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RESEARCH ARTICLE

Species and tissue differences in serotonin glucuronidation

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Abstract

1. Serotonin is a UGT1A6 substrate that is mainly found in the extrahepatic tissues where some UGT1As are expressed. The aim of the present study was to characterize serotonin glucuronidation in various tissues of humans and rodents.
2. Serotonin glucuronidation in the human liver and kidney fitted to the Michaelis–Menten model, and the K_m values were similar to that of recombinant UGT1A6. However, serotonin glucuronidation in the human intestine fitted to the Hill equation, indicating that it is likely catalyzed not only by UGT1A6, but also by another UGT1A isoform. Serotonin glucuronidation in the rat liver, intestine and kidney fitted well to the Michaelis–Menten model and exhibited monophasic kinetics in the kidney, but biphasic kinetics in the liver and intestine. Furthermore, serotonin glucuronidation in the rat brain fitted best to the Hill equation. Serotonin glucuronidation in the mouse tissues fitted to the Michaelis–Menten model and exhibited monophasic kinetics in the liver and intestine microsomes, but biphasic kinetics in the kidney and brain microsomes.
3. In conclusion, we clarified that tissue and species differences exist in serotonin glucuronidation. It is necessary to take these potential differences into account when considering the pharmacodynamics and pharmacokinetics of serotonin.

Keywords

Enzyme kinetic study, serotonin, species differences, tissue differences, UGT

History

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Introduction

UDP-glucuronosyltransferase (UGT) catalyzes the major phase II reaction, which is responsible for catalyzing the conjugation of glucuronic acid with a wide range of xeno- and endobiotics, including drugs (de Leon, 2003; Tukey & Strassburg, 2000), hormones (Court, 2005) and neurotransmitters (Itäaho et al., 2009). An essential neurotransmitter, serotonin, is conjugated by UGT (Krishnaswamy et al., 2003a). Serotonin is mainly found in the extrahepatic tissues such as the intestine (Roth & Gordon, 1990), kidney (Hafdi et al., 1996; Stier & Itskovitz, 1985) and brain (Zheng et al., 2012), where some UGT1As are expressed at a higher level than in the liver (Buckley & Klaassen, 2007; Nakamura et al., 2008; Shelby et al., 2003). A previous study reported that 30% of injected serotonin was excreted in the urine as serotonin glucuronide, and the amount increased to 70% when monoamine oxidase was inhibited by iproniazid in the mouse (Wessbach et al., 1960). These results suggested that glucuronidation represents an important compensatory catabolic pathway of serotonin. Therefore, characterization of the serotonin kinetics in the extrahepatic tissues is of great value to understand the pharmacological effects of serotonin in the body. Accordingly, characterization of serotonin glucuronidation in the extrahepatic tissues would be valuable.

UGTs have overlapping substrate specificities, and most of their substrates are glucuronidated by more than one UGT isoform (Radominska-Pandya et al., 1999). However, unlike 1-naphthol (Ebner & Burchell, 1993; Harding et al., 1988) and acetaminophen (Court et al., 2001; Fisher et al., 2000), serotonin has been shown to be a highly selective substrate for UGT1A6 in the human liver and extrahepatic tissues (King et al., 1999; Krishnaswamy et al., 2003a).

A previous study reported that serotonin glucuronidation was reduced in homozygous mutant (J/J) Gunn rat liver homogenates to about 20% of the activity observed in normal Wistar rat liver homogenates (Leakey, 1978). Another study demonstrated that serotonin glucuronidation in homozygous mutant and heterozygous (J/+) Gunn rats decreased to 13% and 60%, respectively, compared to the activities measured in normal Wistar rats (Krishnaswamy et al., 2003b). These results suggested that serotonin is primarily catalyzed by Ugt1a isoforms (possibly Ugt1a6) in rat liver microsomes. Moreover, Ugt2 isoforms may also contribute to serotonin glucuronidation because of the low but significant activity observed in the homozygous mutant Gunn rat liver. In terms of mice, serotonin glucuronidation was found to be catalyzed by Ugt1a6a and Ugt1a6b with different enzymatic properties (Uchihashi et al., 2013). However, no study has comprehensively evaluated the

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species and tissue differences in the kinetics of serotonin glucuronidation.

Therefore, the aim of the present study was to characterize serotonin glucuronidation in various tissues of humans and rodents and to clarify the species and tissue differences in the kinetics of serotonin glucuronidation.

Materials and methods

Materials

Alamethicin and uridine 5′-diphosphoglucuronic acid trisodium salt were obtained from Sigma-Aldrich (St. Louis, MO). (*p*-Amino diphenyl)methanesulfonyl fluoride and dithiothreitol were purchased from Wako Pure Chemicals (Osaka, Japan). Serotonin β-D-glucuronide and serotonin-d4 β-D-glucuronide were purchased from Toronto Research Chemicals (Toronto, Canada). All other chemicals and solvents were of the highest grade commercially available.

Microsomes from the liver, kidney, intestine and brain

The present study was approved by the Institutional Animal Care and Use Committee of Meijo University. Microsomes from the human liver (pooled, 50 donors), intestine (pooled, 7 donors) and kidney (pooled, 4 donors) were purchased from BD Gentest (Woburn, MA) or KAC (Kyoto, Japan). The pooled microsomes from rat ($n = 10$) and mouse ($n = 10$) tissues were prepared as described previously (Shiratani et al., 2008) and stored at -80 °C until analysis. Protein concentration was determined using Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA).

Recombinant UGT isoforms

Human recombinant UGT1A1, UGT1A6, UGT1A7, UGT1A8, UGT1A10, UGT2B7 and UGT2B15 supersomes were obtained from BD Gentest.

Serotonin glucuronidation

Serotonin glucuronidation was determined using the method described by Mohamed & Frye (2011) with slight modifications. The incubation mixture contained 50 mM Tris–HCl buffer (pH 7.4), 5 mM $MgCl_2$, 25 μg/mL alamethicin, 0.1 mg/mL microsomes from the liver, intestine and kidney, or 0.5 mg/mL microsomes from the brain, and 0.02–40 mM serotonin. The reaction mixture was incubated at 37 °C for 60 min. The reaction was terminated by the addition of 20 μL 60% perchloric acid and 17.8 nmol serotonin-d4 β-D-glucuronide was added as an internal standard. After the mixture was centrifuged at 15 000 *g* for 10 min, 10 μL of the obtained supernatant was subjected to liquid chromatography–tandem mass spectrometry (LC–MS/MS). LC was performed using a Prominence apparatus (Shimadzu, Kyoto, Japan), which was equipped with a Develosil XG-C30 M3 column (2.1 mm × 150 mm; Nomura Chemical, Aichi, Japan). The column temperature was 40 °C, and the flow rate was 0.2 mL/min. The mobile phase consisted of methanol/10 mM ammonium formate (pH 4.5) [5:95 (v/v)]. The LC setup was connected to an API4000 tandem mass spectrometer (Applied Biosystems, Fostercity, CA) operated in the positive electrospray ionization mode. Turbo gas was maintained at 400 °C. Mass/charge (*m*/*z*) ion transitions were recorded in the multiple reaction-monitoring mode, with *m*/*z* 352.8/160.0 for serotonin β-D-glucuronide and *m*/*z* 357.2/164.1 for serotonin-d4 β-D-glucuronide. The retention time of both serotonin β-D-glucuronide and serotonin-d4 β-D-glucuronide was 4.5 min. The limit of detection for serotonin β-D-glucuronide was 0.28 pmol. The limit of quantification in the reaction mixture was 28 nM with a coefficient of variation of less than 10%. In a preliminary study, we confirmed the linearity of the protein concentrations and incubation times.

Inhibition study for serotonin glucuronidation in human intestine microsomes

1-Naphthol, a substrate for UGT1A6 (Court et al., 2001; Fisher et al., 2000; Uchaipichat et al., 2004), emodin, a substrate for UGT1A8 and UGT1A10 (Watanabe et al., 2002), estrone, a substrate for UGT1A10 (Kallionpää et al., 2015), androstanediol and 5-(4′-hydroxyphenyl)-5-phenylhydantoin (HPPH), substrate for UGT2B15 (Nakajima et al., 2007; Turgeon et al., 2001), were used as UGT inhibitors to clarify their inhibitory effects on serotonin glucuronidation in pooled human intestinal microsomes.

Enzyme kinetic analyses

Kinetic parameters were estimated from the fitted curves using the KaleidaGraph computer program (Synergy Software, Reading, PA) designed for nonlinear regression analysis. The following equations were applied for the Michaelis–Menten (Equation 1), Hill (Equation 2) or substrate inhibition (Equation 3) kinetics model:

$$V = \frac{V_{max} \cdot S}{K_m + S}, \quad (1)$$

$$V = \frac{V_{max} \cdot S^n}{S_{50}^n + S^n}, \quad (2)$$

$$V = \frac{V_{max} \cdot S}{K_m + S + S^2/K_i}, \quad (3)$$

where K_m is the Michaelis–Menten constant and V_{max} is the maximum velocity. S_{50} is the substrate concentration showing the half-V_{max}, n is the Hill coefficient and K_i is the substrate inhibition constant. Intrinsic clearance (CL_{int}) was calculated as V_{max}/K_m for the Michaelis–Menten kinetics. For sigmoidal kinetics, maximum clearance (CL_{max}) was calculated as $V_{max} \cdot (n-1)/(S_{50} \cdot n(n-1)^{1/n})$ to estimate the highest clearance (Houston & Kenworthy, 2000). Data are presented as the mean of three independent determinations using pooled microsomes from each tissue.

Results

Serotonin glucuronidation in microsomes from human tissues

As shown in Figure 2, serotonin glucuronidation in the human liver and kidney fitted to the Michaelis–Menten model. Eadie–Hofstee plots for serotonin glucuronidation in the human liver and kidney microsomes exhibited monophasic kinetics, and the K_m values were similar in both tissues.

However, the kinetics of serotonin glucuronidation in the human intestine fitted to the Hill equation with a coefficient of 1.30 (Table 1). The values of V_{max} (29.67 nmol/min/mg protein) and CL_{int} (4.66 μL/min/mg protein) for human liver microsomes were higher than those for the intestine and kidney (Table 1, Figure 2).

Serotonin glucuronidation by human recombinant UGT isoforms

Serotonin glucuronidation was determined at a concentration of 4 mM serotonin using recombinant UGT isoforms, including UGT1A1, UGT1A6, UGT1A7, UGT1A8, UGT1A10, UGT2B7 and UGT2B15 (Figure 3), which are mainly expressed in the human intestine. UGT1A6 resulted in the highest level of serotonin glucuronidation (1092 pmol/min/mg protein). The other isoforms also catalyzed serotonin glucuronidation, but these activities were much lower than that of UGT1A6. Furthermore, enzyme kinetic studies were performed using these recombinant human UGT isoforms (Table 2 and Figure 4) except for UGT1A1 and UGT2B7, whose glucuronidation levels were not high enough to detect for kinetics studies, especially at lower serotonin concentrations. Serotonin glucuronidation by UGT1A6, UGT1A7 and UGT1A10 fitted to the Michaelis–Menten model, whereas that by UGT1A8 and UGT2B15 fitted to the substrate inhibition model.

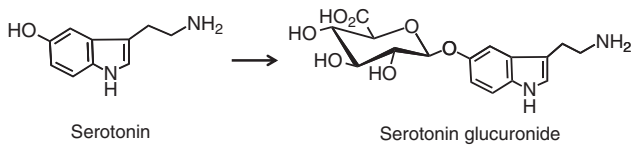

Figure 1. Serotonin glucuronidation.

Inhibition study for serotonin glucuronidation in human intestine microsomes

To elucidate the UGT isoforms responsible for serotonin glucuronidation in human intestine, inhibition study was performed using UGT substrates (Figure 5). Serotonin glucuronidation in human intestinal microsomes decreased by 34% in the presence of 10 μM 1-naphthol (UGT1A6). Serotonin glucurotnidation in human intestine decreased to 40% in the presence of 100 μM emodin (UGT1A8 and UGT1A10). Serotonin glucuronidation decreased to less than 20% in the presence of 1000 μM HPPH (UGT2B15).

Serotonin glucuronidation in microsomes from rat tissues

Serotonin glucuronidation in the rat liver, intestine and kidney fitted to the Michaelis–Menten model. Eadie–Hofstee plots exhibited biphasic kinetics for serotonin glucuronidation in the rat liver and intestine microsomes, and the K_m values were similar in both tissues. By contrast, the rat kidney microsomes exhibited monophasic kinetics, and the K_m value (3.53 mM) differed from those in the rat liver and intestine microsomes. However, serotonin glucuronidation in the rat brain fitted to the Hill equation with a coefficient of 1.49. The value of V_{max} for rat kidney microsomes was highest among all rat tissues examined. Similarly, the value of CL_{int} for the rat liver microsomes was highest among all rat tissue microsomes (Figure 6).

Serotonin glucuronidation in microsomes from mouse tissues

Serotonin glucuronidation in all four mice tissues examined fitted to the Michaelis–Menten model. Eadie–Hofstee plots for serotonin glucuronidation in the mouse liver and intestine microsomes exhibited monophasic kinetics. The values of K_m for the mouse liver and intestine microsomes were 1.47 and

Table 1. Kinetics parameters of serotonin glucuronidation in the human, rat and mouse.

Species	Tissue	Affinity	K_m (S_{50}) (mM)	V_{max} (nmol/min/mg)	CL_{int} (CL_{max}) (μL/min/mg)	n[a]
Human	Liver		6.37 ± 0.31	29.67 ± 0.58	4.66 ± 0.16	
	Intestine		4.47 ± 0.61[b]	0.76 ± 0.06	0.17 ± 0.01[c]	1.30
	Kidney		6.13 ± 1.10	5.00 ± 0.36	0.83 ± 0.10	
Rat	Liver	High	0.30 ± 0.01	45.26 ± 0.54	151.27 ± 4.15	
		Low	0.83 ± 0.02	61.81 ± 0.31	72.09 ± 0.10	
	Intestine	High	0.31 ± 0.01	15.49 ± 0.24	50.48 ± 1.98	
		Low	0.73 ± 0.03	20.85 ± 0.16	28.38 ± 1.12	
	Kidney		3.53 ± 0.21	153.33 ± 5.77	43.45 ± 1.90	
	Brain		1.29 ± 0.06[b]	0.08 ± 0.02	0.07 ± 0.01[c]	1.49
Mouse	Liver		1.47 ± 0.13	68.00 ± 2.50	48.57 ± 2.36	
	Intestine		2.83 ± 0.13	67.00 ± 1.41	23.65 ± 0.59	
	Kidney	High	0.05 ± 0.00	0.49 ± 0.02	9.92 ± 0.25	
		Low	0.91 ± 0.02	2.45 ± 0.01	0.54 ± 0.03	
	Brain	High	1.28 ± 0.07	14.40 ± 0.44[d]	11.25 ± 0.60[e]	
		Low	3.60 ± 0.08	25.24 ± 0.30[d]	7.01 ± 0.00[e]	

Each value represents the mean ± SD.
[a]Hill coefficient.
[b]S_{50}.
[c]CL_{max}.
[d]pmol/min/mg.
[e]nL/min/mg.

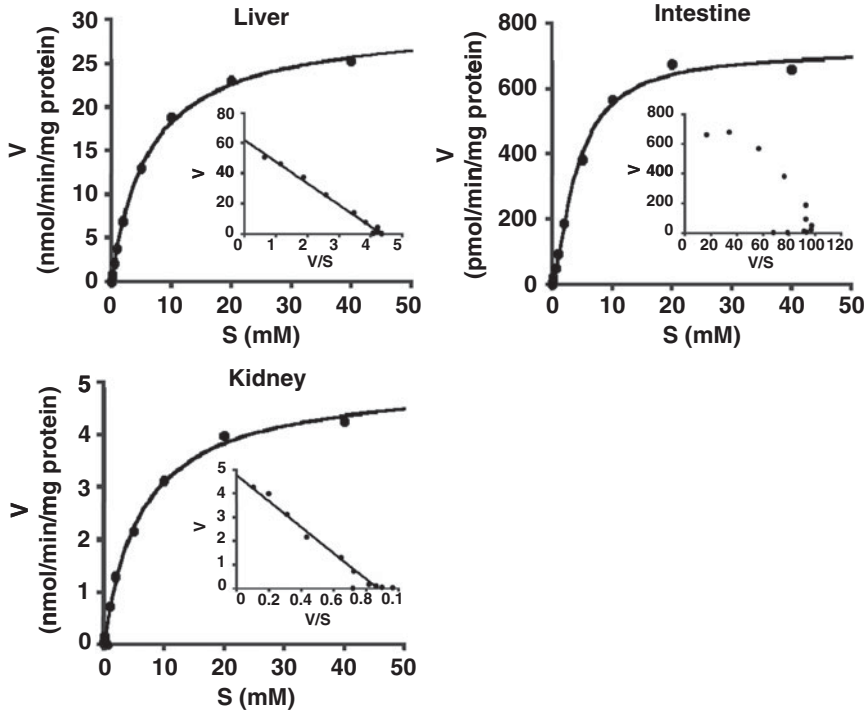

Figure 2. Kinetic study of serotonin glucuronidation by recombinant human UGT isoforms. Serotonin glucuronidation was measured with various serotonin concentrations ranging from 0.1 to 40 mM. Eadie–Hofstee plots are shown as insets in each graph. The points represent the means of three independent determinations.

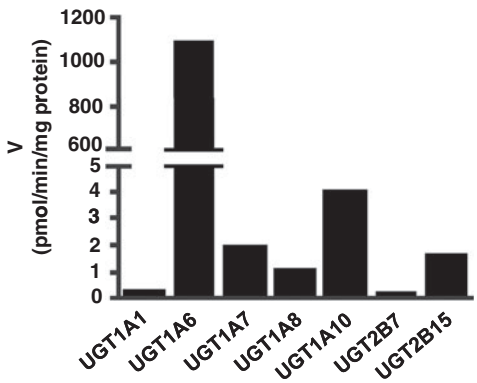

Figure 3. Serotonin glucuronidation by recombinant human UGT isoforms. Serotonin glucuronidation was measured at a serotonin concentration of 4 mM. Each column represents the mean of duplicate determinations.

2.83 mM, respectively. However, Eadie–Hofstee plots for serotonin glucuronidation in the mouse kidney and brain microsomes were biphasic, and the K_m values for serotonin glucuronidation differed between the mouse kidney and brain. The value of CL_{int} for the mouse liver microsomes was highest among the microsomes of all four mice tissues examined (Figure 7).

Discussion

In this study, we conducted kinetic evaluations of serotonin glucuronidation to elucidate whether tissue and species differences exist. The values of CL_{int} for serotonin glucuronidation in human tissues were lower than those in the rat and mouse tissues (Table 1). Moreover, the K_m values in both the rat and mouse tissues were lower than those in human tissues, suggesting that the affinities of UGT isoforms to serotonin in rats and mice are higher than those in humans.

Serotonin glucuronidation in the human liver and kidney fitted to the Michaelis–Menten model, and Eadie–Hofstee plots exhibited monophasic kinetics, with K_m values similar to those obtained in a previous study (liver: 5.2–8.8 mM; kidney: 6.5 mM) (Krishnaswamy et al., 2003a). However, serotonin glucuronidation in the human intestine fitted to the Hill equation. Serotonin glucuronidation in the human intestine exhibited different kinetics from that in the kidney or liver. A previous study reported that serotonin was mainly catalyzed by UGT1A6, but UGT1A7, UGT1A8, UGT1A10, and UGT2B7 also showed quantifiable activities (Krishnaswamy et al., 2003a; Kurkela et al., 2007), which is similar to the results of the present study. Besides these UGT isoforms, UGT1A1 and UGT2B15 also catalyzed serotonin glucuronidation in the present study. The difference in these results is most likely owing to the enhanced detection sensitivity and the difference of the UGT content in the enzyme sources. In the inhibition study for serotonin glucuronidation in human intestinal microsomes, 1-naphthol (UGT1A6) prominently inhibited serotonin glucuronidation. Since serotonin glucuronidation also decreased upon the addition of emodin and HPPH, serotonin glucuronidation in the human intestinal microsomes appears to be catalyzed not only by UGT1A6, but also by other UGT isoforms that are expressed in the intestine with different affinities to serotonin; indeed, the expression level of UGT1A6 protein in the human intestine is much lower than that in the liver or the kidney (Krishnaswamy et al., 2003a). However, in our kinetic study, we could not identify the main recombinant UGT isoform that catalyzes serotonin glucuronidation in the human intestine, because none of the UGT isoforms evaluated fit to the Hill equation.

Table 2. Kinetics parameters of serotonin glucuronidation in recombinant human UGT isoforms.

Isoform	K_m (mM)	V_{max} (pmol/min/mg)	CL_{int} (μL/min/mg)	K_i (mM)
UGT1A6	8.07 ± 0.95	3466.70 ± 251.66	431.32 ± 19.80	
UGT1A7	10.10 ± 2.65	7.10 ± 1.00	0.72 ± 0.08	
UGT1A8	8.33 ± 2.81	4.47 ± 1.36	0.54 ± 0.05	14.30 ± 6.56
UGT1A10	7.80 ± 0.66	6.80 ± 0.36	0.87 ± 0.03	
UGT2B15	3.87 ± 0.31	2.47 ± 0.15	0.64 ± 0.02	

Each value represents the mean ± SD.

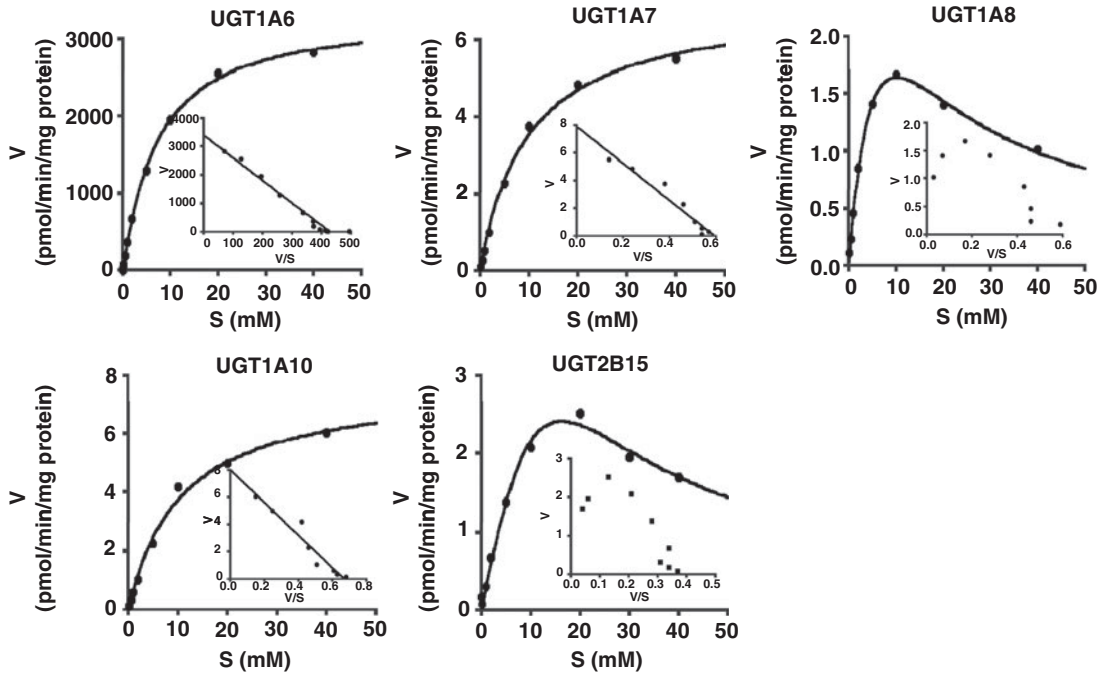

Figure 4. Kinetic study of serotonin glucuronidation in human microsomes. Serotonin glucuronidation was measured in the human liver, intestine and kidney microsomes with serotonin concentrations ranging from 0.02 to 40 mM. Eadie–Hofstee plots are shown as insets in each graph. The points represent the means of three independent determinations.

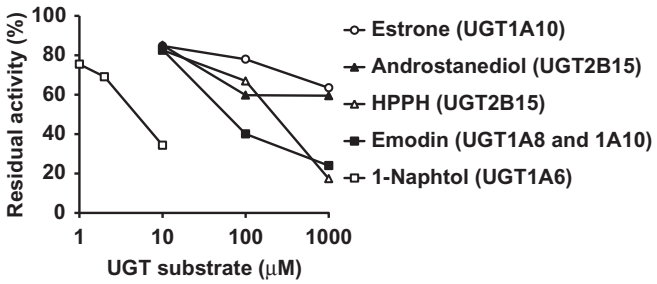

Figure 5. Inhibition study for serotonin glucuronidation in human intestine microsomes. Serotonin glucuronidation was determined at 4 mM serotonin in the presence of some UGT substrates. Each data point represents the mean of triplicate determinations.

The Eadie–Hofstee plot exhibited monophasic kinetics in the rat kidney, but biphasic kinetics in the rat liver and intestine. These results suggested that serotonin glucuronidation is catalyzed by more than two common isoforms in the rat liver and intestine. Moreover, serotonin glucuronidation in the rat brain fitted to the Hill equation, which suggests that the Ugt isoforms catalyzing serotonin glucuronidation in the rat brain might differ from those in the rat liver, intestine and kidney. The previous report using Gunn rats indicated that serotonin glucuronidation was primarily catalyzed by Ugt1a isoforms, with a potential partial contribution from Ugt2 isoforms (Krishnaswamy et al., 2003b). Therefore, serotonin glucuronidation in rats could be catalyzed by several isoforms that have different affinities for serotonin. The value of V_{max} in the rat kidney was higher than that in the liver, unlike the results observed in humans or mice. This result is consistent with a previous study showing that 1-naphthol glucuronidation in the rat kidney was higher than that in the liver (Ito et al., 2005). The difference in the expression of Ugt isoforms in each tissue may contribute to the observed differences between the tissues in the kinetic analysis of serotonin glucuronidation. It has been reported that the mRNA expression levels of Ugt1a and Ugt2b isoforms varied among different rat tissues (Shelby et al., 2003). However, further studies are needed to clarify the Ugt isoforms that are primarily responsible for catalyzing serotonin glucuronidation in each rat tissue.

The Eadie–Hofstee plots for serotonin glucuronidation in the mouse liver and intestine microsomes exhibited monophasic kinetics. However, those in the mouse kidney and brain microsomes were biphasic, and the K_m values were different. These results suggested that serotonin glucuronidation is catalyzed by more than two isoforms in the mouse kidney and brain. It has been reported that Ugt1a6a and Ugt1a6b are the main isoforms responsible for catalyzing serotonin

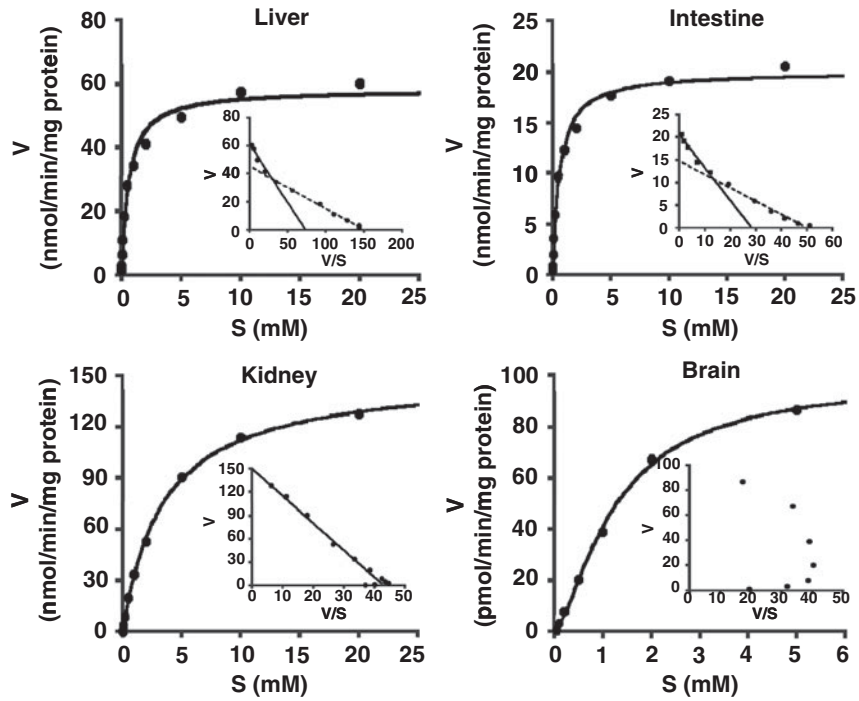

Figure. 6 Kinetic study of serotonin glucuronidation in rat microsomes. Serotonin glucuronidation was measured in the rat liver, intestine, kidney and brain microsomes with serotonin concentrations ranging from 0.02 to 40 mM. Eadie–Hofstee plots are shown as insets in each graph. The points represent the means of three independent determinations.

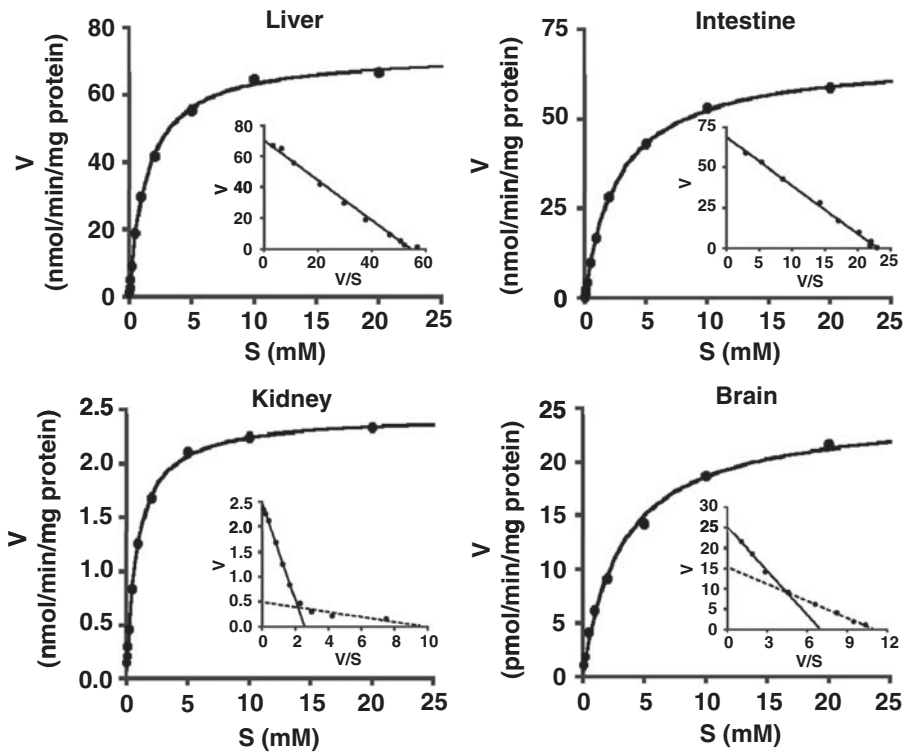

Figure 7. Kinetic study of serotonin glucuronidation in mouse microsomes. Serotonin glucuronidation was measured in the rat liver, intestine, kidney and brain microsomes with serotonin concentrations ranging from 0.02 to 40 mM. Eadie–Hofstee plots are shown as insets in each graph. The points represent the means of three independent determinations.

glucuronidation with K_m values of 2.6 and 11.0 mM, respectively (Uchihashi et al., 2013). In the present study, the K_m values for serotonin glucuronidation in the mouse kidney and brain were lower than those reported previously. These results indicated that serotonin glucuronidation in the mouse may be not only catalyzed by Ugt1a6a and Ugt1a6b, but also by other Ugt isoforms. Although different Ugt isoforms were found to be expressed in the liver and intestine (Buckely & Klaassen, 2009), the specific Ugt isoforms involved in serotonin glucuronidation remain unclear. Therefore, the expression levels in various tissues should be elucidated to determine the cause of the tissue differences observed in the kinetic analysis of serotonin glucuronidation.

Conclusion

In conclusion, we clarified that species and tissue differences exist in serotonin glucuronidation. The present study thus

provides useful information for *in vivo* UGT studies using serotonin as the UGT probe. Moreover, this study is expected to contribute to advancing understanding of the pharmacokinetics of serotonin as an endogenous substrate of UGT and the physiological significance of UGT. It is necessary to take these potential differences into account when considering serotonin pharmacodynamics and pharmacokinetics.

Declaration of interest

This work was supported partly by JSPS KAKENHI Grant Number 25460200. The authors report no declarations of interest.

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