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In Vitro Characterization of Glucuronidation of Vanillin: Identification of Human UDP-Glucuronosyltransferases and Species Differences

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Vanillin is a food flavoring agent widely utilized in foods, beverages, drugs, and perfumes and has been demonstrated to exhibit multiple pharmacological activities. Given the importance of glucuronidation in the metabolism of vanillin, the UDP-glucuronosyltransferase conjugation pathway of vanillin was investigated in this study. Vanillin glucuronide was identified by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and a hydrolysis reaction catalyzed by β-glucuronidase. The kinetic study showed that vanillin glucuronidation by HLMs and HIMs followed Michaelis-Menten kinetics and the kinetic parameters were as follows: 134.9 ± 13.5 μM and 81.3 ± 11.3 μM for K_m of HLMs and HIMs, 63.8 ± 2.0 nmol/min/mg pro and 13.4 ±2.0 nmol/min/mg pro for V_{max} of HLMs and HIMs. All UDP-glucuronosyltransferase (UGT) isoforms except UGT1A4, 1A9, and 2B7 showed the capability to glucuronidate vanillin, and UGT1A6 exerted the higher V_{max}/K_m values than other UGT isoforms for the glucuronidation of vanillin when assuming expression of isoforms is similar in recombinant UGTs. Kinetic analysis using liver microsomes from six studied speices indicated that vanillin had highest affinity for the monkey liver microsomes enzyme (K_m = 25.6 ± 3.2 μM) and the lowest affinity for the mice liver microsomes enzyme (K_m = 149.1 ± 18.4 μM), and intrinsic clearance was in the following order: monkey > dog > minipig > mice > rat ~ human. These data collectively provided important information for understanding glucuronidation of vanillin. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: vanillin; glucuronidation; species difference.

Supporting information may be found in the online version of this article (Supplementary Material)

INTRODUCTION

Glucuronidation, catalyzed by the UDP-glucuronosyltransferases (UGTs), represents a major phase II conjugation reaction of substantial compounds including drugs from all therapeutic classes, dietary chemicals, environmental pollutants, and endogenous compounds (e.g. bilirubin, bile acids, hydroxysteroids) (King *et al.*, 2000). By adding the glycosyl group of uridine-5′-diphosphoglucuronic acid (UDPGA), such compounds become more hydrophilic and are therefore more readily excreted through bile and urine. To date, 19 human UGT proteins have been identified based on sequence homologies (Radominska-Pandya *et al.*, 1999; Mackenzie *et al.*, 2005). Nevertheless, several UGT isoforms (UGT1A5, 2B4, 2B10, 2B11, and 2B18) seem to exhibit low or no activity with xenobiotic compounds. Increasing evidences indicate that the active UGTs exhibit broad and overlapping substrate specificities (Tukey and Strassburg, 2000; Miners *et al.*, 2004). Additionally, tissue-specific expression is also a significant feature of UGTs. For example, UGT 1A7, 1A8, and 1A10 are only expressed in the gastrointestinal tract; whereas UGT2A1 seems to be mainly involved in olfactory signal termination.

Vanillin (Fig. 1), a food flavoring agent widely utilized in foods, beverages, drugs, and perfumes, has been generally regarded as safe status by the Flavor and Extract Manufacturers Association and recognized to be suitable for food use by the Food and Drug Administration (Opdyke, 1975). Meanwhile, vanillin has also been demonstrated to exhibit multiple pharmacological activities, including inhibition of mutagenesis (Keshava *et al.*, 1998; Shaughnessy *et al.*, 2001), chemoprevention (Tsuda *et al.*, 1994; Akagi *et al.*, 1995), treatment of sickle cell anemia disease (Abraham *et al.*, 1991), and antioxidant activity (Kumar *et al.*, 2004). The knowledge related to vanillin's metabolism can be summarized as follows: For phase I metabolism, vanillin mainly undergoes two pathways of biotransformation (oxidation and reduction) to generate its corresponding acids and/or alcohols. Both the parent compound and its metabolites are extensively conjugated with glucuronic acid *in vivo* (Strand and Scheline, 1975). Among known human UGTs, UGT1A3, UGT1A6, UGT1A7, UGT1A8, UGT1A10, and UGT2A1 were previously demonstrated to be able to glucuronidate vanillin (Jedlitschky *et al.*, 1999; Li *et al.*, 2000; Basu *et al.*, 2004). However, comprehensive study to identify human UGTs involved in vanillin glucuronidation remained to be achieved. The same fundamental biochemical machinery exists in various species. However, different species have their own unique characteristics, and their capabilities of metabolizing drugs are different.

The aims of this study were: (i) to determine the kinetics of vanillin glucuronidation by human liver microsomes (HLMs) and human intestinal microsomes (HIMs); (ii) to identify the human UGT isoforms responsible for

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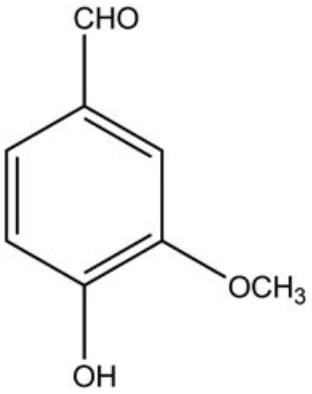

Figure 1. The structure of vanillin.

the vanillin glucuronidation; and (iii) to compare the species differences of vanillin glucuronidation by liver microsomes from human, dog, rat, mice, minipig, and cynomolgus monkey.

MATERIALS AND METHODS

Chemicals and reagents. Alamethicin, magnesium chloride, D-saccharic acid 1,4-lactone, β-D-glucuronidase, and UDPGA were purchased from Sigma-Aldrich (St. Louis, MO, USA). Vanillin (purity > 98%) was obtained from Sichuan Weikeqi Bio-technology Co.Ltd (Sichuan, China). HLMs, cDNA-expressed recombinant UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, and UGT2B17 derived from baculovirus-infected insect cells were purchased from BD Gentest (Woburn, MA). All other reagents were of high-performance liquid chromatography (HPLC) grade or the highest purity commercially available.

Procedures involving animals complied with the Laboratory Animal Management Principles of China. C57BL/6 mice ($n = 20$, male, 6 weeks old, 18 to 20 g) and Sprague–Dawley rats ($n = 10$, male, 180–220 g) were purchased from Shanghai SLAC Laboratory Animals Co. (Shanghai, China). The animals had free access to tap water and pellet diet. All the mice and rats were euthanized by decapitation, and livers were rapidly excised and pooled for preparation of microsomes.

Colony-bred Chinese Bama minipigs weighing 10 to 12 kg ($n = 3$, male, 6 months old) and beagle dogs weighing about 10 kg ($n = 3$, male, 12 months old) were obtained from the Department of Animal Science, Third Military Medical University, China. These animals were euthanized by i.v. injection of pentobarbital sodium (150 mg/kg); Tissue samples were taken from the left medial lobe of the liver within 5 min after death.

Cynomolgus monkeys ($n = 3$, male, 4 years old) weighing 2.7 to 3.0 kg were provided by the animal center of Chinese Academy of Military Medical Sciences, China. All the Cynomolgus monkeys were euthanized by decapitation, and livers were rapidly excised and pooled for preparation of microsomes.

Microsomes were prepared from liver tissue by differential ultracentrifugation. Protein concentrations of microsomal fractions were determined by the Lowry method using bovine serum albumin as a standard (Lowry *et al.*, 1951).

Glucuronidation activity assay. Vanillin (500 μM) was incubated in 0.2 ml reaction mixtures of containing 50 mM Tris–HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 25 μg/ml alamethicin, 10 mM D-saccharic acid 1,4-lactone, and HLM or HIM (0.25 mg of protein/ml) at 37 °C for 60 min in the presence of 5 mM UDPGA. The reaction was terminated by addition of 0.1 ml of methanol, followed by centrifugation at 20000 *g* for 10 min to obtain the supernatant for ultra-fast liquid chromatography spectrometry (UFLC) analysis. Control incubations without UDPGA or without substrate or without microsomes were performed to ensure that the metabolites formation were microsomes and UDPGA dependent. All incubations were performed in duplicate, and results were expressed as mean ± S.D.

UFLC analysis. The separation and quantification of vanillin glucuronide were carried out using a Shimadzu (Kyoto, Japan) Prominence UFLC system, which was equipped with a CBM-20A communications bus module, an SIL-20ACHT autosampler, two LC-20 AD pumps, a DGU-20A3 vacuum degasser, and a CTO-20 AC column oven, as well as a diode array detector (DAD) detector. A Shim-pack XR-ODS column (75 mm × 2.0 mm, 2.2 μm, Shimadzu) was kept at 40 °C. The mobile phase consisted of CH_3CN (A) and 0.5 % (v/v) formic acid (B). The following gradient condition was used: 0–8 min, 98–55 % B; 8–10.5 min, 5% B; 10.5–14 min, 98% B. The flow rate was set at 0.3 ml/min. The injection volume was 10 μl, and the scan wavelength was set at 277 nm.

Hydrolysis with β-glucuronidase. β-glucuronidase was used to identify glucuronide. For this purpose, D-saccharic acid 1,4-lactone was omitted, and an aliquot of the incubation mixture was mixed with an equal volume of 0.15 M acetate buffer (pH 5.0) containing 1800 Fishman units of β-D-glucuronidase and incubated for 2 h at 37 °C before UFLC analysis. The other part containing no β-glucuronidase was treated in parallel as control.

Analysis of vanillin glucuronide by UPLC-Q-TOF-MS/MS analysis. The incubation of vanillin with HLMs for structure elucidation was performed as described above. Detection of vanillin glucuronide was carried out with injection of 20 μl of centrifugal supernatant onto the UPLC-Q-ToF-MS/MS system. An acquity BEH-C8 column (100 mm × 2.0 mm, 1.7 μm, waters) was kept at 40 °C. The mobile phase consisted of CH_3CN (A) and 0.5 % (v/v) formic acid (B). The following gradient condition was used: 0–5 min, 70–20% B; 5–7.5 min, 5% B; 7.5–11 min, 70% B. The flow rate was set at 0.3 ml/min. Spectra were recorded in negative-ion modes from m/z 100 to 600 with a 1.0 s integration time per spectrum. Nitrogen was used as both drying and nebulizing gas with flow rates of 450 and 50 l/h, respectively. The ESI conditions were as follows: source temperature, 100 °C; desolvation temperature, 190 °C; capillary, 2.3 kV; extraction cone, 2 V. For tandem mass detection, argon was used as collision gas, the collision gas pressure was set at 9 psi for negative mass detection, and the collision voltage ranged from 5 V to 33 V by steps of 2 V.

Assay with recombinant UGTs. Vanillin glucuronidation was measured in reaction mixtures containing recombinant human UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8,

1A9, 1A10, 2B4, 2B7, 2B15, and 2B17. The incubations were carried out as described for the HLM study. 100 μM of substrate and 0.1 mg of protein/ml were used. All of the isoforms were left to react at 37 °C for 20 min. UFLC with DAD detection was used to monitor possible metabolites.

Kinetic parameters analysis. The kinetic studies were carried out using liver microsomes obtained from human, rat, mice, dog, minipig, monkey, HIMs, and recombinant UGT isoforms expressed in the homogenate. The incubation time was 20 min. The protein concentrations were 0.1 mg/ml for recombinant UGT isoforms and 0.025 mg/ml for liver microsomes and HIM. These concentrations were within the linear range of the glucuronide formation. To determine the kinetic parameters, the vanillin concentration ranged from 5 to 2000 μM. Due to the difficulty to obtain the standard of vanillin glucuronide, the quantification of vanillin glucuronide was carried out using the standard curve of vanillin assuming the same absorbance index between vanillin and its glucuronide.

Data analysis. Kinetic constants for vanillin glucuronidation by liver microsomes, HIMs and recombinant UGT isoforms were calculated by fitting experimental data to the Michaelis-Menten Kinetics using Origin (OrginLab Corporation, Northampton, MA). The Michaelis-Menten equation is $v = (V_{max} + [S])/(K_m + [S])$, where v is the rate of reaction, V_{max} is the maximum velocity, K_m is the Michaelis constant (substrate concentration at 0.5 V_{max}), and [S] is the substrate concentration. Kinetic constants are reported as the value ± S.E. of the parameter estimate.

RESULTS

Identification of vanillin metabolites

When vanillin was incubated with HLMs in the presence of UDPGA, a new peak was eluted at 4.85 min (Fig. 2). When treated with β-glucuronidase before HPLC analysis, this product was absent (data not shown). The electrospray ionization mass spectra (Supplement Fig. 1)

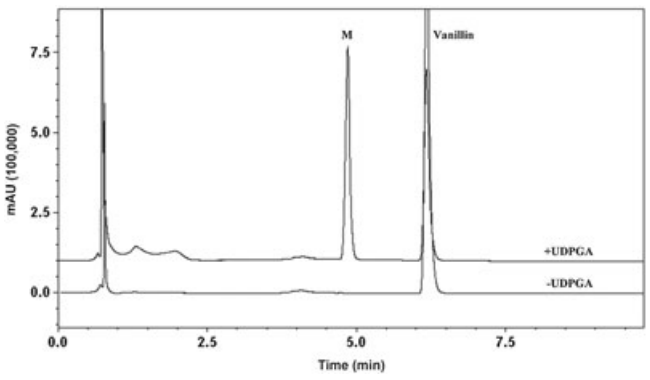

Figure 2. Representative UFLC-DAD profile of vanillin and its metabolite. 0.25 mg/ml HLM was incubated with vanilline (500 μM) at 37 °C for 60 min in the presence or absence of UDPGA (5 mM) as described under Materials and Methods.

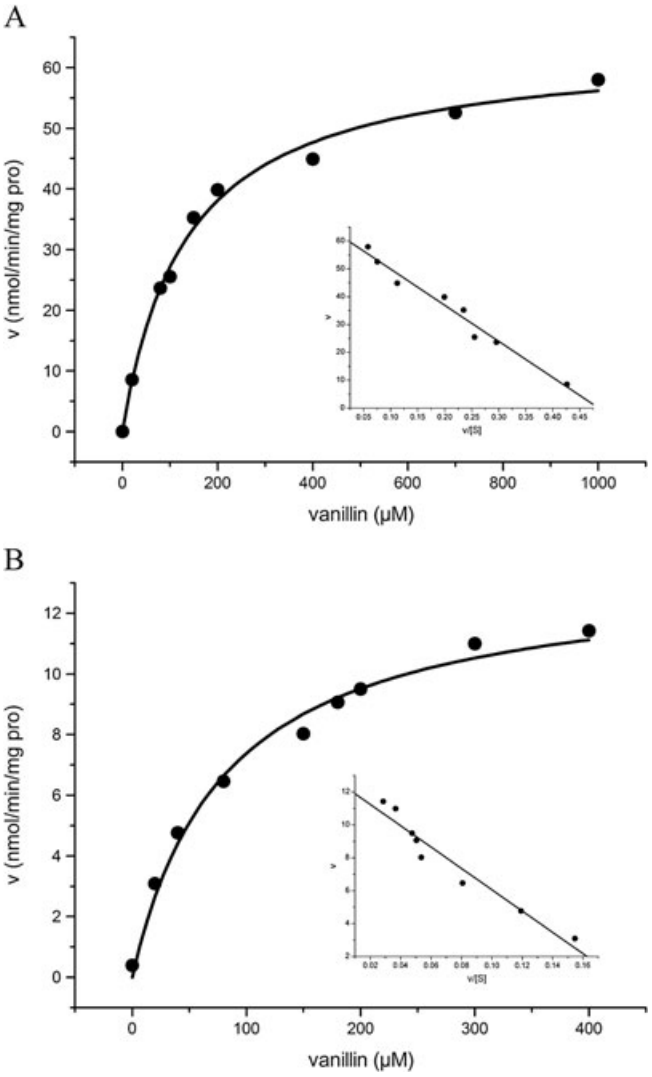

Figure 3. Enzyme kinetics of vanillin glucuronidation by HLM (A) and HIM (B). Various concentrations of vanillin were incubated with HLM (0.025 mg/ml) and HIM (0.025 mg/ml) at 37 °C for 20 min. An Eadie-Hofstee plot is shown as an inset to illustrate monophase kinetics.

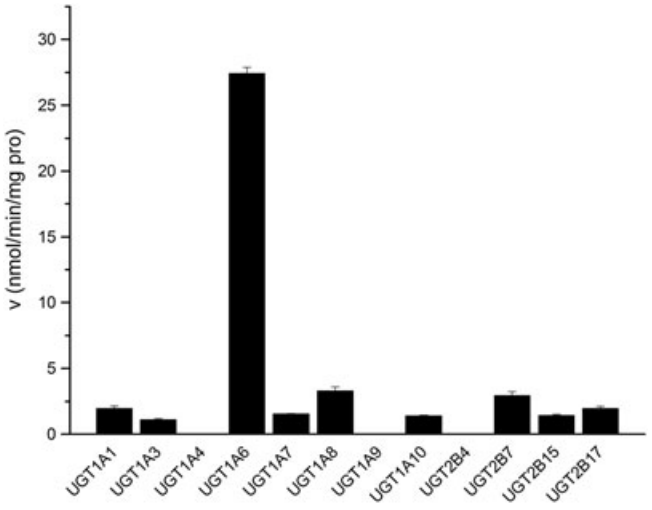

Figure 4. Screening of the UGT isoforms catalyzing the formation of vanillin glucuronide. Vanillin (100 μM) was incubated with various recombinant human UGTs 0.1 mg/ml) at 37 °C for 20 min. Data represent the mean of duplicate incubations.

showed an [M-H]$^-$ ion at m/z 327, corresponding to vanillin glucuronide and a fragment ion at m/z 151, corresponding to the parent compound vanillin with loss of glucuronic acid moiety (176 amu). The same metabolite, vanillin glucuronide, was detected in HIMs and experimental animal liver microsomes (figure not shown).

Kinetics of vanillin glucuronidation in HLMs and HIMs

Pooled HLMs and HIMs were utilized to investigate the kinetics of vanillin glucuronidation. As shown in Fig. 3, the substrate concentration–glucuronidation velocity curves followed typical Michaelis-Menten Kinetics. Incubation of various concentrations of vanillin with HLMs demonstrated that the K_m, V_{max} and Cl_{int} (V_{max}/K_m) values for vanillin glucuronide were 134.9 ± 13.5 μM, 63.8 ± 2.0 nmol/min/mg protein and 472.9 μl/min/mg protein, respectively. Under the same condition, when HIMs were employed, the kinetic parameters were $K_m = 81.3 \pm 11.3$ μM, $V_{max} = 13.4 \pm 0.6$ nmol/min/mg protein and $Cl_{int} = 164.8$ μl/min/mg protein, respectively.

Vanillin glucuronidation in recombinant UGT isoforms

The commercially available recombinant human UGT isoforms expressed in baculovirus-infected insect cells were selected to investigate the glucuronidation of vanillin. Among the 12 UGT isoforms tested, UGT 1A1, 1A3, 1A6, 1A7, 1A8, 1A10, 2B7, 2B15, and 2B17 catalyzed the glucuronidation of 100 μM vanillin, with UGT 1A6 being the most active. However, no metabolite production was detected with UGT 1A4, 1A9, 2B4, and control Supersomes (Fig. 4).

For subsequent studies, the kinetic parameters of vanillin-glucuronide production were determined for all the competent UGT isoforms. As shown in Fig. 5, each Eadie-Hofstee plot was monophasic, which suggested that vanillin glucuronidation by all involved UGT isoforms displayed typical Michaelis-Menten kinetics. Fitting the data points to the Michaelis-Menten equation yielded the kinetic parameters listed in Table 1. The results showed that the metabolic activity (V_{max}/K_m) of all the competent UGT isoforms was as follows: UGT1A6 > UGT2B15 > UGT1A7 > UGT2B7 > UGT1A8 > UGT1A1 > UGT2B17 > UGT1A10 > UGT1A3.

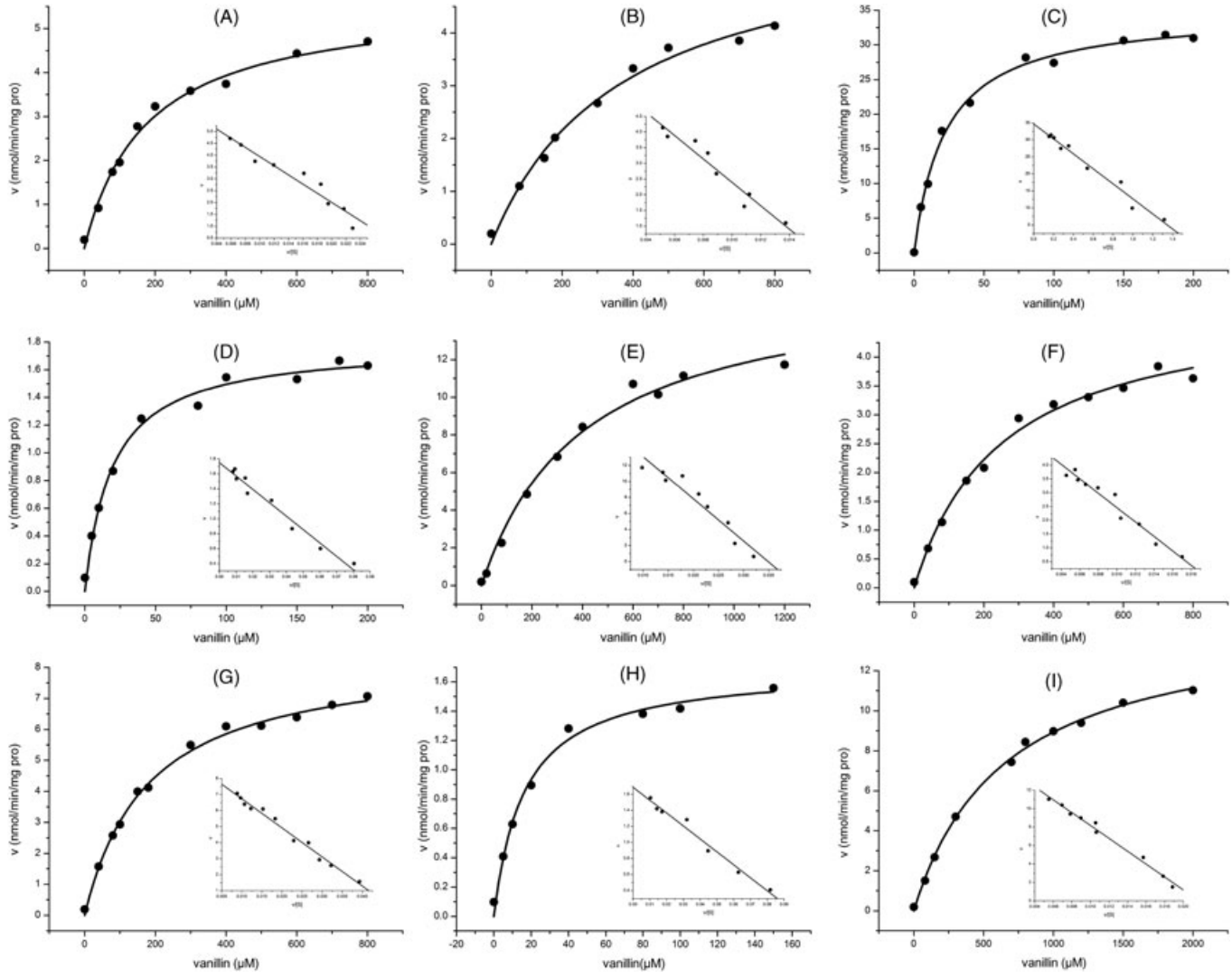

Figure 5. Enzyme kinetics of vanillin glucuronidation by UGT1A1 (A), UGT1A3 (B), UGT1A6 (C), UGT1A7 (D), UGT1A8 (E), UGT1A10 (F), UGT2B7 (G), UGT2B15 (H), and UGT2B17 (I). Vanillin (5–2000 μM) was incubated with 0.1 mg/ml recombinant UGT isoforms at 37 °C for 20 min.

Table 1. Kinetic parameters for the glucuronidation of vanillin by recombinant UGT enzymes.

Allozymes	Apparent Km μM	Observed Vmax nmol/min/mg	Vmax/Km μl/min/mg
rUGT1A1	175.7 ± 18.0	5.7 ± 0.2	32.4
rUGT1A3	366.3 ± 50.1	6.7 ± 0.4	18.3
rUGT1A6	22.3 ± 1.7	34.9 ± 0.7	1565.0
rUGT1A7	19.4 ± 2.2	1.8 ± 0.0	92.8
rUGT1A8	402.6 ± 57.5	16.4 ± 1.0	40.7
rUGT1A10	250.4 ± 28.9	5.0 ± 0.2	20.0
rUGT2B7	179.2 ± 11.4	8.5 ± 0.2	47.4
rUGT2B15	16.3 ± 2.0	1.7 ± 0.1	104.3
rUGT2B17	651.4 ± 41.3	14.7 ± 0.4	22.6

Results are expressed as mean ± standard deviation of two independent experiments performed in triplicates.

Kinetics of vanillin glucuronidation in liver microsomes obtained from experimental animals

Kinetic analysis of vanillin glucuronidation was carried out in liver microsomes obtained from experimental animals. All Eadie-Hofstee plots were monophasic, suggesting that vanillin glucuronidation catalyzed by liver microsomes from dog, rat, mice, minipig, and monkey displayed typical Michaelis-Menten kinetics (data not shown). Fitting the data points to the Michaelis-Menten equation produced the kinetic constants listed in Table 2. Incubation of various concentrations of vanillin with dog, rat, mice, minipig, and monkey liver microsomes demonstrated that the Cl_{int} values for vanillin glucuronide were 6286.7, 488.1, 1667.3, 3721.0, and 8820.3 μl/min/mg protein, respectively.

DISCUSSION

The formation of vanillin glucuronide in our *in vitro* system was confirmed by the requirement for UDP-glucuronic acid to produce a more polar metabolite that was converted back to the corresponding aglycone when treated with β-glucuronidase. The kinetic analysis results showed that vanillin had the highest affinity for the monkey liver microsomal enzyme ($K_m = 25.6 \pm 3.2$ μM) and the lowest affinity for the mice liver microsomal enzyme ($K_m = 149.1 \pm 18.4$ μM) in the tested six species. The metabolic activity (V_{max}/K_m) of vanillin was in the following order: monkey > dog > minipig > mice > rat ~ human. Thus, rat might potentially be a better model than other species for the assessment of vanillin glucuronidation.

Table 2. Kinetic parameters of vanillin glucuronidation in microsomes obtained from human and experimental animals and human intestinal microsomes (HIMs).

Enzyme source	Apparent Km μM	Observed Vmax nmol/min/mg	Vmax/Km μl/min/mg
HLMs	134.9 ± 13.5	63.8 ± 2.0	472.9
HIMs	81.3 ± 11.3	13.4 ± 0.6	164.8
Dog liver	74.3 ± 11.0	467.1 ± 24.3	6286.7
Rat liver	117.6 ± 15.4	57.4 ± 2.5	488.1
Mice liver	149.1 ± 18.4	248.6 ± 11.2	1667.3
Minipig liver	42.3 ± 7.3	157.4 ± 7.6	3721.0
Monkey liver	25.6 ± 3.2	225.8 ± 7.5	8820.3

Results are expressed as mean ± standard deviation of two independent experiments performed in triplicates.

Although the liver plays a central role in metabolism, extrahepatic tissues such as the gastrointestinal tract and kidney also play a significant role in metabolism (Miners *et al.*, 2006). In this study, the kinetics of vanillin glucuronidation by HLMs and HIMs were compared, and the results showed that the intrinsic clearance value of HIMs was about one-third of the corresponding value of HLM, which suggested that the intestine might not be the main glucuronidation metabolic organ. Nevertheless, the systemic bioavailability of vanillin is likely to be reduced due to pre-systemic elimination to which the intestinal glucuronidation could contribute. It should be noted that we could not measure the exact contribution because no HLMs and HIMs were obtained from the same donor. Additionally, the role of intestinal glucuronidation of vanillin might be underestimated due to relatively higher concentration of vanillin in the intestine and the larger surface area of intestine. Thus, the intestinal glucuronidation of vanillin remained to be explored.

Systematic study was carried out to characterize human UGTs involved in vanillin glucuronidation using *in vitro* recombinant human UGT isoforms. Our observations demonstrated that UGT 1A1, 1A3, 1A6, 1A7, 1A8, 1A10, 2B7, 2B15, and 2B17 catalyzed the glucuronidation of vanillin, whereas metabolism by UGT 1A4, UGT 1A9, and UGT 2B4 could not be detected under the assay conditions employed in this work. The kinetic profiles of all involved UGT isoforms followed typical Michaelis-Menten (i.e. hyperbolic) kinetics and the kinetic parameters varied among the UGT isoforms tested (K_m values ranging from 16.3 ± 2.0 to 651.4 ± 41.3 μM and V_{max} values ranging from 1.7 ± 0.1 to 34.9 ± 0.7 nmol/min/mg). In vitro clearance for vanillin glucuronidation was assessed as CL_{int}, and the results showed that vanillin clearance by UGT 1A6 was more than 15-fold higher than for any other UGT isoforms. This result agreed with previous findings that UGT 1A6 is the UGT isoform responsible for conjugating phenols and phenolic compounds, especially for catalyzing the glucuronidation of phenolic substrates with steric requirements of small size and planarity (Ebner and Burchell, 1993; Orzechowski *et al.*, 1994; Bock and Kohle, 2005). These results are also consistent with the previous results that glucuronidation of protocatechuic aldehyde (O-demethyl vanillin) is mainly catalyzed by UGT1A6 (Liu *et al.*, 2008).

In summary, vanillin was efficiently glucuronidated by liver microsomes obtained from various species and HIMs. Multiple recombinant UGT isoforms were demonstrated to be involved in the glucuronidation of vanillin, with UGT1A6 exerting the higher Vmax/Km values than other UGT isoforms for glucuronidation of vanillin, assuming expression of isoforms is similar in recombinant UGTs. At present, we cannot clearly determine whether any of these UGT isoforms may be dominant for vanillin glucuronidation due to the limited knowledge of the relative expression of UGT isoforms in human tissues. However, the apparent participation of multiple UGT isoforms in vanillin glucuronidation

indicates that possible drug-drug interactions with individual UGTs might not significantly affect in vivo glucuronidation of vanillin in humans.

Conflict of Interest

The authors have declared that there is no conflict of interest.

REFERENCES

Abraham DJ, Mehanna AS, Wireko FC, Whitney J, Thomas RP, Orringer EP. 1991. Vanillin, a potential agent for the treatment of sickle cell anemia. *Blood* **77**: 1334–1341.

Akagi K, Hirose M, Hoshiya T, Mizoguchi Y, Ito N, Shirai T. 1995. Modulating effects of ellagic acid, vanillin and quercetin in a rat medium-term multiorgan carcinogenesis model. *Cancer Lett* **94**: 113–121.

Basu NK, Kubota S, Meselhy MR, *et al*. 2004. Gastrointestinally-distributed UDP-glucuronosyltransferase 1A10, which metabolizes estrogens and nonsteroidal antiflammatory drugs, depends upon phosphorylation. *J Biol Chem* **279**: 28320–28329.

Bock KW, Kohle C. 2005. UDP-glucuronosyltransferase 1A6: Structural, functional, and regulatory aspects. *Methods Enzymol* **400**: 57–75.

Ebner T, Burchell B. 1993. Substrate specificities of 2 stably expressed human liver UDP-glucuronosyltrasferases of the UGT1 gene family. *Drug Metab Dispos* **21**: 50–55.

Jedlitschky G, Cassidy AJ, Sales M, Pratt N, Burchell B. 1999. Cloning and characterization of a novel human olfactory UDP-glucuronosyltransferase. *Biochem J* **340**: 837–843.

Keshava C, Keshava N, Ong TM, Nath J. 1998. Protective effect of vanillin on radiation-induced micronuclei and chromosomal aberrations in V79 cells. *Mutat Res* **397**: 149–159.

King CD, Rios GR, Green MD, Tephly TR. 2000. UDP-glucuronosyltransferases. *Curr Drug Metab* **1**: 143–161.

Kumar SS, Priyadarsini KI, Sainis KB. 2004. Inhibition of peroxynitrite-mediated reactions by vanillin. *J Agric Food Chem* **52**: 139–145.

Li Q, Lamb G, Tukey RH. 2000. Characterization of the UDP-glucuronosyltransferase 1A locus in lagomorphs: Evidence for duplication of the UGT1A6 gene. *Mol Pharmacol* **58**: 89–97.

Liu HX, Liu Y, Zhang JW, Li W, Liu HT, Yang L. 2008. UDP-glucuronosyltransferase 1A6 is the major isozyme responsible for protocatechuic aldehyde glucuronidation in human liver microsomes. *Drug Metab Dispos* **36**(8): 1562–1569.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265–275.

Mackenzie PI, Bock KW, Burchell B, *et al*. 2005. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenet Genomics* **15**: 677–685.

Miners JO, Knights KM, Houston JB, Mackenzie PI. 2006. *In vitro-in vivo* correlation for drugs and other compounds eliminated by glucuronidation in humans: Pitfalls and promises. *Biochem Pharmacol* **71**: 1531–1539.

Miners JO, Smith PA, Sorich MJ, McKinnon RA, Mackenzie PI. 2004. Predicting human drug glucuronidation parameters: Application of in vitro and in silico modeling approaches. *Annu Rev Pharmacol Toxicol* **44**: 1–25.

Opdyke DL. 1975. Monographs on fragrance raw materials. *Food Cosmet Toxicol* **13**: 449–457.

Orzechowski A, Schrenk D, Bock-Hennig BS, Bock KW. 1994. Glucuronidation of carcinogenic arylamines and their N-hydroxy derivatives by rat and human phenol UDP-glucuronosyltransferases of the UHT1 gene complex. *Carcinogenesis* **15**: 1549–1553.

Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, Mackenzie PI. 1999. Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev* **31**: 817–899.

Shaughnessy DT, Setzer RW, Demarini DM. 2001. The antimutagenic effect of vanillin and cinnamaldehyde on spontaneous mutation in Salmonella TA104 is due to a reduction in mutations at GC but not AT sites. *Mutat Res* **480**: 55–69.

Strand LP, Scheline RR. 1975. Metabolism of vanillin and isovanillin in rat. *Xenobiotica* **5**: 49–63.

Tsuda H, Uehara N, Iwahori Y, *et al*. 1994. Chemopreventive effects of beta-carotene, alpha-tocopherol and 5 naturally-occurring antioxidants on initiation of hepatocarcinogenesis by 2-amino-3-methylimidzo [4,5-f] quinoline in the rat. *Jpn J Cancer Res* **85**: 1214–1219.

Tukey RH, Strassburg CP. 2000. Human UDP-glucuronosyltransferases: Metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40**: 581–616.