs) are enzymes that catalyze a transfer of glucuronic acid from a co-substrate UDP-glucuronic acid to substrates [1]. More than 20% of clinically used drugs are glucuronidated by UGTs; therefore, UGTs have been recognized as the major drug-metabolizing enzymes [2]. UGT1 and UGT2 family enzymes are primarily involved in drug glucuronidation, while contribution of two other UGT families UGT3 and UGT8 to drug metabolism is minimum and they rather play a role in glucuronidation and glycosylation of endogenous substances such as bile acids [3,4]. When UGT-catalyzed glucuronidation is the sole or major metabolic pathway of drugs, inhibition or induction of UGT can result in an increase or decrease in blood drug concentration [5,6]. Genetic polymorphisms on the *UGT* genes can also result in an increase in blood drug concentration [7]. To avoid drug-drug interactions and adverse drug reactions in individuals, therefore, it is important to understand whether UGTs are involved in metabolism of drugs and drug candidates.

UGTs catalyze the transfer of glucuronic acid to oxygen, nitrogen, or sulfur atom of their substrates. While most forms of glucuronides are less biologically active, acyl-glucuronide, which is formed by glucuronidation of the carboxylic group of substrates, has been known as a reactive metabolite [8]. Accumulating evidences indicate that the formation of acyl-glucuronide can be involved in the carboxylic acid-containing drug-induced toxicity [9]. UGTs glucuronidate not only xenobiotics but also various endogenous substances including a thyroid hormone thyroxine [3,5,3',5'-*I*-tetraiodothyronine (T_4)], which plays a crucial role in brain development during the perinatal period [10]. Induction of T_4 -glucuronidating UGT1A1 by co-administered drugs can accelerate the metabolism of T_4 and cause a delay in brain development [11]. In the meantime, inhibition of bilirubin glucuronidation by co-administered drugs can result in increased blood level of neurotoxic bilirubin [12]. Importantly, certain drug-induced toxicity where UGT-mediated glucuronidation is involved can be uniquely observed in humans. These data indicate that a deeper understanding of drug glucuronidation and regulation of UGTs by drugs in humans is needed to prevent the drug-induced toxicity.

There are several tools that are used to understand the glucuronidation of drugs in humans. Recombinant human UGTs expressed in cells such as Sf9 insect cells and HEK293 cells are powerful tools to determine UGT isoforms that are involved in glucuronidation of substrates [13]. However, it should be noted that the enzyme activities in cells overexpressing UGTs can be lower than those supposed to be in a physiological condition [14]. UGT proteins are highly glycosylated and the protein glycosylation regulates the folding and activity of UGTs [15], indicating that inadequate post-translational modification might be attributed to the underlying mechanism of the lowered activity in UGT-overexpressed cells especially in non-mammal cells. In addition, inadequate protein interaction, which is another key post-translational modification of UGT proteins, might also contribute to the low enzyme activity

in the UGT-overexpressed cells [16–20]. Human tissue microsomes, such as liver and small intestine microsomes, are frequently used to estimate the human glucuronidation in the tissues. Although there is a positive correlation between clearance values of drugs that are estimated from drug glucuronidation in human liver microsomes and observed hepatic clearances of drugs *in vivo*, the data indicate that drug glucuronidation in liver microsomes is somewhat lower than that observed *in vivo* [21]. Meanwhile, it has been shown that clearances estimated from drug glucuronidation in primary cultured hepatocytes well correlate with observed drug clearances [22]. However, there is a wide inter-laboratory variability in drug glucuronidation in hepatocytes. In addition to genetic polymorphisms and health condition of donors, storage and culture conditions of hepatocytes and time lag from the death of donors to the preparation of hepatocytes (freshness) and cryopreservation might be dominant to explain the inter-batch difference in the enzymatic activities of hepatocytes.

It has been demonstrated that animal scale-up is a relatively reliable method for predicting *in vivo* human drug metabolism [23]. Among a variety of animals, rodents are still most often used in pharmacokinetics studies and toxicity testing. However, there are marked species differences between humans and rodents in the expression and function of drug-metabolizing enzymes including UGTs [24–26]. To overcome the species differences, chimeric mice with humanized livers were established by transplanting human hepatocytes into a urokinase-type plasminogen activator^{+/+}/severe combined immunodeficient transgenic mouse line [27]. The replacement of their livers with human hepatocytes ranged from 80% to 90% in the chimeric mice and they exhibited human-like drug metabolism and disposition in the livers. Importantly, tissues such as intestine and kidneys also contribute to glucuronidation of drugs *in vivo* as a number of UGT1 and UGT2 family enzymes are highly expressed in extrahepatic tissues [28–30], indicating that alternative animal models are needed to reliably estimate *in vivo* drug glucuronidation in humans.

Recently, animals in which certain drug-metabolizing enzymes are humanized have been developed. Cytochrome P450s (CYPs) are the major phase I drug metabolizing enzymes. Humanized CYP3A4 mice, humanized CYP2E1 mice, and many other humanized CYP mice have been utilized to understand drug oxidation in humans [31]. Humanized *UGT1* (*hUGT1*) mice were created in 2010 by crossing *Ugt1*-null mice with human *UGT1* transgenic mice in a C57BL/6 background [32–34]. *hUGT1* mice can be promising tools to predict drug glucuronidation and drug-induced toxicity in humans. In this review article, studies of drug metabolism and toxicity in the *hUGT1* mice are summarized. We further discuss research and strategic directions to advance the understanding of drug glucuronidation in humans.

2. Species differences in the expression and function of UDP-glucuronosyltransferase 1 (UGT1)

The human *UGT1* gene is located on chromosome 2q37, producing nine functional enzymes, UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10, by exon sharing (Fig. 1) [35]. UGT1A1 is the most extensively studied UGT1 family enzyme due to its involvement in bilirubin metabolism. Substrates of

UGT1A1 are relatively bulky like bilirubin, but it also glucuronidates smaller molecules such as 1-naphthol and 4-methylumbelliferone. Although UGT1A1 is predominantly expressed in the liver, which is the main tissue in metabolism of xenobiotics, it is also expressed in many other tissues including small and large intestine, kidneys, lung, skin, and brain [36]. UGT1A3, UGT1A4, UGT1A6, and UGT1A9 are also UGT1 family enzymes that are expressed in the liver. These hepatic UGT1 family enzymes except for UGT1A4 are largely involved in *O*-glucuronidation of various drugs including SN-38, which is an active metabolite of irinotecan (CPT-11) [2,37]. In contrast, UGT1A4 as well as UGT1A3 are the major UGT1 isoforms that can glucuronidate tertiary amines [38,39]. While contribution of these hepatic UGT isoforms to glucuronidation of drugs is greater, UGT isoforms expressed in extrahepatic tissues such as small intestine and kidneys are also important in drug metabolism. UGT1A8 and UGT1A10 are mainly expressed in the small intestine and large intestine. These two isoforms are critically involved in intestinal metabolism of raloxifene [40,41].

The human *UGT1* locus is conserved in mice [3] and the mouse *Ugt1* gene generates 8 functional *Ugt1a* isoforms, *Ugt1a1*, *Ugt1a2*, *Ugt1a5*, *Ugt1a6a*, *Ugt1a6b*, *Ugt1a7c*, *Ugt1a9*, and *Ugt1a10* (Fig. 1). Since *Ugt1a4* is a pseudogene in mice and rats (Fig. 1), they exhibit extremely low *N*-glucuronidation toward tertiary amines [42]. The tissue distribution of the *Ugt1a* family isoforms is generally similar to that observed in humans. In mouse livers, *Ugt1a1* and *Ugt1a6* are predominantly expressed, while *Ugt1a5* and *Ugt1a9* are moderately expressed in the tissue [43]. Although mouse *Ugt1a2* was highly expressed in the kidneys, such expression was only observed in female mice. Human UGT1A7 has been identified as an isoform expressed in stomach and lung [36]. Meanwhile, mouse *Ugt1a7* is highly expressed in various extrahepatic tissues such as kidneys, small intestine, and large intestine, as well as stomach [43]. *Ugt1a10* is not intestine specific isoforms in mice, but is expressed in a wide variety of tissues including liver, small and large intestines, kidney, lung, heart, brain, and placenta. Similarly, expression of *Ugt1a8* in various tissues is reported [43]; however, the examined isoform might be different from *Ugt1a8* (Fig. 1).

Except for *N*-glucuronidation of tertiary amines, most of the substrates of human UGTs are glucuronidated by mouse *Ugt* family enzymes. However, kinetic parameters of certain glucuronidation reactions are different between humans and mice. In human liver microsomes, furosemide *O*-glucuronidation follows the Hill equation, showing $S_{50} = 681 \mu\text{M}$, $V_{\text{max}} = 576 \text{ pmol/min/mg}$, Hill coefficient = 1.3, and $CL_{\text{max}} = 0.50 \mu\text{L/min/mg}$ [44]. In contrast, the furosemide *O*-glucuronidation in mouse liver microsomes follows the Michaelis–Menten equation with a substrate inhibition and exhibits $K_m = 405 \mu\text{M}$, $V_{\text{max}} = 998 \text{ pmol/min/mg}$, $K_i = 12.2 \text{ mM}$, and $CL_{\text{int}} = 2.47 \mu\text{L/min/mg}$, indicating that the clearance of furosemide is 5-fold greater in mouse liver microsomes. Similarly, clearances of *S*-naproxen and ibuprofen *O*-glucuronidations in mouse liver microsomes are approximately 3-fold greater than those in human liver microsomes [44]. In contrast, the clearance value of etodolac *O*-glucuronidation in mouse liver microsomes is 3-fold lower than that in human liver microsomes [45]. These differences in the clearance parameters are mainly attributed to different K_m and V_{max} values between the species. In the ibuprofen *O*-glucuronidation, human liver microsomes exhibit a higher K_m value. Meanwhile, human liver microsomes show lower K_m values in etodolac and diclofenac *O*-glucuronidations [45].

There is also a species difference in glucuronidation of endogenous substances. Bilirubin, which is an end product of heme catabolism, is specifically glucuronidated by UGT1A1 [46]. Human neonates physiologically develop mild unconjugated hyper-bilirubinemia due to an inadequate UGT1A1 expression in the liver [28]. In contrast, serum bilirubin level is extremely low in neonatal mice due to a high hepatic expression of Ugt1a1, indicating that developmental regulation of hepatic UGT1A1 expression is quite different between humans and mice. Serum bilirubin levels in normal human adults are ranging from 0.2 to 1.0 mg/dL, which is still higher than the level in mice, even though the expression of hepatic UGT1A1 is matured. This observation suggests that UGT1A1-mediated bilirubin glucuronidation is more efficient in mice. Efficient glucuronidation of a thyroid hormone thyroxine (3,5,3',5'-L-tetraiodothyronine, T₄) by mouse Ugt1a1 might be attributed to a short half-life of T₄ in rodents compared to humans [47].

3. Humanized *UGT1* (*hUGT1*) mice and expression of human UGT1s in the mice

There are several techniques to create humanized animal models, but humanized *UGT1* (*hUGT1*) mice were created by simply crossing *Ugt1*-null mice with human *UGT1* transgenic mice in a C57BL/6 background [32–34]. Currently both of *Ugt1*-null mice and human *UGT1* transgenic mice are commercially available mice from Jackson Laboratory. Since the original transgenic mice were introduced with the whole human *UGT1* gene spanning more than 200 kbp, all of the UGT1A family enzymes were functional in the original *hUGT1* mice [34]. It was further characterized that the introduced human *UGT1* gene was carrying a gene polymorphism *UGT1A1**28. *hUGT1* mice with *UGT1A1**1 were subsequently created, although a slight disadvantage of the *hUGT1* mice with *UGT1A1**1 is that the introduced human *UGT1* gene does not contain the exon 1 of *UGT1A8* [34].

In the liver of *hUGT1* mice, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 are highly expressed, which is the same expression pattern observed in human livers [32]. As reported in human livers, the genetic polymorphism *UGT1A1**28 results in a significantly lowered expression of hepatic UGT1A1 in *hUGT1* mice [48]. In the small intestine, UGT1A1 is greatly expressed in the both *hUGT1* mice with *1 and *28 allele [34], indicating that the effect of the promoter polymorphism on the expression is restricted in the tissue. UGT1A10 is greatly expressed in the small intestine of *hUGT1* mice, which is very similar to the tissue distribution of the isoform in the human small intestine. UGT1A7 is expressed in the lung and stomach in the human *UGT1* gene-introduced mice as observed in humans [32]. In human brain, UGT1A1 and UGT1A6 are notably and UGT1A3 and UGT1A10 are slightly expressed. *hUGT1* mice completely mimicked the expression pattern of UGT1As in brain [49]. These observations indicate that tissue distribution of human UGT1A family enzymes is highly preserved in *hUGT1* mice.

Due to a difficulty in obtaining specific regions of human brains, there is limited information on the UGT expression in brain regions. In a study where brains were isolated from neonatal and adult *hUGT1* mice and were separated to 5 regions (cerebellum, olfactory bulbs, midbrain, hippocampus, and cerebral cortex), it was found that UGT1A1 expression was

much higher in all of the brain regions in adult *hUGT1* mice compared to the expression in neonates [49]. The expression of UGT1A1 in midbrain was much higher than the expression levels in other regions in neonatal *hUGT1* mice. In contrast, the expression level of UGT1A6 in brain was mostly the same between neonatal and adult *hUGT1* mice. In the brain of adult *hUGT1* mice, the expression level of UGT1A6 in the cerebellum was statistically higher than that in the midbrain, hippocampus, and cerebral cortex. These indicate that *hUGT1* mice are tools that can provide insight into the physiological importance of UGTs in specific tissue regions, of which the availability are limited.

A series of *in vitro* and *in vivo* studies have shown that the promoter regions of the *UGT1A* gene are responsive to various transcription factors such as constitutive-active/androgen receptor (CAR), pregnane X receptor (PXR), aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR), peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), liver X receptor (LXR), farnesoid X receptor (FXR), and NF-E2-related factor 2 (Nrf2) in humans [50,51]. The human *UGT1* transgene introduced to *hUGT1* mice contains the whole promoter regions of each UGT1A isoform (Fig. 1). In *hUGT1* mice, it was first demonstrated that hepatic UGT1A isoforms, UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9, were significantly induced by phenobarbital, which is an indirect CAR activator, increasing the rate of SN-38 glucuronidation *in vivo* [48]. Subsequently, it was demonstrated that treatments of *hUGT1* mice with TCDD, PCN, phenytoin, and fatty acids caused induction of human UGT1A1 in the liver, indicating that the promoter region of human *UGT1A1* in *hUGT1* mice is responsive to AhR, PXR, and PPAR activations as well [11,34,52]. It was also shown that human UGT1A isoforms expressed in extrahepatic tissues such as small intestine, skin, and brain were inducible by xenobiotics in *hUGT1* mice [53–55]. *hUGT1* mice were also useful to understand the transcriptional regulation of human UGT1A isoforms in the liver during pregnancy [56]. These data indicate that the promoter regions of human UGT1A isoforms are functional in *hUGT1* mice, enabling us to investigate the transcriptional regulation of human UGT1A isoforms in various tissues.

It needs to be mentioned here that transcriptional factors in *hUGT1* mice are still mouse genes. Although the function of transcriptional factors is generally similar between humans and mice, certain ligands species-specifically activate the transcriptional factors. While CITCO is a relatively specific activator of human CAR, TCPOBOP is a mouse CAR specific activator [57–59]. Similarly, it has been shown that PCN weakly activates human PXR but is a potent activator of mouse PXR [60]. Therefore, transcriptional regulation of human *UGT1* observed in *hUGT1* mice may not be simply extrapolated to humans. Transgenic and humanized mice expressing human xenobiotic receptors might be useful to fully understand the transcriptional regulation of the human *UGT1* gene in mice.

4. Phenotype of *hUGT1* mice

As described earlier, there is a significant species difference in bilirubin glucuronidation between humans and mice. Human neonates develop physiological hyperbilirubinemia called jaundice due to inadequate expression of the bilirubin-glucuronidating enzyme UGT1A1 in the liver. The elevation of serum bilirubin can allow its invasion into the brain, increasing a risk of developing irreversible brain damage kernicterus. Meanwhile, mice do

not develop such neonatal hyperbilirubinemia, which is a result from efficient bilirubin glucuronidation in the liver. The phenotype analysis of *hUGT1* mice revealed that they develop physiological hyperbilirubinemia during the neonatal period the same way as human neonates (Fig. 2) [34]. The serum bilirubin levels gradually increase after birth and the increase continues through 14 days. The serum bilirubin values decline rapidly to adult levels between 14 and 21 days of birth. During the neonatal developmental period, serum bilirubin levels were found to be similar in *hUGT1* mice with the *UGT1A1**1 allele and those with the *UGT1A1**28 allele [34]. It was further demonstrated that the development of neonatal hyperbilirubinemia in *hUGT1* mice was attributed to inadequate expression of hepatic UGT1A1. Five to ten percent of the neonatal *hUGT1* mice exhibit even higher serum bilirubin levels and develop kernicterus. In humans, breast-fed infants have higher serum bilirubin levels than formula-fed infants. As observed in humans, breast-fed neonatal *hUGT1* mice show higher serum bilirubin levels than formula-fed neonatal *hUGT1* mice [53]. Inadequate calorie intake or starvation has been suggested as causes of neonatal jaundice in humans. Supplemental glucose solution led to lowered serum bilirubin levels in *hUGT1* mice through inducing UGT1A1 in the small intestine via activating specificity protein 1 (SP1) [61]. Adult *hUGT1* mice show slight hyperbilirubinemia with serum bilirubin levels ranging from 0.2 to 1.0 mg/dL, which is equivalent to the levels in human adults. These phenotypic observations indicate that developmental expression, regulation of UGT1A1, and bilirubin metabolism are very similar between humans and *hUGT1* mice. Other than higher serum bilirubin levels and development of kernicterus, the basic characteristics of *hUGT1* mice such as growth curve, longevity, and capability of reproducing are comparable to those of wild-type mice.

5. Glucuronidation of drugs in *hUGT1* mice

UGT1A4 plays a crucial role in *N*-glucuronidation of primary, secondary, and tertiary amine-containing compounds. However, rodents lack the human *UGT1A4* homologue gene and therefore they show less or no *N*-glucuronidation activity. One of the biggest advantages of *hUGT1* mice is that the transgene introduced to the mice contains *UGT1A4*. It was demonstrated that UGT1A4 mRNA was expressed in the liver of *hUGT1* mice [32]. Imipramine and trifluoperazine *N*-glucuronides were formed when imipramine and trifluoperazine were incubated in the liver microsomes of *hUGT1* mice [44], indicating that human UGT1A4 is functionally expressed in the liver. *hUGT1* mice would be powerful tools to predict human *N*-glucuronidation of amine-containing compounds.

Kinetic parameters of furosemide *O*-glucuronidation in liver microsomes are different between humans and mice. Especially, the clearance value of furosemide glucuronidation in human liver microsomes is 5-fold lower than the value obtained in mouse liver microsomes. In liver microsomes prepared from *hUGT1* mice, furosemide glucuronidation follows the Hill equation, showing $S_{50} = 715 \mu\text{M}$, $V_{\text{max}} = 673 \text{ pmol/min/mg}$, Hill coefficient = 1.3, and $CL_{\text{max}} = 0.55 \mu\text{L/min/mg}$, all of which are similar to the kinetic parameters observed in human liver microsomes [44] (Table 1). Especially, the clearance values were identical between *hUGT1* and human liver microsomes. In the liver microsomes prepared from *hUGT1* mice, the *S*-naproxen *O*-glucuronidation follows the Michaelis-Menten equation, showing $K_m = 465 \mu\text{mol/l}$, $V_{\text{max}} = 5.6 \text{ nmol/min/mg}$, and $CL_{\text{int}} = 12.0 \mu\text{L/min/mg}$. The K_m

value obtained in the *hUGT1* liver microsomes was closer to the parameter in human liver microsomes than to that in mouse liver microsomes. The ibuprofen *O*-glucuronidation follows the Michaelis-Menten equation in the liver microsomes of *hUGT1* mice, showing $K_m = 319 \mu\text{M}$, $V_{\max} = 4.3 \text{ nmol/min/mg}$, and $CL_{\text{int}} = 13.4 \mu\text{L/min/mg}$ (45). While there was a 3-fold difference in the clearance of ibuprofen glucuronidation between human and mouse liver microsomes, the clearance parameter obtained in the *hUGT1* liver microsomes was very similar to the parameter in human liver microsomes (Table 1). The K_m values of etodolac and diclofenac *O*-glucuronidations were also identical between *hUGT1* and human liver microsomes. However, certain kinetic parameters of glucuronidation of UGT2B substrates in *hUGT1* mouse liver microsomes, such as CL_{int} values of S-naproxen and diclofenac glucuronidation, are rather similar to those in wild-type mice (Table 1). These data indicate that glucuronidations of drugs in human liver microsomes can be closely mimicked in liver microsomes of *hUGT1* mice especially when drugs are substrates of UGT1 family enzymes.

In vitro analysis indicates that the hepatic ibuprofen glucuronidation is relatively slower in *hUGT1* mice compared to in wild-type mice (Table 1). When ibuprofen was orally administered to *hUGT1* mice at 200 mg/kg, the $C_{\max}$ and AUC values were 368 μM and 1285 $\mu\text{M h}$, respectively. These values are much higher than the parameters observed in wild-type mice (270 μM and 721 $\mu\text{M h}$) [45], suggesting that *hUGT1* mice might also be useful to understand *in vivo* pharmacokinetics of drugs in humans.

6. Drug-induced toxicity in *hUGT1* mice

Although rats and mice are frequently used as experimental models for toxicological studies, the data obtained in rodents cannot be directly extrapolated to humans. This is partly because they exhibit different pharmacokinetic profiles compared to humans. Ibuprofen is one of the drugs that induce human specific toxicity. Although ibuprofen generally has a lower risk of developing liver injury in both humans and mice, several cases of ibuprofen-induced serious liver damage have been reported in humans [62,63]. Pharmacokinetics studies show that ibuprofen glucuronidation in humans is much slower than in wild-type mice, suggesting that the species difference in pharmacokinetics of ibuprofen might explain the human-specific toxic effect of the drug [45]. *hUGT1* mice display human-like ibuprofen glucuronidation *in vitro* and *in vivo* (Table 1). When ibuprofen was orally administered to wild-type mice at 750 mg/kg, serum ALT levels did not change at 6 h after the administration. By contrast, the serum ALT level was significantly increased at 6 h after the oral administration of ibuprofen in *hUGT1* mice, indicating that the onset pattern of ibuprofen-induced liver injury is different between *hUGT1* and wild-type mice, and that *hUGT1* mice might be useful to understand the human-specific ibuprofen-induced liver toxicity [45]. *hUGT1* mice will also be useful to understand carboxylic acid-containing drug-induced toxicity, especially when there is species difference in the acylglucuronidation. Further studies are needed to evaluate the usefulness of *hUGT1* mice for predicting acyl glucuronide-induced liver toxicity.

Disruption in homeostasis of endogenous hormones can lead to development of various diseases. T_4 is a very important endogenous substance for brain development during the

perinatal period [64]. It has been reported that lowered serum T₄ level is tightly associated to developmental delay in humans [65]. UGT1A1 is one of the major T₄-metabolizing enzymes and its induction causes accelerated T₄ metabolism, leading lower serum T₄ level in humans [66,67]. Human infants who are exposed to UGT1A1-inducing phenytoin have a higher risk of developing neurodevelopmental toxicity [68]. In experimental wild-type mice, however, the impact of phenytoin treatment on brain development is not so significant. This is basically because the function of Ugt1a1 in neonatal mice is already high and therefore the UGT1A1 inducer phenytoin is not able to induce mouse Ugt1a1 significantly [11]. In contrast, phenytoin dramatically induces UGT1A1 and causes reduction of serum T₄ levels in neonatal *hUGT1* mice, where the basal expression and function of UGT1A1 is much lower compared to those in neonatal wild-type mice [11]. The lowered serum T₄ levels cause developmental delay in *hUGT1* mice. These data therefore indicate that *hUGT1* mice are useful animal models to understand the developmental toxicity of UGT-inducing agents. While decrease in thyroid hormones during perinatal period causes developmental delay in infants, such decrease stimulates the production of thyroid-stimulating hormone, increasing thyroid cancer potential in adults [69]. *hUGT1* mice might also be utilized as a model to investigate thyroid regulation and thyroid carcinogenesis.

7. Gene induction by isothiocyanates in *hUGT1* mice

Epidemiological studies have indicated that consumption of cooked meat can increase a wide variety of diseases including cancers [70,71]. While 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine (PhIP) and 2-amino-3,8-dimethylimidazo [4,5-f]quinoxaline are the most abundant food-derived heterocyclic amines [72–75], accumulating evidences show that PhIP is the potent mutagen and genotoxic in [76–78]. PhIP levels in well done grilled/ barbecued chicken are up to 480 ppb [79] and this carcinogen is highly bioavailable especially to the colon [80]. While administered PhIP can cause tumors in colon due to its higher accumulating potency into the colon [76], it can also cause breast cancers [81], indicating that PhIP might distribute and exhibit toxicity throughout the body.

While UGTs metabolize various endogenous compounds such as estradiol and bilirubin [82], they are also involved in metabolism of exogenous compounds like PhIP, which is mainly glucuronidated by UGT1A1 [83]. Importantly, it has been determined that glucuronidation is the main metabolic pathway of PhIP in humans [84]. The promoter region of UGT1A1 contains various transcription factor-binding sites. Interestingly, previous studies have shown that human subjects who consumed cruciferous vegetables had a higher PhIP-glucuronidation capacity [85]. Cruciferous vegetables such as broccoli and watercress highly contain glucosinolates, which are hydrolyzed by plant myosinase and by microflora in gastrointestinal tract to form isothiocyanates (ITCs) [86,87]. Sulforaphane, phenethyl isothiocyanate (PEITC), allyl isothiocyanate (AITC), and benzyl isothiocyanate (BITC) are major ITCs and they are well known as anticarcinogenic compounds [88]. As ITCs have been reported to induce UGT1A1 [89], it was assumed that ITCs in cruciferous vegetables might have increased the function of PhIP-glucuronidating UGT1A1 in the body. However, little was known about the underlying mechanism. Although contribution of the Keap1-Nrf2 pathway to the up-regulation of UGT1A1 by ITCs was previously hypothesized, the induction was still observed in the Nrf2-null mice [90].

In *hUGT1* mice, oral administration of PEITC induced UGT1A1 expression in liver and small intestine, which led to a decrease in their serum bilirubin levels [91]. It was further demonstrated that the Cyp2b10 was also induced by the PEITC treatment in the liver and small intestine. Cyp2b10 is a target gene of CAR; therefore, it was hypothesized that CAR might be involved in the upregulation of UGT1A1 by PEITC. In *hUGT1/Car^{-/-}* mice, PEITC was not able to induce UGT1A1 in the liver, indicating that CAR was the dominant factor controlling the induction of hepatic UGT1A1. In contrast, PEITC was still able to induce intestinal UGT1A1 in *hUGT1/Car^{-/-}* mice, suggesting that another transcriptional factor was involved in the induction of UGT1A1 in the small intestine. The PEITC treatment also induced oxidative stress in the *hUGT1* mice. Since *N*-acetylcysteine, which counteracts oxidative stress, inhibited the induction of intestinal UGT1A1, oxidative stress might have been mainly involved in the induction of UGT1A1 in the small intestine.

Previous studies have demonstrated that UGT1 expression was inversely associated with cancer development, including colorectal cancer, breast cancer, bladder cancer and biliary cancer [92–98]. Conditional *Ugt1* knockout mice, in which *Ugt1* is not expressed in intestinal epithelial cells, had greater colon tumorigenesis when induced by an azoxymethane plus dextran sodium sulfate treatment [99]. Therefore, in addition to the increased PhIP glucuronidation, induction of UGT1A itself might, at least partially, contribute to the anticarcinogenic function of ITCs. *hUGT1* mice are useful animal models not only to predict human drug glucuronidation, but also to understand the beneficial effect of the *UGT1* gene on carcinogenesis in humans.

8. Conclusions

As described in guidelines for drug development, enzymes involved in metabolism of drug candidates need to be identified prior to clinical trials. In addition to recombinant enzyme systems and CYP specific inhibitors, mice lacking CYPs have been served as useful tools to estimate the contribution of CYPs to metabolism of drugs [100]. Although recently established mice lacking *Ugt2* family enzymes have been used for *in vivo* and *in vitro* kinetic study of UGT substrates [101], such studies have been rarely conducted in *Ugt1*-null mice. This insufficiency in the pharmacokinetic study with *Ugt1*-null mice is largely attributed to the early lethality of *Ugt1*-null mice [33]. Most recently, it was revealed that *Ugt1*-null mice that were treated with zinc protoporphyrin, which is a heme oxygenase-I inhibitor, avoided their extremely early lethality and they all became adults [102]. This methodology will allow the creation of complete *Ugt* knockout mice and completely humanized *UGT* mice. In the future, humanized *UGT*, humanized *UGT1*, and humanized *UGT2* mice, as well as *Ugt*, *Ugt1*, and *Ugt2* knockout mice, can be extensively used to accurately estimate glucuronidation of xenobiotics including drugs in humans. Furthermore, it is very important to understand the impact of the genetic polymorphism of *UGT1A1* on pharmacokinetics of drugs and drug-induced toxicity. However, most studies used either *hUGT1* with *1A1*1* mice or *hUGT1* with *1A1*28* mice. Therefore, further studies are needed to understand the impact of *UGT1A1*28* on pharmacokinetics of drugs and drug-induced toxicity by using *hUGT1* with *1A1*1* mice or *hUGT1* with *1A1*28* mice.

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Fig. 1. Gene structures of human, mouse, and rat *UGT1*

The structures of human, mouse, and rat *UGT1* (*Ugt1*) genes were visualized using the ZENBU genome browser (<http://fantom.gsc.riken.jp/zenbu>) along with information reported by Owens et al. (2005) [103] and Bock (2003) [104]. The *UGT1* (*Ugt1*) genes contain various first exons and common exons 2–5, producing multiple functional UGT1A members. Black boxes represent exons and white boxes represent pseudogenes. Rat Ugt1a9 was previously recognized as Ugt1a10.

Fig. 2. Serum bilirubin levels of neonatal *hUGT1* mice and wild-type mice

Serum bilirubin levels in neonatal wild-type mice are low (filled circles). In contrast, neonatal *hUGT1* mice develop physiological hyperbilirubinemia (open circles).

Table 1

Kinetic parameters of drug glucuronidation in liver microsomes.

Drug	UGT isoforms	Liver microsomes	Equation	Kinetic parameter		
				K _m (or S ₅₀) (mM)	V _{max} (nmol/min/mg)	CL _{int} (mL/min/mg)
Furosemide	1A9 > 2B7 > 1A1, 1A3, 1A6, 1A7, 1A10	<i>hUGT1</i> Human	<i>Hill</i>	715	0.67	0.55
		Mouse	<i>Hill</i>	681	0.58	0.50
S-Naproxen	2B7 > 1A3 > 1A9 > 2B4	<i>hUGT1</i> Human	Substrate inhibition	405	1.0	2.47
		Mouse	Michaelis-Menten	465	5.6	12
		Human	Michaelis-Menten	308	1.7	5.6
		Mouse	Michaelis-Menten	703	10	14
Etodolac	1A9 > 1A10 2B7	<i>hUGT1</i> Human	Michaelis-Menten	483	0.38	0.79
		Mouse	Michaelis-Menten	483	0.25	0.51
		Human	Michaelis-Menten	1170	0.20	0.17
Diclofenac	2B7 > 1A3 > 2B17 > 1A9 > 2B15 > 1A6	<i>hUGT1</i> Human	Michaelis-Menten	32	7.1	222
		Mouse	Substrate inhibition	33	10	313
		Human	Michaelis-Menten	56	12	211
Ibuprofen	2B7 > 1A3 > 1A9 > 2B4	<i>hUGT1</i> Human	Michaelis-Menten	319	4.3	13
		Mouse	Michaelis-Menten	651	6.4	10
		Human	Michaelis-Menten	364	11	31