

Quantitative Analysis of UDP-Glucuronosyltransferase Ugt1a and Ugt2b mRNA Expression in the Rat Liver and Small Intestine: Sex and Strain Differences[§]

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ABSTRACT

UDP-glucuronosyltransferases (UGTs) catalyze the glucuronidation of numerous endogenous and exogenous compounds to facilitate their excretion from the body. Because rats are commonly used in nonclinical studies, information regarding UGT species differences between rats and humans would be helpful for understanding human pharmacokinetics. In this study, we determined the absolute mRNA expressions of Ugt isoforms in the liver and small intestine of male and female Sprague-Dawley, Fischer 344, and Wistar rats. The sum of the mRNA levels of Ugt isoforms expressed in the liver was significantly ($P < 0.005$) higher than that in the small intestine regardless of the strain and sex. Ugt2b mRNA levels represented approximately 80% of total Ugt

mRNA levels in the liver, whereas Ugt1a mRNA levels accounted for almost 90% in the small intestine. Ugt2b2 mRNA was specifically expressed in Wistar rat liver, resulting in 2-fold higher expression of total hepatic Ugt mRNA in Wistar rats than that in the other strains. Wistar rats showed prominently higher Ugt2b3 and Ugt2b8 mRNA levels in the small intestine than the other strains. The difference between sexes was remarkable with regard to hepatic Ugt1a10 in any of the strains, although slight differences between sexes were also observed in multiple Ugt isoforms. Taken together, this study revealed sex and strain differences in mRNA levels of rat Ugt. The data shown here would be useful for the selection of rat strains in nonclinical studies.

Introduction

UDP-glucuronosyltransferases (UGTs) are responsible for the glucuronidation of a wide range of structurally diverse endogenous and exogenous substances (Tukey and Strassburg, 2000; Mackenzie et al., 2005). UGTs conjugate glucuronic acid to substrates to increase their water solubility and enhance their excretion through bile and urine (Dutton, 1980); therefore, glucuronidation is generally considered to be a detoxification reaction. UGT activities significantly affect the pharmacokinetic and pharmacodynamic profiles and toxicities of drugs. In humans, 40%–70% of all clinical drugs are subjected to glucuronidation by UGTs (Wells et al., 2004). UGTs are widely distributed throughout the body and have been found in the intestines, kidney, brain, pancreas, and placenta; however, most UGTs are found in the liver, which is the primary detoxification organ (Strassburg et al., 2000; Collier et al., 2002; Izukawa et al., 2009; Knights et al., 2013).

In humans, 19 functional UGT isoforms comprise two families (UGT1A and UGT2) based on genetic similarity (Guillemette et al., 2010). The *UGT1A* gene consists of multiple unique first exons and common exons 2–5 (Gong et al., 2001). There are nine functional UGT1A isoforms (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10). The *UGT2* gene family

includes three UGT2A isoforms (UGT2A1, UGT2A2, and UGT2A3) and seven UGT2B isoforms (UGT2B4, UGT2B7, UGT2B10, UGT2B11, UGT2B15, UGT2B17, and UGT2B28) (Riedy et al., 2000; Rummel et al., 2008). Similar to UGT1A family members, UGT2A1 and UGT2A2 are encoded by differential first exons that are spliced to common exons 2–6 of a single gene (Mackenzie et al., 2005; Sneitz et al., 2009). UGT2A3 and UGT2B isoforms are encoded by unigenes.

Rats are commonly used in nonclinical drug development studies to investigate the pharmacokinetic profiles or toxicity of new chemical entities. Since Ugt exert a critical influence on drug pharmacokinetics, species differences in Ugt expression profiles between humans and rats should be elucidated. In rats, there are 18 functional Ugt isoforms that are classified into three subfamilies: Ugt1a, Ugt2a, and Ugt2b (Mackenzie and Rodbourn, 1990; Haque et al., 1991). The rat *Ugt1a* gene cluster is located on chromosome 9q35 and consists of multiple unique first exons and common exons 2–5. There are eight Ugt1a isoforms (Ugt1a1, Ugt1a2, Ugt1a3, Ugt1a5, Ugt1a6, Ugt1a7, Ugt1a8, and Ugt1a10) with two pseudogenes (Ugt1a4 and Ugt1a9) (Emi et al., 1995). The rat *Ugt2* gene family located on chromosome 14q21 includes three members of the Ugt2a subfamily and seven members of the Ugt2b subfamily (Klaassen and Parkinson, 2001). Rat Ugt2a1 and Ugt2a2 are formed by alternative splicing of variable first exons and common exons 2–6, whereas the Ugt2a3 and Ugt2b subfamilies (Ugt2b1, Ugt2b2, Ugt2b3, Ugt2b6, Ugt2b8, Ugt2b12, and Ugt2b34) are encoded by unigenes (Emi et al., 1995; Mackenzie et al., 2005). Tissue distribution of rat Ugt1a and Ugt2b mRNA expression was previously reported

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ABBREVIATIONS: F344, Fischer 344; PCR, polymerase chain reaction; SD, Sprague-Dawley; UDPGA, UDP-glucuronic acid; UGT, UDP-glucuronosyltransferase.

(Shelby et al., 2003). However, a quantitative evaluation remains to be performed. Since the glucuronidation potential depends on the UGT isoforms expressed in tissues and their abundance, information about the absolute expression levels of individual Ugts in rat tissues would be useful in estimating the contribution of glucuronidation to pharmacokinetics or toxicity.

Glucuronidation occurs primarily in the liver, but it has become clear that glucuronidation in extrahepatic tissues has a significant impact on the pharmacokinetics and bioavailability of UGT substrates (Fisher et al., 2001). In fact, previous studies reported that rat intestinal UGT contributes to the first-pass effect (Xu et al., 2009; Furukawa et al., 2012). This study aimed to determine the absolute expression levels of rat *Ugt* mRNA in the liver and small intestine and to clarify sex and strain differences in their expression levels among male and female Sprague-Dawley (SD), Fischer 344 (F344), and Wistar rats, which are all commonly used in nonclinical studies.

Materials and Methods

Materials. RNAiso Plus, a random hexamer, and SYBR Premix Ex Taq were purchased from Takara Bio (Shiga, Japan). ROX and ReverTra Ace were from Stratagene (La Jolla, CA) and Toyobo (Tokyo, Japan), respectively. Primers were commercially synthesized at Rikaken (Tokyo, Japan). All other reagents were of the highest grade commercially available.

Animals. Male and female (7-week-old) Sasco SD rats (140–230 g), F344 rats (110–140 g), and Wistar rats (150–230 g) were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed in an institutional animal facility in a controlled environment (25°C temperature and a 12-hour light/dark cycle) with access to food and water ad libitum. Rats were acclimatized for 3 days before use and maintained in accordance with the National Institutes of Health Guide for Animal Welfare of Japan, as approved by the Institutional Animal Care and Use Committee of Kanazawa University.

Total RNA from the Rat Liver and Small Intestine. Total RNA was isolated from 50–100 mg rat liver and small intestinal mucosa using RNAiso Plus according to the manufacturer's instructions (https://takara.co.kr/file/manual/pdf/9108_9109_e.v1301Da.pdf#search=%27RNAiso%27). From the duodenum to upper jejunum were used as the small intestine, because UGT activities in these parts are relatively higher than those in the lower jejunum and ileum (Shiratani et al., 2008). The ratio of the band intensities of the 28S and 18S ribosomal RNAs was used to confirm the integrity of the extracted RNA.

Real-Time Reverse Transcription Polymerase Chain Reaction. cDNA was synthesized from 4 µg total RNA extracted from the rat liver and small intestine using ReverTra Ace with a random hexamer. cDNA samples from each individual rat were pooled within groups (three rats per each strain and sex). A 1-µl portion of the pooled cDNA was added to a polymerase chain reaction (PCR) mixture containing 10 pmol of each primer (Supplemental Table 1), 12.5 µl SYBR Premix Ex Taq solution, and 75 nM ROX in a final volume of 25 µl. For *Ugt1a* mRNAs, sense primers were set at the specific region in the exon of each isoform, and the antisense primer set at exon 2 was common for all *Ugt1a* isoforms except for *Ugt1a5* (Supplemental Table 1). Another antisense primer was designed for *Ugt1a5* because the amplification efficiency with the former antisense primer was low. For *Ugt2b* mRNAs, sense and antisense primers were designed at the specific regions within exon 1 and exon 2, respectively.

PCR conditions were as follows: after an initial denaturation at 94°C for 25 seconds, the amplification was performed by denaturation at 94°C for 7 seconds; the annealing temperature and extension time are provided in Supplemental Table 1 for 40 cycles. Real-time reverse transcription PCR was performed using an Mx3000P instrument with MxPro QPCR software (both from Stratagene). Amplified PCR products were monitored by measuring the increase in SYBR Green I fluorescence intensity. A calibration curve was constructed using known amounts of PCR product. The copy number was calculated for each sample based on the calibration curve. Specific amplification was confirmed by digestion of the PCR amplicon with the appropriate restriction enzymes as needed. The sum of the copy numbers of all *Ugt* isoforms was used to calculate the percentage of the expression level of each *Ugt* isoform.

Statistical Analyses. A three-factor analysis of variance model with factors for sex, strain, tissue, and their interactions was used to compare group means.

Further analyses involving either one-way analysis of variance (followed by the Tukey honest significant difference test for pairwise comparisons) or two-sample *t* tests were performed when significant interactions were observed among the factors in the three-way model. *P* < 0.05 was considered statistically significant.

Results

Absolute Expression Levels of *Ugt1a* mRNAs in the Rat Liver and Small Intestine. Absolute *Ugt1a* mRNA levels in the rat liver and small intestine are shown in Supplemental Table 2 and visualized in Fig. 1A. *Ugt1a1* mRNA levels in the liver were 3- to 29-fold higher than those in the small intestine in any strain. Regardless of the sex, F344 rats and Wistar rats had the highest *Ugt1a1* level in the liver and small intestine, respectively. In SD and F344 rat livers as well as SD and Wistar rat small intestines, *Ugt1a1* mRNA was significantly higher in females than in males. In F344 rat intestines, *Ugt1a1* mRNA was significantly higher in males than in females, and no sex difference was observed in Wistar rat livers. *Ugt1a2* mRNA levels in the small intestine were 46- to 211-fold higher than that in the liver of any strain. The *Ugt1a2* mRNA level was highest in F344 male rat small intestines. In SD and Wistar rat small intestines, *Ugt1a2* mRNA levels were significantly higher in females than in males. Higher expression in females was also observed for hepatic *Ugt1a2* levels. An opposite difference between sexes was observed in F344 rat small intestines. *Ugt1a3* mRNA levels were higher in the small intestine than in the liver in any strain. Hepatic *Ugt1a3* mRNA levels were significantly higher in females than in males. Such sex differences were also observed in SD and Wistar rat intestines, whereas the opposite sex difference was observed in F344 rat small intestines. *Ugt1a5* mRNA levels were 16-fold higher in the liver than in the small intestine in any strain. A difference between sexes was observed in *Ugt1a5* levels in SD rat livers. In females, SD rats showed significantly higher hepatic *Ugt1a5* levels than F344 and Wistar rats.

Ugt1a6 mRNA levels in the small intestine were slightly higher than those in the liver in any strain. The largest difference was observed in female SD rats (2.7-fold). In both the liver and small intestine, *Ugt1a6* mRNA levels were highest in male Wistar rats, with a significant difference between sexes. Similar differences between sexes were observed in F344 rat livers and small intestines, but an opposite difference between sexes was observed in *Ugt1a6* levels in SD rat small intestines. *Ugt1a7* mRNA levels in the small intestine were 13- to 34-fold higher than those in the liver of any strain. The level was highest in male Wistar rats, with a significant difference between sexes. Similar sex differences were observed in F344 rat small intestines and Wistar rat livers, but an opposite difference between sexes was observed in SD rat small intestines. *Ugt1a8* mRNA was only detected in male Wistar rat livers at a very low level. In F344 and Wistar rats, *Ugt1a10* mRNA in the liver was significantly higher in females than in males. The highest level was observed in female SD rats, whereas it was not detected in male SD rats. *Ugt1a10* mRNA was not expressed in the small intestine in any strain.

Absolute Expression Levels of *Ugt2b* mRNAs in the Rat Liver and Small Intestine. *Ugt2b* mRNA levels in the rat liver and small intestine are shown in Supplemental Table 2 and visualized in Fig. 1B. *Ugt2b1* mRNA was expressed in the liver but not in the small intestine. The highest level was observed in the liver of male Wistar rats. In SD and F344 rats, the *Ugt2b1* mRNA level was significantly higher in females than in males, whereas an opposite difference between sexes was observed in Wistar rat livers. *Ugt2b2* mRNA was highly expressed in Wistar rat livers, with a level 1.4-fold higher in males than in females. Trace levels of *Ugt2b2* were detected in SD and F344 rat livers, with no difference between sexes. *Ugt2b2* was not expressed in the rat small intestine. *Ugt2b3* mRNA levels in the liver were higher than in the small intestine in any strain. Levels in SD and Wistar rat livers were significantly higher in males than in females. In the small intestine,

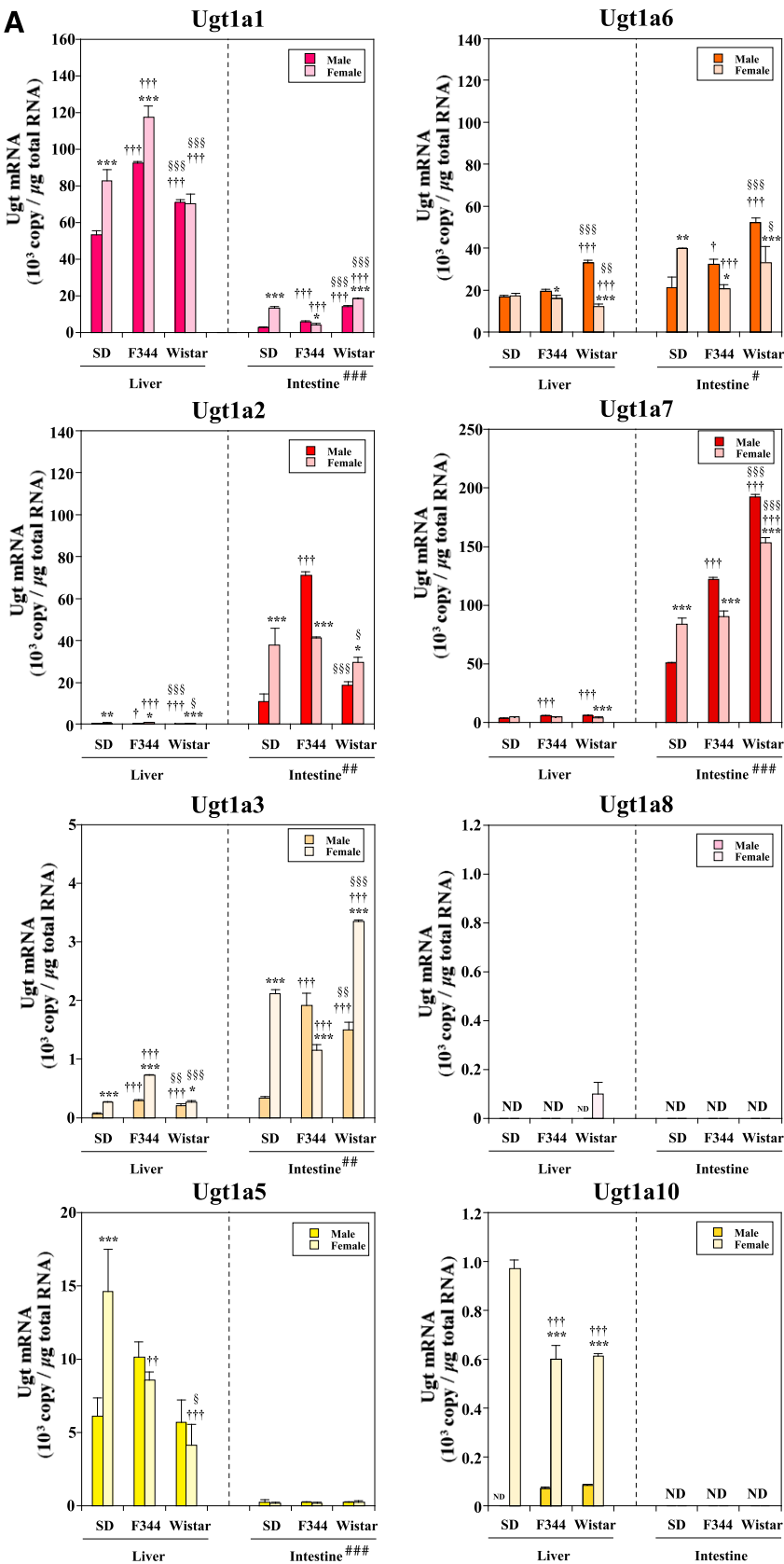


Fig. 1. Absolute quantification of Ugt mRNAs in the liver and small intestine from male and female SD, F344, and Wistar rats. (A) Ugt1a mRNA levels. (B) Ugt2b mRNA levels. The mRNA copy numbers were calculated from the standard curve of each Ugt. Each column represents the mean \pm S.D. of triplicate determinations. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (compared with males); $^{\dagger}P < 0.05$; $^{\ddagger}P < 0.01$; $^{\text{†††}}P < 0.001$ (compared with SD rats); $^{\S}P < 0.05$; $^{\S\S}P < 0.01$; $^{\S\S\S}P < 0.001$ (compared with F344 rats); $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ (compared with liver). Gapdh, glyceraldehyde 3-phosphate dehydrogenase; ND, not detectable.

Ugt2b3 mRNA levels in Wistar rats were 15-fold higher than those in SD and F344 rats regardless of sex. The Ugt2b6 mRNA level was highest in the livers of male SD rats. The hepatic Ugt2b6 mRNA level in

female SD rats was 4-fold lower than that in male SD rats. Levels in F344 and Wistar rats were comparable with those in female SD rats, with no difference between sexes. In the small intestine, a trace level of

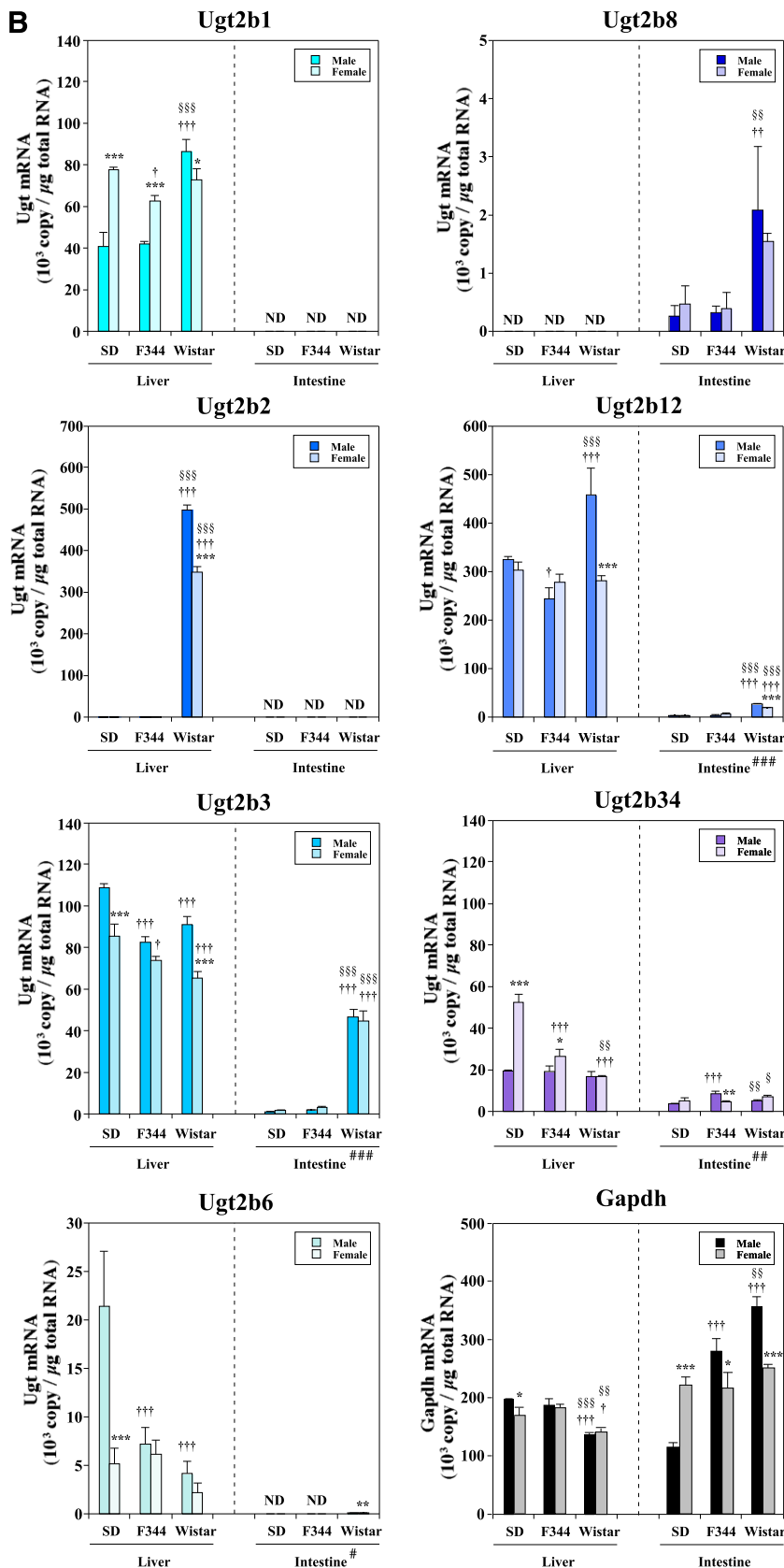


Fig. 1. Continued.

Ugt2b6 expression was detected in Wistar rats, with a difference between sexes (1.5-fold higher in males than females), but not in SD and F344 rats.

Ugt2b8 was expressed in the small intestine but not in the liver. The highest Ugt2b8 level was detected in Wistar rats. Ugt2b8 levels in SD and F344 rats were 3- to 7-fold lower than those in Wistar rats, but no

difference between sexes was observed. Ugt2b12 mRNA levels in the liver were 14- to 116-fold higher than those in the small intestine. In Wistar rats, Ugt2b12 levels in the liver and small intestine were significantly higher in males than those in females. Meanwhile, no difference between sexes was observed in the other strains. Ugt2b34 mRNA levels were higher in the liver than those in the small intestine in any strain. Hepatic Ugt2b34 levels in males were equal in the three strains; in females, levels in SD rats were significantly higher than those in the other strains. In SD and F344 rats, levels were significantly higher in females than in males. In the small intestine, levels in females were equal in the three strains; in males, levels in F344 rats were higher than those in the other strains. In F344 rats, levels were significantly higher in males than in females.

Comparison of Ugt Expression Profiles in the Liver and Small Intestine of the Three Rat Strains. Figure 2A shows the sum of mRNA levels of individual Ugts in the liver and small intestine. The sums of all Ugts in the liver were 2.1- to 6.3-fold higher than those in the small intestine. Wistar male rats showed the highest levels in both the liver and small intestine. In the liver, the sums of all Ugts in Wistar rats were 2-fold higher than those in the other strains, which was due to abundant Ugt2b2 expression. In SD and F344 rat livers, the sums of all Ugts were slightly higher in females than in males. Meanwhile, in Wistar rats, the sums of all Ugts were higher in males than in females. In the small intestine, the sums of all Ugts were higher in Wistar rats than the other strains. In the SD rat small intestine, the sums of all Ugts were slightly higher in females than in males. Meanwhile, the opposite difference between sexes was observed in F344 and Wistar rats.

Percentages of mRNA levels of individual Ugts relative to total Ugt levels were calculated (Supplemental Table 3) and are visualized in Fig. 2B. Ugt2b accounted for approximately 80% of total Ugt levels in the liver. The most abundant isoform was Ugt2b12 (approximately 50% of total Ugts) in SD and F344 rat livers. In Wistar rat livers, Ugt2b2 (40% of total Ugts) was the most abundant, followed by Ugt2b12 (approximately 35% of total Ugts). Ugt2b1, Ugt2b3, and Ugt1a1 accounted for approximately 10% of total Ugts. In the small intestines, Ugt1a accounted for approximately 90% of the total Ugt level. The most abundant isoform was Ugt1a7 (50% of total Ugts) regardless of strain and sex, followed by Ugt1a2 and Ugt1a6. Moderate expression of Ugt2b3 (14% of total Ugts) was characteristic in the Wistar rat small intestine.

Discussion

Glucuronidation is a major conjugation pathway of a large variety of endogenous and xenobiotic compounds. UGTs play a significant role in the control of the pharmacokinetics, bioavailability, and toxicity of drugs. Expression profiles of human UGTs at mRNA and protein levels in several tissues have been well characterized (Strassburg et al., 1997; Tukey and Strassburg, 2001; Nakamura et al., 2008). As for rat Ugts, a previous article reported the tissue distribution (liver, kidney, lung, stomach, duodenum, jejunum, ileum, large intestine, cerebellum, and cerebral cortex) of Ugts at the mRNA level in SD rats by branched DNA signal amplification analysis (Shelby et al., 2003). Quantitative evaluation of rat Ugt expression remains to be performed. In this study, we sought to determine the absolute expression of Ugt1 and Ugt2b mRNA levels in the rat liver and small intestine by quantitative reverse transcription PCR to clarify sex and strain differences.

The sum of the expressions of all Ugts in the liver was significantly ($P < 0.005$) higher than that in the small intestine. This phenomenon was consistent with findings in human Ugts (Ohno and Nakajin, 2009; Court et al., 2012). In the rat liver, Ugt2b accounted for approximately 80% of the total Ugt mRNA level, which was consistent with the

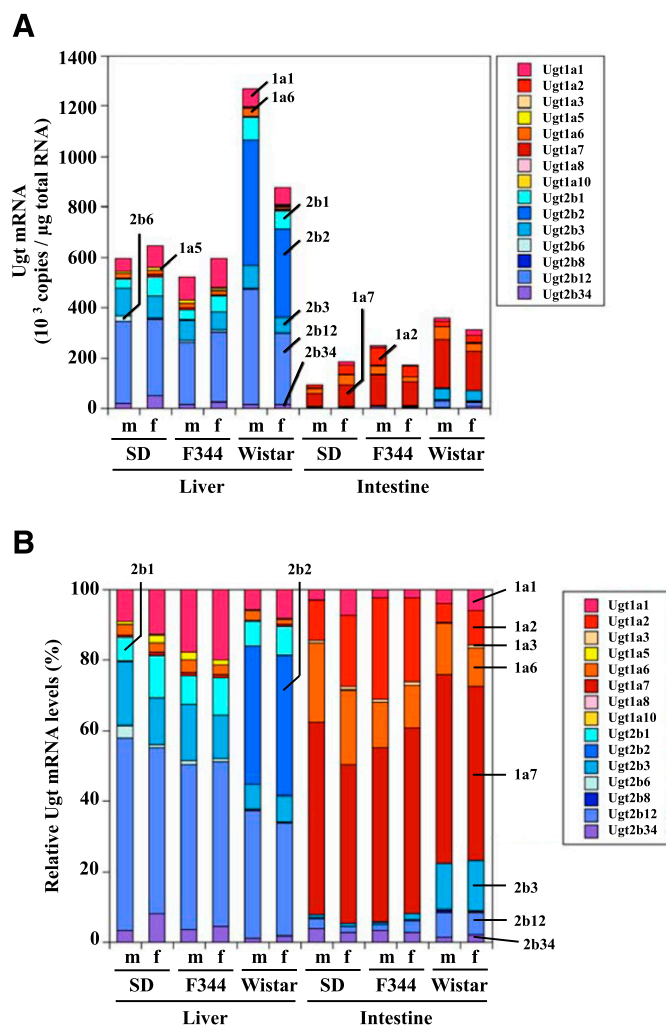


Fig. 2. Comparative evaluation of Ugt mRNA expression levels in the liver and small intestine from male and female SD, F344, and Wistar rats. (A) Sum of the absolute mRNA expression levels of Ugt1a and Ugt2b in rats. (B) Percentage of each Ugt isoform mRNA level in the sum of all of the Ugt levels. f, female; m, male.

abundant expression of Ugt2b in the human liver (70%–90% of total Ugt mRNA) (Izukawa et al., 2009; Ohno and Nakajin, 2009; Court et al., 2012). In the rat small intestines, Ugt1a accounted for approximately 90% of the total Ugt mRNA level. This observation was consistent with a previous report for human Ugt by Court et al. (2012) (approximately 90% of total Ugts), although Ohno and Nakajin (2009) reported that Ugt1a accounts for 30% of total Ugts in the human small intestine. Based on a recent study, expression profiles of UGT1A and UGT2B in the liver and small intestine appear to be similar between humans and rats.

Particularly interesting findings of this study are the strain differences in Ugt expression profiles. Ugt2b2 is abundantly expressed in only Wistar rat livers, leading to 2-fold higher expression of total Ugts in the liver compared with the other strains. As for the strain differences, glucuronidations of androsterone and lithocholic acid, which are substrates of Ugt2b2, were previously detected in liver microsomes from SD rats but were hardly detected in those from F344 rats (Radomska et al., 1994). Richardson and Klaassen (2010) reported that the glucuronidation of triiodothyronine, which is a substrate of Ugt2b2, was detected in liver microsomes from Wistar rats but was hardly detected in those from F344 rats. According to these results, it has been recognized that Ugt2b2 is deficient in F344 rats (Haque et al., 1991). Our study showed that the Ugt2b2 mRNA level in F344 rat livers

was extremely low, supporting the results of previous studies. In a previous study, Wistar rats were divided into two groups on the basis of hepatic microsomal glucuronidation of androsterone: those with high activity and those with low activity (Matsui and Hakozaiki, 1979). The discontinuous variation was due to the genetic heterogeneity of Ugt (Beetstra et al., 1991). It would be plausible that the Wistar rats used in our study belong to the former group. When we measured hepatic Ugt2b2 levels in an additional eight individual Wistar rats (four males and four females) purchased from Charles River (Hino, Japan), the levels were comparable to those shown in Fig. 1B (data not shown). Thus, we could not find individual- and colony-dependent differences in Ugt2b2 expression in the 14 Wistar rats studied. Previous studies (Matsui and Hakozaiki, 1979; Green et al., 1985) reported that discontinuous variation in androsterone glucuronidation was not observed in the SD strain. Shelby et al. (2003) reported substantial expression of Ugt2b2 mRNA in SD rat livers. Meanwhile, our study demonstrated that the Ugt2b2 mRNA level was extremely low in SD rat livers. As for the discrepancy, two reasons are considered. First, the probes to detect Ugt2b2 used in the study by Shelby et al. (2003) might react with other Ugt isoforms such as Ugt2b3 and Ugt2b6 because of their high homologies with Ugt2b2 (approximately 90%). Supporting this suspicion, the tissue distributions of Ugt2b2, Ugt2b3, and Ugt2b6 were similar (Shelby et al., 2003). In this study, we carefully designed primers that can specifically detect only Ugt2b2 mRNA, and we confirmed that the amplicon was surely Ugt2b2 by cleavage with some restriction enzymes. Second, the glucuronidation of androsterone and lithocholic acid observed in SD rat microsomes might be due to other Ugt isoforms, because evaluation using recombinant rat Ugts was not sufficient enough in the past.

In addition to Ugt2b2, a distinct expression profile was observed in intestinal Ugt2b3. Ugt2b3 mRNA levels in the small intestine of Wistar rats were prominently higher than those in the other strains, resulting in higher expression of the total Ugt level in the small intestine of Wistar rats. Rat Ugt2b3 has been reported to catalyze the glucuronidation of aromatic primary amines (Irshaid and Tephly, 1987). Therefore, strain differences in the intestinal Ugt2b3 expression level may contribute to strain differences in the pharmacokinetics of such drugs. Taken together, we demonstrated that Ugt expression profiles in Wistar rats were different from those in other strains. This information may provide a hint for the selection of rat strains.

In humans, UGT1A7, UGT1A8, and UGT1A10 are expressed in the gastrointestinal tract but not in the liver (Tukey and Strassburg, 2001; Nakamura et al., 2008). Our study revealed that Ugt1a7 was highly expressed in the intestine in rats and slightly expressed (approximately 5% of total Ugts) in the liver. Meanwhile, Ugt1a8 was hardly expressed in both the liver and intestine. In the study by Shelby et al. (2003), UGT1A8 mRNA was detected in the liver but at levels barely above the detection limit. Their probes for Ugt1a8 in branched DNA assays might react with other Ugt isoforms showing high sequence similarity with Ugt1a8. Rat Ugt1a10 was not expressed in the intestine but was expressed in the liver at a trace level (0.1%–1% of total Ugts). These results demonstrated that the tissue distributions of UGT1A7, UGT1A8, and UGT1A10 were different between humans and rats. Previous studies reported that the tissue-specific distribution of human UGT1A7, UGT1A8, and UGT1A10 is regulated by transcription factors such as caudal-related homeodomain protein CDX2 and hepatocyte nuclear factor HNF4 α (Gregory et al., 2004; Mubarak et al., 2018). Species differences in tissue distribution might be due to differences in the *trans* or *cis* factors between humans and rats. The mechanisms of tissue-specific expression of rat Ugts are an area for future study.

As for differences between sexes, hepatic Ugt1a10 levels were significantly higher in females than in males in all strains, although the

copy number was much lower than other isoforms. In Wistar rats, the sum of the expression levels of hepatic Ugt2b isoforms was higher in males than in females. In F344 rats, the sum of the expression levels of intestinal Ugt1a isoforms was higher in males than in females. Similar to the previous report by Shelby et al. (2003), hepatic Ugt1a5 and Ugt2b1 mRNA levels in SD rats were 2-fold higher in females than in males. Meanwhile, Emi et al. (1995) reported no remarkable differences between sexes in hepatic Ugt1a mRNA levels. Previous studies reported that sex hormones such as 17 β -estradiol modulate some Ugt expression (Jeong et al., 2008; Chen et al., 2009). Therefore, the menstrual cycle may affect Ugt expression in females.

In conclusion, this is the first study to comprehensively quantify hepatic and intestinal mRNA levels of Ugt isoforms in SD, F344, and Wistar rats, which are commonly used for nonclinical studies. We provided basic information on species differences in hepatic and intestinal UGT expression between rats and humans as well as strain differences. Our results may be helpful for pharmaceutical scientists in choosing rat strains and sex for future studies.

Authorship Contributions

Participated in research design: Furukawa, Fukami, Nakajima.

Conducted experiments: Furukawa.

Contributed new reagents or analytic tools: Furukawa.

Performed data analysis: Kutsukake, Furukawa, Ondo.

Wrote or contributed to the writing of the manuscript: Kutsukake, Gotoh, Nakajima.

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Drug Metabolism and Disposition

Quantitative Analysis of Ugt1a and Ugt2b mRNA Expression in Rat Liver and Small Intestine: Sex and Strain Differences

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Supplementary Table 1. Primer sequences and PCR conditions for real-time RT-PCR analyses.

Gene	Primer	Sequence (5' → 3')	Position ^a	Annealing (°C)	Extension (sec)	Step
Ugt1a1	r1a1 ex1 S	GTG GTT TAC TCC CCC TAT GG	679 - 698	57	20	3
	r1a ex2 AS	ACC ACG ATG CCA TGT TCT CC	898 - 917			
Ugt1a2	r1a2 ex1 S	GCC ATT TAT CAA TCA CTC CC	662 - 681	57	20	3
	r1a ex2 AS		886 - 905			
Ugt1a3	r1a3 ex1 S	AGA TCC TCA GAC ATG CCT C	731 - 749	57	20	3
	r1a ex2 AS		886 - 905			
Ugt1a5	r1a5 ex1 S	ATT CCA GCT CCT TTG ACA TG	416 - 435	66	30	2
	r1a AS ex2 2	AAA GAG AAA ACC ACG ATG CC	895 - 914			
Ugt1a6	r1a6 S	TCC TTG CCT GAG ACC TCC TC	689 - 708	60	20	2
	r1a ex2 AS		883 - 902			
Ugt1a7	r1a7 S	TGA TGC CCA ATG TGA TCC AC	796 - 816	60	20	2
	r1a ex2 AS		886 - 905			
Ugt1a8	r1a8 ex1 S	TCG AAC TGA CTT TGT GCT GG	759 - 778	60	20	2
	r1a ex2 AS		883 - 902			
Ugt1a10	r1a10 ex1 S	AGT TTC AAG GAG CGA TTG TG	604 - 623	60	30	2
	r1a ex2 AS		883 - 902			
Ugt2b1	r2b1 ex1 S	AAG AGT GAA GAA TAT GTT GC	630 - 649	55	20	3
	r2b1 ex2 AS	TTG GCT GGT TTG CAA TGT AG	841 - 860			
Ugt2b2	r2b2 ex1 S	TCG AGA GAC TTA GAC ACA AG	674 - 693	55	20	3
	r2b2 ex2 AS	CCA ATA GGA TCT AAT AAG CC	770 - 789			
Ugt2b3	r2b3 ex1 S	GGG ATC CAT TTT ACA GTG AG	698 - 717	57	20	3
	r2b3 ex2 AS	GGC AAG GGT TTA GGA GGT C	851 - 869			
Ugt2b6	r2b6 ex1 S	GAA GAT TTA TAC TAC CTC CC	557 - 576	62	30	2
	r2b6 ex2 AS	GCA AGG GTT TAG CAG GTT TG	849 - 868			
Ugt2b8	r2b8 ex1 S	TGG TTC GAG TCA TTT CCT GC	670 - 689	64	20	2
	r2b8 ex2 AS	AAC TCA ATG TTT GGC AAG G	812 - 830			
Ugt2b12	r2b12 ex1 S	GGT TCC AAA CAT TTA GAG AG	671 - 690	59	20	2
	r2b12 ex2 AS	CAA CAT TTG GCG ATA TTG GG	807 - 826			
Ugt2b34	r2b34 ex1 S	GTC TCT GTA AAG ATG TAG TC	383 - 402	45	20	3
	r2b34 ex2 AS	CTC TGA GAG TGT AGT GGG TC	734 - 753			

The primer sequence is given from the 5'-3' DNA strand. ^a Nucleotide position on cDNA when the A in the start codon is 1.

Supplementary Table 2. Absolute expression levels of Ugt mRNAs in the liver and small intestine from male and female SD, F344, and Wistar rats.

Isoform	Liver					
	SD		F344		Wistar	
	Male	Female	male	female	male	female
Ugt1a1	53.3 ± 2.4	82.4 ± 6.6	92.2 ± 0.9	117.3 ± 6.4	70.9 ± 1.5	70.0 ± 5.3
Ugt1a2	0.2 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.9 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
Ugt1a3	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0	0.3 ± 0.0
Ugt1a5	6.1 ± 1.2	14.6 ± 2.9	10.1 ± 1.1	8.6 ± 0.6	5.7 ± 1.5	4.1 ± 1.5
Ugt1a6	16.9 ± 0.6	17.3 ± 0.9	19.5 ± 0.9	16.1 ± 1.6	33.2 ± 0.9	12.3 ± 0.8
Ugt1a7	3.8 ± 0.2	4.5 ± 0.2	5.6 ± 0.4	4.8 ± 0.3	6.2 ± 0.1	4.5 ± 0.5
Ugt1a8	ND	ND	ND	ND	ND	ND
Ugt1a10	ND	1.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.1	0.1 ± 0.0	0.6 ± 0.0
Ugt2b1	40.8 ± 6.7	77.8 ± 1.2	41.9 ± 1.2	62.8 ± 2.6	86.4 ± 5.8	72.8 ± 5.5
Ugt2b2	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	497.8 ± 11.4	348.3 ± 14.0
Ugt2b3	108.8 ± 2.2	85.7 ± 5.6	82.5 ± 2.5	73.7 ± 2.2	91.1 ± 3.7	65.2 ± 3.1
Ugt2b6	21.4 ± 5.7	5.1 ± 1.6	7.1 ± 1.8	6.1 ± 1.4	4.1 ± 1.3	2.2 ± 1.0
Ugt2b8	ND	ND	ND	ND	ND	ND
Ugt2b12	325.3 ± 7.1	302.7 ± 17.4	243.8 ± 23.0	278.1 ± 16.1	457.5 ± 56.0	280.9 ± 10.2
Ugt2b34	19.6 ± 0.4	52.7 ± 3.8	18.8 ± 2.8	26.6 ± 3.2	16.9 ± 2.3	16.7 ± 0.6
	Intestine					
Ugt1a1	2.8 ± 0.1	13.5 ± 0.6	5.7 ± 0.6	4.0 ± 0.8	14.4 ± 0.3	18.5 ± 0.3
Ugt1a2	10.8 ± 3.5	37.7 ± 8.2	71.3 ± 1.4	41.1 ± 0.5	18.7 ± 1.4	29.7 ± 2.0
Ugt1a3	0.3 ± 0.0	2.1 ± 0.1	1.9 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	3.3 ± 0.0
Ugt1a5	0.2 ± 0.2	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
Ugt1a6	21.2 ± 4.7	39.7 ± 0.5	32.3 ± 2.3	20.6 ± 2.1	52.2 ± 2.3	33.2 ± 7.7
Ugt1a7	50.9 ± 0.7	84.0 ± 4.7	122.3 ± 1.5	90.5 ± 4.7	192.6 ± 2.0	153.4 ± 4.5
Ugt1a8	ND	ND	ND	ND	ND	ND
Ugt1a10	ND	ND	ND	ND	ND	ND
Ugt2b1	ND	ND	ND	ND	ND	ND
Ugt2b2	ND	ND	ND	ND	ND	ND
Ugt2b3	0.9 ± 0.2	1.7 ± 0.1	1.8 ± 0.2	3.0 ± 0.5	46.8 ± 3.5	44.9 ± 4.5
Ugt2b6	ND	ND	ND	ND	0.1 ± 0.0	0.1 ± 0.0
Ugt2b8	0.3 ± 0.2	0.5 ± 0.3	0.3 ± 0.1	0.4 ± 0.3	2.1 ± 1.1	1.5 ± 0.1
Ugt2b12	2.8 ± 0.2	3.4 ± 0.3	3.8 ± 0.3	6.0 ± 1.9	25.8 ± 2.0	19.1 ± 0.4
Ugt2b34	3.6 ± 0.4	4.9 ± 1.8	8.6 ± 0.9	4.5 ± 0.4	5.2 ± 0.4	7.0 ± 0.6

Data are the mean ± SD of triplicate determinations. Unit is $\times 10^3$ copies/ μ g total RNA. ND: Not detected.

Supplementary Table 3. Percentage of the mRNA levels of each Ugt in the liver and small intestine from male (m) and female (f) SD, F344, and Wistar rats.

Isoform	Liver						Intestine					
	SD		F344		Wistar		SD		F344		Wistar	
	m	f	m	f	m	f	m	f	m	f	m	f
Ugt1a1	8.9	12.8	17.7	19.7	5.6	8.0	3.0	7.2	2.3	2.3	4.0	6.0
Ugt1a2	0.0	0.1	0.1	0.1	0.0	0.0	11.5	20.1	28.7	24.0	5.2	9.5
Ugt1a3	0.0	0.0	0.1	0.1	0.0	0.0	0.4	1.1	0.8	0.7	0.4	1.1
Ugt1a5	1.0	2.3	1.9	1.4	0.4	0.5	0.2	0.1	0.1	0.1	0.1	0.1
Ugt1a6	2.8	2.7	3.7	2.7	2.6	1.4	22.6	21.1	13.0	12.0	14.5	10.7
Ugt1a7	0.6	0.7	1.1	0.8	0.5	0.5	54.2	44.8	49.2	52.8	53.6	49.3
Ugt1a8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ugt1a10	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Ugt2b1	6.8	12.1	8.0	10.5	6.8	8.3	0.0	0.0	0.0	0.0	0.0	0.0
Ugt2b2	0.0	0.0	0.0	0.0	39.2	39.7	0.0	0.0	0.0	0.0	0.0	0.0
Ugt2b3	18.2	13.3	15.8	12.4	7.2	7.4	0.9	0.9	0.7	1.8	13.0	14.4
Ugt2b6	3.6	0.8	1.4	1.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Ugt2b8	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.1	0.2	0.6	0.5
Ugt2b12	54.6	46.9	46.7	46.6	36.0	32.0	3.0	1.8	1.5	3.5	7.2	6.1
Ugt2b34	3.3	8.2	3.6	4.5	1.3	1.9	3.8	2.6	3.5	2.6	1.4	2.2
Total	100	100	100	100	100	100	100	100	100	100	100	100

The mean values of triplicate determinations were adopted.