

Glucuronidation of thyroxine in human liver, jejunum, and kidney microsomes

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¹Abbreviations used are: HLM, human liver microsomes; HJM, human jejunum microsomes; HKM, human kidney microsomes; LC-MS/MS, liquid chromatography-mass/mass spectrometry; UGT, UDP-glucuronosyltransferase; UDPGA, UDP-glucuronic acid.

Abstract

Glucuronidation of thyroxine is a major metabolic pathway facilitating its excretion. In this study, we characterized the glucuronidation of thyroxine in human liver, jejunum, and kidney microsomes, and identified human UDP-glucuronosyltransferase (UGT) isoforms involved in the activity. Human jejunum microsomes showed a lower K_m value (24.2 μM) than human liver (85.9 μM) and kidney (53.3 μM) microsomes. Human kidney microsomes showed a lower V_{max} value (22.6 pmol/min/mg) than human liver (133.4 pmol/min/mg) and jejunum (184.6 pmol/min/mg) microsomes. By scaling-up, the *in vivo* clearances in liver, intestine, and kidney were estimated to be 1440, 702, and 79 $\mu\text{l}/\text{min}/\text{kg}$ of body weight, respectively. Recombinant human UGT1A8 (108.7 pmol/min/unit), UGT1A3 (91.6 pmol/min/unit), and UGT1A10 (47.3 pmol/min/unit) showed high, and UGT1A1 (26.0 pmol/min/unit) showed moderate thyroxine glucuronosyltransferase activity. The thyroxine glucuronosyltransferase activity in microsomes from 12 human livers was significantly correlated with bilirubin *O*- ($r = 0.855$, $p < 0.001$) and estradiol 3-*O*- ($r = 0.827$, $p < 0.0001$) glucuronosyltransferase activities catalyzed by UGT1A1, indicating that the activity in human liver is mainly catalyzed by UGT1A1. Kinetic and inhibition analyses suggested that the thyroxine glucuronidation in human jejunum microsomes was mainly catalyzed by UGT1A8 and UGT1A10 and to a lesser extent UGT1A1, and the activity in human kidney microsomes was mainly catalyzed by UGT1A7, UGT1A9 and UGT1A10. The changes of activities of these UGT1A isoforms via inhibition and induction by administered drugs as well as genetic polymorphisms may be a causal factor of interindividual differences in the plasma thyroxine concentration.

Introduction

Thyroid hormones mediate many physiological processes including embryonic development, cellular differentiation, metabolism, and the regulation of cell proliferation (Hulbert, 2000, Wu and Koenig, 2000; Zhang and Lazar, 2000). The plasma concentrations of thyroid hormones are strictly controlled by thyroid stimulating hormone, which is subjected to negative feedback regulation by thyroid hormones. The major form of thyroid hormone secreted from thyroid gland is thyroxine. In hypothyroidism, thyroxine is orally administered to keep the plasma thyroid hormone level normal. Thyroxine has little biological activity, and is converted to the active form, triiodothyronine (T3) mainly in human liver and kidney (Leonard and Koehrle, 1996). In human, about 80% of the total plasma T3 is produced by thyroxine via outer ring deiodination (Fig. 1). In addition to the deiodination, thyroxine is metabolized to sulfate and glucuronide by sulfotransferases and UDP-glucuronosyltransferases (UGTs), respectively (Fig. 1). The thyroxine sulfate hardly appears in bile, urine or serum, because it is rapidly degraded by inner ring deiodination in the liver. In contrast to the sulfate, thyroxine glucuronide is readily excreted into bile, and subsequently hydrolyzed by β -glucuronidases in the intestine and may affect the enterohepatic circulation of thyroxine (Visser, 1994). It has been reported that administration of UGT inducers such as phenytoin, carbamazepine (Isojarvi et al., 1992), and rifampicin (Ohnhaus and Studer, 1983) markedly decreased the serum thyroxine levels in patients. Thus, the glucuronidation is a major metabolic pathway to control the serum thyroxine level.

In humans, UGTs are divided into two families: UGT1 and UGT2 (Mackenzie et al., 2005). The *UGT1* and *UGT2* genes appear to be structurally different in that the UGT1 proteins result from alternate splicing of the unique first exon with four common exons encoded by the *UGT1* gene complex, whereas UGT2 proteins appear to be encoded by unique genes. In humans, there are nine functional UGT1A and seven UGT2B proteins (Mackenzie et al., 2005). Among them, human UGT1A1 and UGT1A9 have been reported to catalyze thyroxine glucuronidation (Fendlay et al., 2000). However, a limitation of the study was that

only two UGT isoforms were investigated. The first purpose of the present study is to investigate the catalytic activity of thyroxine glucuronidation by all human UGT isoforms for which recombinant proteins are currently available.

Human UGTs are expressed in a tissue-specific manner. Table 1 summarizes the UGT isoforms expressed in human liver, intestine, and kidney (King et al., 2000; Tukey and Strassburg, 2000; Fisher et al., 2001; Levesque et al., 2001; Basu et al., 2004, Finel et al., 2005). Generally, liver plays an important role in the glucuronidation of most drugs, but extrahepatic tissues also contribute significantly to the glucuronidation of certain drugs in human (Krishna and Klotz, 1994). It is conceivable that the glucuronidation of thyroxine occurs not only in liver but also in intestine and kidney. The second purpose of the present study is to characterize thyroxine glucuronidation in human liver, jejunum, and kidney microsomes, and to identify the UGT isoforms involved in the glucuronidation in each tissue.

Materials and Methods

Materials. Thyroxine, UDP-glucuronic acid (UDPGA), and alamethicin were purchased from Sigma-Aldrich (St. Louis, MO). Bilirubin, chenodeoxycholic acid, serotonin, imipramine hydrochloride, and propofol were purchased from Wako Pure Chemicals (Osaka, Japan). Morphine hydrochloride was from Takeda Chemical Industries (Osaka, Japan). Pooled human liver microsomes (Lot H161), microsomes from 12 individual human livers (H003, H023, H030, H043, H056, H064, H066, H070, H089, H093, H112, and HK23), recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10, UGT2B4, UGT2B7, UGT2B15, UGT2B17 expressed in baculovirus-infected insect cells (Supersomes), and UGT control Supersomes were purchased from BD Gentest (Woburn, MA). The human jejunum (Lot HJM0023) or kidney (Lot 045290170002) microsomes from an individual donor were purchased from KAC (Kyoto Japan). All other chemicals and solvents were of analytical or the highest grade commercially available.

Thyroxine glucuronidation assay. A typical incubation mixture (100 μ l total volume) contained 100 mM Tris-HCl buffer (pH 7.4), 5 mM $MgCl_2$, 5 mM UDPGA, 25 μ g/ml alamethicin, 0.4 mg/ml human liver, jejunum, or kidney microsomes or recombinant UGTs, and 50 μ M thyroxine. Thyroxine was dissolved in dimethyl sulfoxide/0.05 M sodium hydroxide (50:50). The final concentration of the organic solvents in the incubation mixture was 1% (v/v). The reaction was initiated by the addition of UDPGA. After incubation at 37°C for 90 min, the reaction was terminated by adding 100 μ l of ice cold 94% acetonitrile/6% formic acid. After the centrifugation at 12,000 rpm for 5 min, the supernatant was filtered with a 0.22 μ m filter (Ultrafree®-MC centrifugal filter unit, Millipore, Eschborn, Germany). Aliquots of 10 μ l were injected into the LC-MS/MS system.

LC-MS/MS analysis for thyroxine glucuronides. LC was performed using an HP1100 system including a binary pump, an automatic sampler, and a column oven (Agilent

Technologies, Waldbronn, Germany), which was equipped with a Mightysil RP-18 GP (4.6 x 150 mm; 5 μ m) column (Kanto Chemical, Tokyo, Japan). The column temperature was 35°C. The mobile phase was 0.1% formic acid (A) and acetonitrile including 0.1% formic acid (B). The conditions for elution were as follows: 25% B (0 – 1 min); 25 – 70% B (1 – 4 min); 70% B (4 – 10 min); 70 – 25% B (10 – 11 min). Linear gradients were used for all solvent changes. The flow rate was 0.5 ml/min. The LC was connected to a PE Sciex API2000 tandem mass spectrometer (Applied Biosystems, Langen, Germany) operated in the positive electrospray ionization mode. The turbo gas was maintained at 450°C. Nitrogen was used as the nebulizing, turbo, and curtain gas at 50, 80, 20 psi, respectively. Parent and/or fragment ions were filtered in the first quadrupole and dissociated in the collision cell using nitrogen as the collision gas. The collision energy was 20 V and 26 V for thyroxine and thyroxine glucuronides, respectively. Two mass/charge (m/z) ion transitions were recorded in the multiple reaction monitoring (MRM) mode: m/z 778 and 778 for thyroxine; m/z 954 and 778 for thyroxine glucuronide. The retention times of thyroxine glucuronide and thyroxine were 7.1 min and 8.9 min, respectively. For the quantification of thyroxine glucuronide, the eluate of the HPLC from the incubation mixture including thyroxine glucuronide was collected, referring to the retention time. A part of the eluate was incubated with 800 U/ml of β -glucuronidase at 37°C for 24 hr. The produced thyroxine was quantified by LC-MS/MS. Once we determined the peak area per known content of thyroxine glucuronide, the ratio was applied to the calculation of the thyroxine glucuronide formed in the incubation mixtures.

Kinetic analyses of thyroxine glucuronidation in human liver, jejunum, and kidney microsomes or recombinant UGTs. Thyroxine glucuronosyltransferase activities were determined as described above with substrate concentrations from 2 μ M to 100 μ M. Kinetic parameters were estimated from the fitted curve using a computer program (KaleidaGraph, Synergy Software, Reading, PA) designed for non-linear regression analysis. The following equations were applied for Michaelis-Menten kinetics (1) or substrate inhibition kinetics (2) (Houston and Kenworthy, 2000):

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

$$V = V_{\max} \cdot S / (K_m + S + S^2/K_{si}) \quad (2)$$

where V is the velocity of the reaction, S is the substrate concentration, K_m is Michaelis-Menten constant, V_{\max} is the maximum velocity, and K_{si} is the substrate inhibition constant. Data are expressed as mean \pm SD of three independent determinations.

Estimation of tissue clearances from in vitro data. In vivo clearance was scaled-up by an equation (Obach et al., 1997; Soars et al., 2002):

$$CL = \frac{V_{\max}}{K_m} \times \frac{\text{Microsomal protein}}{\text{Tissue}} \text{ (mg/g)} \times \frac{\text{Tissue}}{\text{Body weight}} \text{ (g/kg)}$$

According to a previous study (Soars et al., 2002), 45, 3, and 45 mg/g tissue were used as the contents of the microsomal protein in liver, intestine, and kidney, respectively. Twenty, 30, and 4.4 g/kg body weight were used as the weights of liver, intestine, and kidney, respectively.

Immunoblot analysis of recombinant human UGT1A isoforms. SDS-polyacrylamide gel electrophoresis and immunoblot analysis of recombinant UGT1A isoforms were performed according to Laemmli (1970). The microsomes from baculovirus-infected insect cells (0.5 μ g) were separated on 10% polyacrylamide gel and transferred electrophoretically to a PVDF membrane Immobilon-P (Millipore, Bedford, MA). Rabbit anti-human UGT1A polyclonal antibodies (BD Gentest) react with all human UGT1A isoforms, since the antibodies recognize the conserved C-terminal region of UGT1A isoforms (Malfatti and Felton, 2004). Biotinylated anti-rabbit IgG and VECTASTAIN ABC kit (Vector Laboratories, Burlingame, CA) were used for diaminobenzidine staining. The densities of the bands were determined using an ImageQuant (GE Healthcare Bio-Science, Piscataway, NJ). For each isoform, the densities of multiple bands, possibly owing to variability in the glycosylation (Malfatti and Felton, 2004), were summed for the quantification. The expression level of UGT1A was defined based on a standard curve using the recombinant UGT1A1 (1 unit per 1 mg protein).

Other glucuronidation assays. Bilirubin *O*- (Kato et al., 2007), imipramine *N*- (Nakajima et al., 2002), and serotonin *O*- (Fujiwara et al., 2007) glucuronosyltransferase activities in microsomes from 12 human livers were determined according to methods established in our laboratory. Chenodeoxycholic acid 24-*O*-glucuronosyltransferase activities in these human liver microsomes were determined according to the method by Trottier et al. (2006) with slight modifications. Estradiol 3-*O*-, propofol *O*-, and morphine 3-*O*-glucuronosyltransferase activities in these human liver microsomes were provided by the manufacturer.

Correlation analyses. Correlation between thyroxine glucuronidations, and the other glucuronosyltransferase activities were determined by unpaired Student's *t*-test. A *p* value of less than 0.05 was considered statistically significant.

Inhibition analysis of thyroxine glucuronosyltransferase activities in human liver, jejunum, and kidney microsomes or recombinant UGTs. Bilirubin is a typical substrate of UGT1A1 (King et al., 2000). Imipramine is a substrate of UGT1A3 and UGT1A4 (Green and Tephly, 1998). Emodin is a substrate of UGT1A1, UGT1A3, UGT1A8 and UGT1A9 (King et al., 2000). Propofol is a substrate of UGT1A8 and UGT1A9 (King et al., 2000). Troglitazone is a substrate of UGT1A1, UGT1A8, and UGT1A10 (Watanabe et al., 2002). Bilirubin was dissolved in 0.1 M sodium hydroxide. Imipramine hydrochloride was dissolved in water. Emodin and troglitazone were dissolved in dimethyl sulfoxide. Propofol was dissolved in methanol. These compounds were added to the incubation mixtures described above to investigate their inhibitory effects on the thyroxine glucuronosyltransferase activities in human liver, jejunum, and kidney microsomes or recombinant UGTs. The final concentration of the organic solvents in the incubation mixture was < 2% (v/v). The substrate concentration was 20 μ M.

Results

Thyroxine glucuronosyltransferase activities in human liver, jejunum, or kidney

microsomes. The formation of thyroxine glucuronide increased in microsomal protein concentration- and time-dependent manners. The formations were linear at least for 0.5 mg/ml microsomal protein and 120 min incubation (data not shown). Unless specified, the typical incubation mixture containing 0.4 mg/ml microsomal protein was incubated at 37°C for 90 min. The kinetics of thyroxine glucuronosyltransferase activities in human liver, jejunum, and kidney microsomes were fitted to the Michaelis-Menten equation (Fig. 2A). The Eadie-Hofstee plots were monophasic (Fig. 2B). The apparent K_m and V_{max} values are summarized in Table 2. Human jejunum microsomes showed a lower K_m value (24.2 μM) than human liver (85.9 μM) and kidney (53.3 μM) microsomes. Human kidney microsomes showed a lower V_{max} (22.6 pmol/min/mg) value than human liver (133.4 pmol/min/mg) and jejunum (184.6 pmol/min/mg) microsomes. The in vitro intrinsic clearances (V_{max}/K_m) of thyroxine glucuronidation in human liver, jejunum, and kidney microsomes were 1.6, 7.8, and 0.4 $\mu\text{l}/\text{min}/\text{mg}$, respectively. By scaling-up, the in vivo clearances in liver, intestine, and kidney were estimated to be 1440, 702, and 79 $\mu\text{l}/\text{min}/\text{kg}$ of body weight, respectively.

Thyroxine glucuronosyltransferase activities by recombinant UGT isoforms. Eleven recombinant UGT isoforms expressed in baculovirus-infected insect cells were used to determine their thyroxine glucuronosyltransferase activities. As shown in Fig. 3A, UGT1A8 exhibited the highest thyroxine glucuronosyltransferase activity (87 pmol/min/mg), followed by UGT1A1 (26 pmol/min/mg), UGT1A10 (20 pmol/min/mg), UGT1A3 (18 pmol/min/mg), UGT1A9 (5 pmol/min/mg), and UGT1A7 (5 pmol/min/mg). To quantify the expression level of UGT1A in each expression system, immunoblot analysis was performed (Fig. 3B). In accordance with previous studies (Malfatti and Felton, 2004; Fujiwara et al., 2007), the recombinant UGT1A isoforms showed multiple bands, possibly owing to variability in the glycosylation. Based on a UGT1A1 level of 1.00 unit/mg, the expression levels of the other

isoforms were determined as follows: UGT1A3, 0.20 unit/mg; UGT1A4, 0.38 unit/mg; UGT1A6, 0.95 unit/mg; UGT1A7, 0.60 unit/mg; UGT1A8, 0.80 unit/mg; UGT1A9, 0.68 unit/mg; UGT1A10, 0.42 unit/mg. By normalization with the UGT expression levels (Fig. 3C), UGT1A8 (108.7 pmol/min/unit) and UGT1A3 (91.6 pmol/min/unit) exhibited the highest thyroxine glucuronosyltransferase activities, followed by UGT1A10 (47.3 pmol/min/unit) and UGT1A1 (26.0 pmol/min/unit). The activities by UGT1A7 (7.6 pmol/min/unit) and UGT1A9 (7.1 pmol/min/unit) were low.

Kinetics of thyroxine glucuronosyltransferase activities by recombinant UGT1A isoforms. For six UGT isoforms showing thyroxine glucuronosyltransferase activity, kinetic analyses were performed. The kinetics of thyroxine glucuronosyltransferase activities by recombinant UGT1A1, UGT1A7, and UGT1A9 fitted to the Michaelis-Menten kinetics (Fig. 4A). The activities by recombinant UGT1A3, UGT1A8, and UGT1A10 fitted to the substrate inhibition kinetics (Fig. 4B). The apparent K_m and corrected V_{max} values are summarized in Table 2. The K_m values varied from 24.1 μM to 104.9 μM . UGT1A8 (6.6 $\mu\text{l}/\text{min}/\text{unit}$) exhibited the highest clearance, followed by UGT1A3 (6.2 $\mu\text{l}/\text{min}/\text{unit}$) and by UGT1A10 (3.7 $\mu\text{l}/\text{min}/\text{unit}$). UGT1A1 (0.7 $\mu\text{l}/\text{min}/\text{unit}$), UGT1A9 (0.4 $\mu\text{l}/\text{min}/\text{unit}$), and UGT1A7 (0.4 $\mu\text{l}/\text{min}/\text{unit}$) exhibited low intrinsic clearance.

Interindividual variability of thyroxine glucuronosyltransferase activities in microsomes from 12 human livers and correlation analyses. The thyroxine glucuronosyltransferase activity in microsomes from 12 human livers ranged from 23.7 to 84.8 pmol/min/mg of protein, representing 3.6-fold variability. Correlation analyses were performed between the thyroxine glucuronosyltransferase activity and typical activities including bilirubin *O*-glucuronidation catalyzed by UGT1A1, estradiol 3-*O*-glucuronidation catalyzed by UGT1A1 and UGT1A8 (Lepine et al., 2004), chenodeoxycholic acid 24-*O*-glucuronidation catalyzed by UGT1A3 (Trottier et al., 2006), imipramine *N*-glucuronidation catalyzed by UGT1A3 and UGT1A4, serotonin *O*-glucuronidation catalyzed by UGT1A6, propofol

O-glucuronidation catalyzed by UGT1A8 and UGT1A9, or morphine 3-*O*-glucuronidation catalyzed by UGT2B7 (Table 3). Since UGT1A8 is not expressed in human liver, the estradiol 3-*O*- and propofol *O*-glucuronosyltransferase activities represented are the UGT1A1 and UGT1A9 activities, respectively. The thyroxine glucuronosyltransferase activities in 12 human liver microsomes were significantly correlated with bilirubin ($r = 0.855$, $p < 0.001$), estradiol ($r = 0.827$, $p < 0.0001$), and serotonin ($r = 0.522$, $p < 0.05$) glucuronidations.

Inhibitory effects of typical substrates for UGT isoforms on thyroxine glucuronosyltransferase activities in human liver, jejunum, and kidney microsomes or recombinant UGTs. The inhibitory effects of bilirubin, imipramine, emodin, propofol and troglitazone on the thyroxine glucuronosyltransferase activity were investigated. These inhibitors were used at effective concentrations according to the previous studies (Watanabe et al., 2002; Kuehl et al., 2005; Yamanaka et al., 2005). The thyroxine glucuronosyltransferase activity in human liver microsomes was strongly inhibited by bilirubin (29% of control) and emodin (15% of control), and was moderately inhibited by troglitazone (51% of control), but was activated by propofol (121% of control) (Fig. 5A). The thyroxine glucuronosyltransferase activity in human jejunum microsomes was strongly inhibited by emodin (45% of control), and was moderately inhibited by bilirubin (68% of control). The thyroxine glucuronosyltransferase activity in human kidney microsomes was strongly inhibited by troglitazone (46% of control), and was moderately inhibited by emodin (55% of control). Thus, the effects of these compounds on the activity were different between the tissues. The inhibitory effects on thyroxine glucuronidation were also investigated for recombinant UGT1A isoforms (Fig. 5B). Bilirubin strongly inhibited the activity by UGT1A1 (16% of control), and moderately inhibited the activities by UGT1A7 (68% of control). Imipramine moderately inhibited the activity by UGT1A3 (68% of control) and UGT1A10 (64% of control). Emodin strongly inhibited the activities by all UGT1A isoforms. Propofol moderately inhibited the activities by UGT1A9 (63% of control) and UGT1A10 (66% of control), and activated the activities by UGT1A1 (349% of control) and UGT1A3 (324% of

control). Troglitazone strongly inhibited the activities by the 5 UGT1A isoforms other than UGT1A3.

Discussion

In this study, we extensively investigated the thyroxine glucuronidation in human liver, jejunum, and kidney microsomes and recombinant UGTs. The tissue clearances in liver, intestine, and kidney were estimated to be 1440, 702, and 79 $\mu\text{l}/\text{min}/\text{kg}$ of body weight, respectively. Although the UGT activities are not the same in the region of intestine (Strassburg et al., 2000), it was first demonstrated that intestine exhibited approximately one half the clearance of liver for thyroxine glucuronidation. The glucuronidation in intestine may affect the enterohepatic circulation of thyroxine. In kidney, thyroxine is highly converted to T3 with $K_m = 3.0 \mu\text{M}$, $V_{\text{max}} = 26.1 \text{ pmol}/\text{min}/\text{mg}$, and $V_{\text{max}}/K_m = 8.7 \mu\text{l}/\text{min}/\text{mg}$ (Boye, 1986). In addition to the outer ring deiodination, this study demonstrated that thyroxine is glucuronidated in kidney.

This is the first study demonstrating that human UGT1A8, UGT1A10, and UGT1A3 have high, and UGT1A1 has moderate catalytic activity toward thyroxine glucuronidation. Since UGT isoforms are differently expressed in human liver, jejunum, and kidney, it was conceivable that UGT isoform(s) responsible for the thyroxine glucuronidation might be different between these tissues. If the absolute protein levels of each UGT isoform in human tissues are available, quantitative estimation of the contribution of each isoform to the concerned activity can be accomplished like cytochrome P450 (Becquemont et al., 1998). Unfortunately, for UGT, a methodology of quantification is lacking. In addition, specific antibodies against each UGT1A isoform are limited. In the present study, we qualitatively identified the UGT isoforms that are responsible for the thyroxine glucuronidation in human tissues by kinetic, correlation, and inhibition analyses as well as tissue distribution of each UGT isoform.

The Eadie-Hofstee plots of thyroxine glucuronosyltransferase activities in human liver, jejunum, and kidney microsomes were monophasic, suggesting that one or more UGT isoform(s) would be involved in the activities. In human liver microsomes, the apparent K_m value was similar to that of recombinant UGT1A1. The activity in a panel of human liver

microsomes was significantly correlated with the bilirubin *O*- and estradiol 3-*O*-glucuronosyltransferase activities catalyzed by UGT1A1. Although the thyroxine glucuronosyltransferase activity was also correlated with the serotonin *O*-glucuronosyltransferase activity catalyzed by UGT1A6, it might be a fortuitous result, because the serotonin *O*-glucuronosyltransferase activity was significantly ($r = 0.627$, $p < 0.005$) correlated with the estradiol 3-*O*-glucuronosyltransferase activity in the panel of human liver microsomes. These results suggested that a major UGT isoform responsible for the thyroxine glucuronidation in human liver microsomes would be UGT1A1. We could not exclude the possibility that UGT1A9 might also contribute to the thyroxine glucuronosyltransferase activity in human liver microsomes. Interestingly, propofol activated the thyroxine glucuronosyltransferase activity in human liver microsomes. It has been reported that propofol activated 4-methylumbelliferone glucuronidation by recombinant UGT1A1 (Mano et al., 2004). Thus, the result supported the finding that a major isoform responsible for thyroxine glucuronidation in human liver microsomes would be UGT1A1. We used propofol as an inhibitor of UGT1A8 and UGT1A9. However, our previous study found that propofol can activate *trans*-3'-hydroxicotinine *O*-glucuronidation by recombinant UGT1A9 (Yamanaka et al., 2005). Propofol might not be an appropriate inhibitor. The contribution of UGT1A3 to the thyroxine glucuronidation in human liver microsomes would be negligible, because the thyroxine glucuronosyltransferase activity in the panel of human liver microsomes was not correlated with the chenodeoxycholic acid 24-*O*-glucuronosyltransferase activity catalyzed by UGT1A3 (Table 3), and imipramine did not inhibit the thyroxine glucuronosyltransferase activity (Fig. 5) although it inhibited the chenodeoxycholic acid 24-*O*-glucuronosyltransferase activity by 48% of control (data not shown).

In human jejunum microsomes, the apparent K_m value was similar to that of recombinant UGT1A8. The thyroxine glucuronidation in human jejunum microsomes was inhibited by emodin, and was moderately inhibited by bilirubin. These results suggested that the thyroxine glucuronidation in human jejunum microsomes might be catalyzed mainly by

UGT1A8 and UGT1A10, and to a minor extent by UGT1A1. In human kidney microsomes, the apparent K_m value was similar to that of recombinant UGT1A7. The thyroxine glucuronidation in human kidney microsomes was inhibited by troglitazone and emodin. It was suggested that the thyroxine glucuronidation in human kidney microsomes might be catalyzed by UGT1A7, UGT1A9, and UGT1A10. In summary, the contribution of each UGT1A isoform would vary between human tissues, depending on the relative abundance of each isoform.

In conclusion, we characterized the thyroxine glucuronidation in human liver, intestine, and kidney microsomes, and found that UGT1A1 in the liver, UGT1A8 and UGT1A10 in the intestine, and UGT1A7, UGT1A9, and UGT1A10 in the kidney mainly contribute to the activity. The change of activities of these UGTs via inhibition and induction by administered drugs (Kiang et al., 2005) as well as genetic polymorphisms (Miners et al., 2002) may be a causal factor of interindividual differences in the plasma thyroxine concentration.

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Footnote

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Figure legends

Fig. 1. Major metabolic pathways of thyroxine in human.

Fig. 2. Kinetic analyses of thyroxine glucuronosyltransferase activities in human liver, jejunum, or kidney microsomes. Michaelis-Menten plots (A) or Eadie-Hofstee plots (B) are shown. Microsomes were incubated with 2 to 100 μ M thyroxine and 5 mM UDPGA at 37°C for 90 min. Each data point represents the mean of triplicate determinations. HLM, human liver microsomes; HJM, human jejunum microsomes; HKM, human kidney microsomes.

Fig. 3. Thyroxine glucuronosyltransferase activities by recombinant human UGTs expressed in baculovirus-infected insect cells. The substrate concentration was 50 μ M. Each column represents the mean of duplicate determinations. The activities were expressed as pmol/min/mg (A) or pmol/min/unit (C) normalized with the UGT1A expression levels determined by immunoblot analysis (B). Immunoblot analysis of the recombinant UGT1A isoforms (0.5 μ g) was performed using rabbit anti-human UGT1A antibodies. Control represents the UGT control Supersomes (0.5 μ g). The expression levels were defined, assuming that UGT1A1 was 1.00 unit/mg. ND: not detected.

Fig. 4. Kinetic analyses of thyroxine glucuronosyltransferase activities by recombinant UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, and UGT1A10. Recombinant UGT1As were incubated with 2 to 100 μ M thyroxine and 5 mM UDPGA at 37°C for 90 min. The activities by recombinant UGT1A1, UGT1A7, and UGT1A9 fitted to the Michaelis-Menten kinetics (A). The activities by recombinant UGT1A3, UGT1A8, and UGT1A10 fitted to the substrate inhibition kinetics (B). Each data point represents the mean of triplicate determinations.

Fig. 5. Effects of typical substrates for UGTs on thyroxine glucuronosyltransferase activities

in human liver, jejunum, and kidney microsomes (A) or recombinant UGTs (B). Bilirubin (UGT1A1), imipramine (UGT1A3 and UGT1A4), emodin (UGT1A1, UGT1A3, UGT1A8 and UGT1A9), propofol (UGT1A8 and UGT1A9), and troglitazone (UGT1A1, UGT1A8, and UGT1A10) were used as inhibitors. (A) HLM, human liver microsomes; HJM, human jejunum microsomes; HKM, human kidney microsomes. Control activities in HLM, HJM, and HKM at 20 μ M thyroxine were 26.7 pmol/min/mg, 90.9 pmol/min/mg, and 6.2 pmol/min/mg, respectively. (B) Control activities for 20 μ M thyroxine by recombinant UGT1A1, UGT1A3, UGT1A7, UGT1A8, UGT1A9, and UGT1A10 were 11.1 pmol/min/unit, 69.2 pmol/min/unit, 4.7 pmol/min/unit, 81.5 pmol/min/unit, 4.7 pmol/min/unit, and 42.6 pmol/min/unit, respectively. Each data point represents the mean \pm SD of triplicate determinations. ND: not detected.

Table 1. Tissue distribution of human UGT mRNAs.

Tissue	UGT isoform															
	1A1	1A3	1A4	1A5	1A6	1A7	1A8	1A9	1A10	2B4	2B7	2B10	2B11	2B15	2B17	2B28
Liver	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+
Intestine	+	+	+	+	+	-	+	-	+	+	+	+	-	+	-	+
Kidney	-	-	-	ND	+	+	-	+	+	+	+	+	+	+	+	+

Data are from King et al. (2000), Tukey and Strassburg (2000), Fisher et al. (2001), Levesque et al. (2001), Basu et al. (2004), and Finel et al. (2005). ND, no data; +, present; -, not detectable.

Table. 2. Kinetic parameters of thyroxine glucuronosyltransferase activities in human liver, jejunum and kidney microsomes, and by recombinant UGTs.

Enzyme	Km	Vmax	Vmax/Km		
	μM	$pmol/min/mg$	$\mu l/min/mg$		
HLM	85.9 \pm 6.9	133.4 \pm 9.8	1.6 \pm 0.0		
HJM	24.2 \pm 4.1	184.6 \pm 7.0	7.8 \pm 1.0		
HKM	53.3 \pm 5.4	22.6 \pm 1.8	0.4 \pm 0.0		

Enzyme	Km	Vmax	Corrected Vmax	Ksi	Corrected Vmax/Km
	μM	$pmol/min/mg$	$pmol/min/unit$	μM	$\mu l/min/unit$
UGT1A1	104.9 \pm 6.6	75.7 \pm 0.7	75.7 \pm 0.7		0.7 \pm 0.1
UGT1A3	33.2 \pm 2.2	40.8 \pm 2.4	204.0 \pm 11.9	83.5 \pm 14.3	6.2 \pm 0.1
UGT1A7	38.1 \pm 2.5	8.2 \pm 0.3	13.7 \pm 0.4		0.4 \pm 0.0
UGT1A8	45.4 \pm 8.4	239.9 \pm 38.2	299.9 \pm 47.8	56.4 \pm 22.5	6.6 \pm 0.2
UGT1A9	24.1 \pm 1.2	7.1 \pm 0.9	10.4 \pm 1.4		0.4 \pm 0.0
UGT1A10	96.3 \pm 27.3	146.7 \pm 26.3	349.4 \pm 62.6	9.0 \pm 1.5	3.7 \pm 0.4

Data are mean \pm SD of three independent experiments.

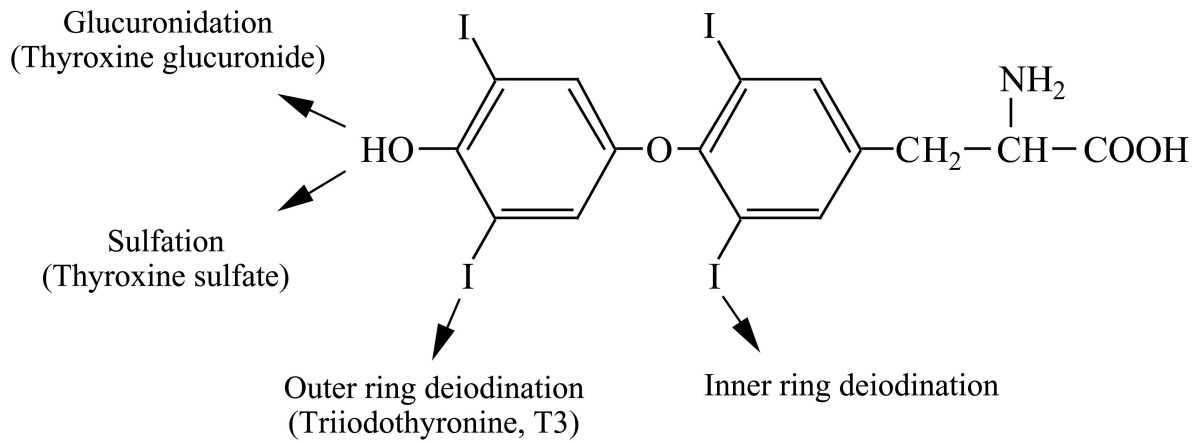
HLM, human liver microsomes; HJM, human jejunum microsomes; HKM, human kidney microsomes

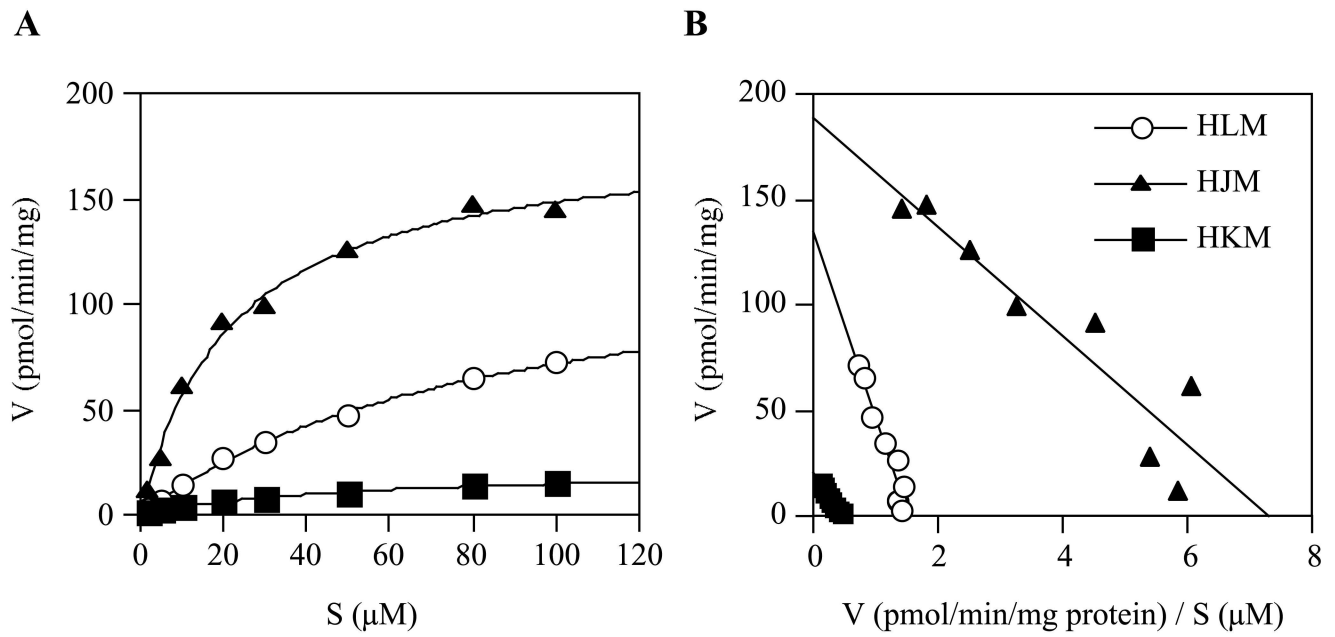
Table. 3. Correlation coefficients between thyroxine glucuronidation and other glucuronidations in microsomes from 12 human livers.

Activity (isoform)	Substrate concentration	<i>r</i>	<i>p</i>
	<i>μM</i>		
Bilirubin <i>O</i> -glucuronidation (UGT1A1)	10	0.855	< 0.001
Estradiol 3- <i>O</i> -glucuronidation (UGT1A1)	100	0.827	< 0.0001
Chenodeoxycholic acid 24- <i>O</i> -glucuronidation (UGT1A3)	10	-0.080	NS
Imipramine <i>N</i> -glucuronidation (UGT1A3 and UGT1A4)	500	0.135	NS
Serotonin <i>O</i> -glucuronidation (UGT1A6)	1000	0.522	< 0.05
Propofol <i>O</i> -glucuronidation (UGT1A9)	30	0.330	NS
Morphine 3- <i>O</i> -glucuronidation (UGT2B7)	250	-0.035	NS

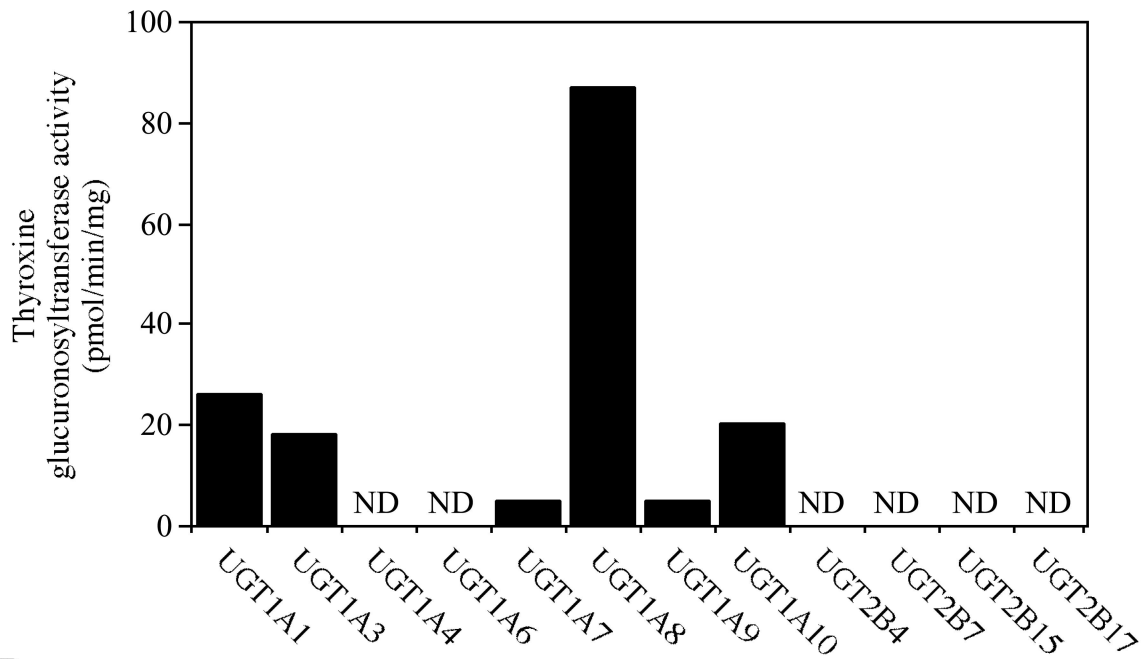
NS, not significant.

Thyroxine (T4)

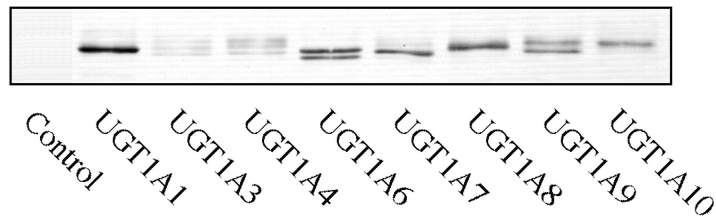




A



B



C

