Sequential Metabolism of Sesamin by Cytochrome P450 and UDP-Glucuronosyltransferase in Human Liver

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ABSTRACT:

Our previous study revealed that CYP2C9 played a central role in sesamin monocatecholization. In this study, we focused on the metabolism of sesamin monocatechol that was further converted into the dicatechol form by cytochrome P450 (P450) or the glucuronide by UDP-glucuronosyltransferase (UGT). Catecholization of sesamin monocatechol enhances its antioxidant activity, whereas glucuronidation strongly reduces its antioxidant activity. In human liver microsomes, the glucuronidation activity was much higher than the catecholization activity toward sesamin monocatechol. In contrast, in rat liver microsomes, catecholization is predominant over glucuronidation. In addition, rat liver produced two isomers of the glucuronide, whereas human liver produced only one glucuronide. These results suggest a significant species-based difference in the metabolism of sesamin between humans and rats. Kinetic studies using recombinant human UGT isoforms identified UGT2B7 as the most important UGT isoform for glucuronidation of sesamin monocatechol. In addition, a good correlation was observed between the glucuronidation activity and UGT2B7-specific activity in vitro studies using 10 individual human liver microsomes. These results strongly suggest that UGT2B7 plays an important role in glucuronidation of sesamin monocatechol. Interindividual difference among the 10 human liver microsomes is approximately 2-fold. These results, together with our previous results on the metabolism of sesamin by human P450, suggest a small interindividual difference in sesamin metabolism. We observed the methylation activity toward sesamin monocatechol by catechol O-methyl transferase (COMT) in human liver cytosol. On the basis of these results, we concluded that CYP2C9, UGT2B7, and COMT played essential roles in the metabolism of sesamin in the human liver.

Introduction

Sesamin is a major lignan in sesame, and its biological effects such as cholesterol and lipid-lowering (Hirose et al., 1991; Ogawa et al., 1995; Hirata et al., 1996; Kiso, 2004), anticarcinogenic effects (Hirose et al., 1992; Miyahara et al., 2000), and suppression of hypertension (Miyawaki et al., 2009) have been extensively studied by many researchers. Among them, antioxidant effects (Ikeda et al., 2003; Nakai et al., 2003) are attributed to its metabolites because sesamin itself has few antioxidative properties.

Nakai et al. (2003) demonstrated that sesamin was converted to its mono- and dicatechol forms by demethylation of methylenedioxyphenyl (MDP) groups by cytochromes P450 (P450s) in rat livers (Kumagai et al., 1991; Murray, 2000). The resultant catechols had much higher antioxidative activity compared with sesamin, and sesamin dicatechol showed the highest antioxidative activity (Nakai et al., 2003; Miyake et al., 2005). In addition, studies suggested that sesamin monocatechol had the ability to induce enhancement of endothelium-dependent vasorelaxation (Nakano et al., 2006) and neuronal differentiation (Hamada et al., 2009). Thus, the conversion of sesamin to its mono- or dicatechol form is considered to be an important reaction in the production of antioxidants and other bioactive compounds in the human body. Liu et al. (2006) tentatively proposed metabolic pathways of sesamin containing these catechols in vivo (Peñalvo et al., 2005; Liu et al., 2006). The plasma concentrations of sesamin monocatechol and dicatechol in sesamin-administered rats were much higher than those of sesamin itself (Nakano et al., 2006), suggesting biological effects of these catechols in rats.

Very little is known about the plasma concentrations of sesamin mono- and dicatechols in humans. Moazzami et al. (2007) reported the presence of glucuronide of sesamin monocatechol in human urine, but other metabolites such as dicatechol were not detected. Recently, we examined the sesamin catecholization by P450s in human liver microsomes (Yasuda et al., 2010). Sesamin monocatechol was detected as a metabolite of sesamin, but the dicatechol form was not detected. Our results are consistent with those of Moazzami et al. (2007) and suggest a species-based difference in sesamin metabolism by P450 between humans and rats. To understand the biological significance of the sesamin metabolites, especially their antioxidative

ABBREVIATIONS: MDP, methylenedioxyphenyl; P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; Glc-UA, glucuronic acid; COMT, catechol O-methyl transferase; MBI, mechanism-based inhibition; HPLC, high-performance liquid chromatography; SAM, S-adenosyl methionine; AZT, 3’-azido-3’-deoxythymidine; TFA, trifluoroacetic acid; CLint, intrinsic clearance.
properties, it is essential to understand what controls the ratio of conversion of sesamin to its mono- or dicatechol or glucuronide forms. In addition, it is important to identify the P450 and UGT isoforms that are responsible for sesamin metabolism from the viewpoint of drug-sesamin interaction. Our previous study demonstrated that the most essential P450 isoform for sesamin metabolism is CYP2C9 and secondly CYP1A2 in human liver. We also found a weak mechanism-based inhibition (MBI) of CYP2C9 by sesamin (Yasuda et al., 2010) and other MDP compounds (Nakajima et al., 1999; Murray, 2000; Chatterjee and Franklin, 2003; Usia et al., 2005). Sesamin monocatechol still has another MDP group; thus, it is possible that monocatechol is also a mechanism-based inhibitor of CYP2C9 or other P450s.

In this study, we focused on the metabolism of sesamin that occurs after its monocatecholization to reveal overall metabolic pathways of sesamin in humans. We identified the UGT isoform responsible for sesamin glucuronidation. We also observed methylolation of sesamin monocatechol by catechol-O-methyl transferase (COMT). Finally, we describe a species-based difference between humans and rats in sesamin metabolism.

### Materials and Methods

**Materials**

Sesamin was purchased from Sigma-Aldrich (St. Louis, MO). NADPH and NADH were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). UDP-GlcUA, *Escherichia coli* β-glucuronidase, and D-aenosyl methionine were purchased from Sigma-Aldrich. Human single-donor liver microsomes (HG43, HH47, HH18, HH74, HH77, HG95, HH715, HH581, HG3, and HH74), a 50-donor human liver microsome pool, human liver cytosol pool, human small intestinal microsomes, mouse Sprague-Dawley rat liver microsomes and cytosol, and recombinant human UGTs (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, or 2B17) microsomes, 10 μM sesamin monocatechol, 2 mM UDP-GlcUA, and 1 mM MgCl2 in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 0 to 60 min at 37°C, and then the metabolites were analyzed as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.

**Preparation of Sesamin Monocatechol by Recombinant Yeast Cells Expressing Human CYP2C19**

Our previous study revealed that sesamin was efficiently converted to its monocatechol form by whole-cell fraction of recombinant *S. cerevisiae* cells expressing human CYP2C19 (Yasuda et al., 2010). Thus, we used these cells for preparing sesamin monocatechol. The recombinant yeast cells were cultivated in 50 ml of synthetic minimal medium containing 2% glucose, 0.67% yeast nitrogen base without amino acids, and 20 mg/ml l-histidine at 30°C for 24 h. The culture was harvested by centrifugation at 5000g at 4°C for 10 min. The cell pellet was suspended in 5 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 4% glucose and 100 μM sesamin and incubated at 37°C under shaking conditions. Glucose was added again to 4% of the final concentration at 24 h after addition of sesamin. After 48 h of incubation, the metabolites were extracted with four volumes of chloroform/methanol (3:1, v/v). The organic phase was recovered and dried and then dissolved in dimethyl sulfoxide to be used as a substrate as follows.

**Metabolism of Sesamin Monocatechol by P450 or UGT, or COMT in the Liver, Small Intestinal Microsomes, or Liver Cytosol**

In P450-dependent catecholization of sesamin monocatechol, the reaction mixture containing 0.5 mg protein/ml of the liver or small intestinal microsomes, 1 to 50 μM sesamin monocatechol, and 1 mM NADPH in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10 to 30 min at 37°C, and the metabolite was analyzed as described below.

In UGT-dependent glucuronidation of sesamin monocatechol, the reaction mixture containing 0.5 mg protein/ml of the liver or small intestinal microsomes, 1 to 50 μM sesamin monocatechol, and 1 mM MgCl2 in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 15 to 30 min at 37°C, and the metabolite was analyzed by HPLC as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT. To confirm that the metabolite was the glucuronide, the aliquot of the reaction mixture was further incubated for 60 min at 37°C in the presence of 0.1 mg/ml β-glucuronidase in 20 mM potassium phosphate buffer (pH 7.4). In COMT-dependent methylolation, the reaction mixture containing 0.5 mg protein/ml of the liver cytosol, 1 to 50 μM sesamin monocatechol, 200 μM SAM, 2 mM MgCl2, and 1 mM dithiothreitol in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 5 to 20 min at 37°C, and then the metabolites were analyzed by HPLC as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.

**Metabolism of Sesamin by P450 and UGT in Human Liver Microsomes or Small Intestinal Microsomes in the Presence of NADPH and UDP-GlcUA**

The reaction mixture containing 0.5 mg protein/ml human liver microsomes or small intestinal microsomes, 1 to 50 μM sesamin, 1 mM NADPH, 1 mM UDP-GlcUA, and 1 mM MgCl2 in 100 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 30 min, and then the metabolites were analyzed by HPLC as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.

**Metabolism of Sesamin Monocatechol by Recombinant Human UGTs.**

The reaction mixture containing 0.1 mg protein/ml recombinant human UGT (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, or 2B17) microsomes, 10 μM sesamin monocatechol, 2 mM UDP-GlcUA, and 1 mM MgCl2 in 100 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 15 min at 37°C, and then the metabolites were analyzed as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.

**Inhibition of AZT Glucuronidation in Human Liver Microsomes by Sesamin Monocatechol.**

AZT glucuronidation reactions in a 50-donor human liver microsome pool were performed by the same methods described under Metabolism of 3'-Azido-3'-Deoxythymidine in Human Liver Microsomes in the presence of 0 to 100 μM sesamin monocatechol. Metabolites were analyzed by HPLC as described under HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.

**HPLC Analysis of Metabolites of Sesamin, Sesamin Monocatechol, and AZT.**

Each reaction was terminated by addition of an equal volume of ice-cold methanol. After centrifugation at 14,500g for 15 min, the supernatant was applied to HPLC under the following conditions: column, YMC-pack ODS-AM (4.6 × 300 mm) (YMC, Inc., Wilmington, NC); UV detection, 267 nm; flow rate, 1.0 ml/min; column temperature, 40°C; and linear gradients of 10 to 90% methanol aqueous solution containing 0.05% trifluoroacetic acid (TFA) for 30 min and 90 to 100% methanol containing 0.05% TFA for 5 min for metabolites of sesamin and sesamin monocatechol. On the other hand, AZT metabolites were analyzed under the following conditions: column, YMC-pack ODS-AM (4.6 × 300 mm) (YMC, Inc., Wilmington, NC); UV detection, 280 nm; flow rate, 1.0 ml/min; column temperature, 40°C; and linear gradients of 10 to 65% methanol aqueous solution containing 0.05% TFA for 20 min.

**Liquid Chromatography-Mass Spectrometric Analysis of Metabolized Metabolite of Sesamin Monocatechol.**

The metabolites of sesamin monocatechol in the presence of SAM were isolated by HPLC and subjected to mass spectrometric analysis using a Finnigan LCQ Advantage Mix (Thermo Fisher Scientific, Waltham, MA) with atmospheric pressure chemical ionization, positive mode. The conditions of liquid chromatography were as follows: column, reverse-phase ODS column (2 × 150 mm, Developol ODS-HG-3; Nomura Chemical Co., Ltd., Aichi, Japan); mobile phase, acetonitrile/methanol/water = 3:4:3; flow rate, 0.2 ml/min; and UV detection, 280 nm.

**Kinetic Analysis.**

The kinetic studies for P450-dependent oxidation, UGT-dependent glucuronidation, and COMT-dependent methylation were performed...
incubated with NADPH for 30 min. Human liver microsomes (Ab) were incubated with UDP-GlcUA from 0 to 50 μM. The kinetic parameters, K_m and V_max, were calculated by nonlinear regression analysis using KaleidaGraph (Synergy Software, Reading, PA). The equation was applied for Michaelis-Menten kinetics.

**Results**

**HPLC Analysis of the Metabolites of Sesamin Monocatechol.** Figure 1A shows the HPLC profiles of sesamin monocatechol and its metabolite in human and rat liver microsomes in the presence of NADPH. One major metabolite (M1) was observed at a retention time of 17.5 min in human and rat. Cocrhromatography of M1 with the authentic sesamin dicatechol strongly suggested that M1 was sesamin dicatechol. It should be noted that the conversion ratio in rat liver microsomes was much higher than that in human liver microsomes.

Figure 1B shows the HPLC profiles of sesamin monocatechol and its metabolite in human and rat liver microsomes in the presence of UDP-GlcUA. Only one major metabolite (M2) was observed at a retention time of 23.4 min in human microsomes (Fig. 1Ba). In rats, another metabolite in addition to M2 was detected at a retention time of 22.4 min (M3) (Fig. 1Bc). M2 and M3 were converted to sesamin monocatechol upon incubation with β-glucuronidase (Fig. 1B, b and d), suggesting that M2 and M3 were glucuronide isomers of sesamin monocatechol.

Figure 1C shows the HPLC profiles of sesamin monocatechol and its metabolites in human and rat liver cytosol in the presence of SAM. Two major metabolites were detected at retention times of 26.9 min (M4) and 27.4 min (M5) in human and rat liver cytosol. To identify the chemical structures of the metabolites, we collected M4 and M5 in the HPLC effluents and subjected them to mass spectrometric analysis. Relative intensities (percentage) of major ion fragments of the authentic standard of sesamin monocatechol and its metabolites (M4 and M5) were as follows: sesamin monocatechol, m/z 307 (M+H-2H2O), 4%; m/z 325 (M+H-H2O), 100%; m/z 343 (M+H) 43%; M4: m/z 321 (M+H-2H2O), 8%; m/z 339 (M+H-H2O), 100%; m/z 357 (M+H) 50%. M5: m/z 321 (M+H-2H2O), 8%; m/z 339 (M+H-H2O), 100%; m/z 357 (M+H) 46%. These results strongly suggest that M4 and M5 are methylated isomers of sesamin monocatechol.

We conclude that sesamin monocatechol can be converted to sesamin dicatechol by P450s, glucuronide forms by UGTs, and methylated forms by COMT (Fig. 2). Among these enzymes, P450s and UGTs are localized in microsomal membranes, whereas COMT is localized in the cytosol.

**Kinetic Analysis of Sesamin Monocatechol Oxidation, Glucuronidation, and Methylation.** Table 1 shows the kinetic parameters for sesamin monocatechol glucuronidation and oxidation in liver microsomes and methylation in liver cytosol. In human samples, the V_max value for UGT-dependent glucuronidation of sesamin monocatechol (4.35 nmol/mg protein/min) was 18 times higher than that of P450-dependent oxidation of sesamin monocatechol (0.24 nmol/mg protein/min). UGT and P450 activities had almost the same K_m values. Thus, the V_max/K_m value for glucuronidation was 18 times higher than that for oxidation. It is noted that P450 and UGT reactions depend on NADPH and UDP-glucuronic acid, respectively. Thus, their activities cannot be compared directly. However, if enough amounts of NADPH and UDP-glucuronic acid are present under physiological conditions, both activities could be compared. Our results indicate that sesamin monocatechol is predominantly converted to the glucuronide form rather than the dicatechol form in human liver microsomes. It is noted that the V_max/K_m value for the oxidation of sesamin monocatechol is 9 times lower than that of sesamin (Table 1), which indicates that sesamin monocatechol is a poor substrate for human liver P450s compared with sesamin itself. The kinetic parameters of COMT for the methylation of sesamin monocatechol in human liver cytosol were determined. Because P450 and UGT reactions occur in microsomes, and the COMT reaction occurs in the cytosol, the kinetic parameters of COMT cannot be compared with those of the P450 and UGT reactions. However, it is clear that methylation by COMT is also an important reaction for the metabolism of sesamin monocatechol in human liver.

We also examined the same activities in rat liver microsomal and cytosolic fractions to reveal species-based differences between rats and humans in the metabolism of sesamin and sesamin monocatechol (Table 1). To our surprise, a drastic difference was observed between human and rat liver microsomes. In rat liver microsomes, P450-dependent catecholization activity toward sesamin and sesamin monocatechol is predominant over glucuronidation (Table 1). In contrast, the V_max/K_m value of COMT-dependent methylation in the cytosolic fraction of rat liver was not so different from that of human liver. The observed differences in the metabolism of sesamin suggest that the potential health benefits of sesamin also differ between rats and humans.

**Metabolism of Sesamin in the Presence of NADPH and UDP-GlcUA by Human Liver Microsomes or Human Small Intestinal
**Microsomes.** Orally administered sesamin is first metabolized in small intestinal epithelial cells, so we examined the metabolism of sesamin in small intestinal microsomes. P450s and UGTs are expressed in human small intestine, thus we are able to compare the metabolism of sesamin in human small intestinal microsomes with the metabolism in liver microsomes. Liver and small intestinal microsomes were incubated with NADPH and UDP-GlcUA, and the metabolites produced were assayed. As shown in Fig. 3Aa, most of the substrate remained intact and the glucuronide form was observed as a single metabolite in small intestinal microsomes. On the contrary, most of the substrate was converted to the metabolites consisting of sesamin monocatechol (7.2%) and its glucuronide (82%) in human liver microsomes.

**TABLE 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Reaction</th>
<th>Fraction</th>
<th>$V_{\text{max}}$ (nmol/mg protein/min)</th>
<th>$K_m$ (μM)</th>
<th>$V_{\text{max}}/K_m$</th>
<th>Relative $V_{\text{max}}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Sesamin</td>
<td>Cat (P450)</td>
<td>mic</td>
<td>0.58 ± 0.05</td>
<td>7.7 ± 1.7</td>
<td>0.075</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sesamin monocatechol</td>
<td>Cat (P450)</td>
<td>mic</td>
<td>0.24 ± 0.04</td>
<td>28.2 ± 8.4</td>
<td>0.0085</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glu (UGT)</td>
<td>mic</td>
<td>4.35 ± 0.62</td>
<td>27.0 ± 7.9</td>
<td>0.16</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met (COMT)</td>
<td>cyt</td>
<td>0.93 ± 0.02</td>
<td>8.4 ± 0.8</td>
<td>0.11</td>
<td>1.5</td>
</tr>
<tr>
<td>Rat</td>
<td>Sesamin</td>
<td>Cat (P450)</td>
<td>mic</td>
<td>3.49 ± 0.32</td>
<td>7.1 ± 1.8</td>
<td>0.49</td>
<td>6.5</td>
</tr>
<tr>
<td></td>
<td>Sesamin monocatechol</td>
<td>Cat (P450)</td>
<td>mic</td>
<td>1.99 ± 0.19</td>
<td>7.9 ± 2.1</td>
<td>0.25</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glu (UGT)</td>
<td>mic</td>
<td>0.76 ± 0.17</td>
<td>30.5 ± 10.2</td>
<td>0.025</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met (COMT)</td>
<td>cyt</td>
<td>2.41 ± 0.23</td>
<td>13.8 ± 1.0</td>
<td>0.17</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Cat, catecholization; Glu, glucuronidation; Met, methylation; mic, microsomal fraction; cyt, cytosolic fraction.
We calculated the CLint values for the glucuronidation of sesamin monocatechol from human liver microsomes (45 mg microsomal protein/g liver and 20 g liver/kg b.w.t.) and the human small intestinal microsomes (3 mg microsomal protein/g intestine and 30 g intestine/kg b.w.t.) (Soars et al., 2002). The CLint values for monocatecholization in human liver and human intestine were 68 and 3.1, respectively, and the CLint values for glucuronidation were 144 and 16, respectively (Table 2). For monocatecholization and glucuronidation, CLint values in human liver were much higher than those in human small intestine.

**Metabolism of Sesamin Monocatechol by the Recombinant Human UGTs.** Glucuronidation of sesamin monocatechol is essential for the metabolism of sesamin in the human body (Fig. 3; Table 2). Thus, identification of the UGT isoforms responsible for sesamin glucuronidation is required from the viewpoint of drug-sesamin interaction. We examined the metabolism of sesamin monocatechol by recombinant human UGTs expressed in baculovirus-infected insect cells. Among the 12 UGT isoforms, UGT2B7 and UGT2B17 showed glucuronidation activity toward sesamin monocatechol. The Vmax/Km of UGT2B7 was 3.4 times higher than that of UGT2B17, whereas both Km values were almost identical (Table 3). Because the levels of UGT2B7 and UGT2B17 in the recombinant microsomes were similar (0.6–0.8 nmol/mg protein) as described in our previous report (Uchihashi et al., 2011), the enzymatic activity of UGT2B7 appears to be significantly higher than that of UGT2B17. In addition, UGT2B7 mRNA is higher than UGT2B17 mRNA in the human liver (Congiu et al., 2002; Court, 2005; Izuikawa et al., 2009). Thus, the contribution of UGT2B7 to glucuronidation of sesamin monocatechol in the human liver appears to be small.

**Correlation between Sesamin Monocatechol Glucuronidation Activity and UGT2B7-Specific Activity.** Kinetic analysis suggests that UGT2B7 plays a central role in the glucuronidation of sesamin monocatechol in the human body. Thus, we examined the correlation between sesamin monocatechol glucuronidation activity and AZT glucuronidation activity, which is known as UGT2B7-specific activity, using single-donor human liver microsomes. There was a 2.1-fold activity range of sesamin monocatechol glucuronidation in human liver microsomes from 10 different individuals (Fig. 4A). Thus, the interindividual difference of sesamin monocatechol glucuronidation was not so large. A good correlation (r = 0.80) was observed between sesamin monocatechol glucuronidation activity and AZT glucuronidation activity (Fig. 4B), suggesting that UGT2B7 is the major isoform involved in the glucuronidation of sesamin monocatechol in human liver.

**Inhibition of UGT2B7-Specific Activity in Human Liver Microsomes by Sesamin Monocatechol.** Figure 5 shows the inhibitory effects of sesamin monocatechol on AZT glucuronidation activity in pooled human liver microsomes. The UGT2B7-specific activity was significantly inhibited by sesamin monocatechol, and the IC50 value was estimated to be 47.5 μM.

**TABLE 2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Reaction</th>
<th>Vmax (nmol/mg protein/min)</th>
<th>Km (μM)</th>
<th>Vmax/Km (μM)</th>
<th>CLint (ml·min⁻¹·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesamin</td>
<td>Cat (P450) Liver</td>
<td>0.58 ± 0.05²</td>
<td>7.7 ± 1.7²</td>
<td>0.075³</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16 ± 0.01</td>
<td>4.7 ± 0.9</td>
<td>0.034</td>
<td>3</td>
</tr>
<tr>
<td>Sesamin Monocatechol</td>
<td>Glu (UGT)</td>
<td>4.35 ± 0.62²</td>
<td>27.0 ± 7.9²</td>
<td>0.16³</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.60 ± 0.02</td>
<td>9.1 ± 0.3</td>
<td>0.18</td>
<td>16</td>
</tr>
</tbody>
</table>

Cat, catecholization; Glu, glucuronidation.

²The values were identical to those in Table 1.
Discussion

Sesamin, one of the major lignans in sesame, has recently gained attention because of its potential health benefits. Many of the health benefits associated with sesamin may actually be achieved by the action of sesamin metabolites. For example, the antioxidative properties of sesamin seem to be originated from its mono- or dicatechol forms. The plasma concentrations of sesamin mono- and dicatechol (13 and 3 μM, respectively) were higher than the concentration of sesamin (0.2 μM) in sesamin-administered rats (Nakano et al., 2006). Because the catechol metabolites are present in the plasma, they are expected to perform biological functions in rats. However, very little has been published on the serum levels of such metabolites after sesamin uptake in humans. Peñalvo et al. (2005) reported the conversion of sesamin into enterolactone after uptake of sesame seeds. Fecal fermentation of sesamin strongly suggested that enterolactone was produced by the intestinal microflora. However, Moazami et al. (2007) detected glucurono- and/or sulfoconjugated forms of sesamin monocatechol as a sole urine metabolite after uptake of commercial sesamin capsules. Thus, the metabolism of sesamin by the intestinal microflora may not be essential for the supplemental use of pure sesamin. Our previous study demonstrated that sesamin was metabolized to its monocatechol form mainly by CYP2C9 in the human liver with a weak MBI of CYP2C9 (Yasuda et al., 2010), which is attributed to the MDP group of sesamin (Nakajima et al., 1999; Murray, 2000; Chatterjee and Franklin, 2003; Usia et al., 2005). Sesamin monocatechol still has one MDP group; thus, it is possible that sesamin monocatechol could result in MBI of CYP2C9. However, the extent of MBI of CYP2C9 by sesamin monocatechol appeared to be much less than the inhibition by sesamin. The reduced inhibition may have resulted because sesamin monocatechol is not a good substrate for CYP2C9 or the other P450 isoforms compared with sesamin itself.

When sesamin is administered orally, intestinal P450s may play important roles in the initial metabolism. However, the conversion rate of sesamin to its monocatechol by intestinal P450s is very slow, as shown in Fig. 3A and Table 2. Thus, most sesamin appears to be converted to its monocatechol by P450s in the liver and then converted to its glucuronide by UGTs. In this study, we focused on the metabolism of sesamin monocatechol. As shown in Table 1, the V_max/Km value of glucuronidation of sesamin monocatechol was approximately 20 times higher than the oxidation of sesamin monocatechol in human liver microsomes. This result suggests that sesamin moncatechol could be rapidly conjugated by UGTs, whereas oxidation by P450s to yield dicatechol hardly occurs in human liver. Most sesamin was actually metabolized to its glucuronide via sesamin monocatechol in the human liver microsomes. The sulfoconjugated form of sesamin monocatechol was not detected in this study (data not shown).

We identified UGT isoforms that may be responsible for the glucuronidation of sesamin monocatechol. First, we examined the conversion of sesamin monocatechol in each of 12 recombinant human UGT isoforms expressed in baculovirus-infected insect cells. Although UGT2B7 and 2B17 showed the activity, UGT2B7 appears to be responsible for glucuronidation of sesamin monocatechol on the basis of their molecular activity and expression levels in the human liver. We conclude that UGT2B7 is the most important UGT isoform on the basis of the following three lines of evidence: 1) our kinetic analysis; 2) the correlation between the glucuronidation activity toward sesamin monocatechol and the UGT2B7-specific activity in in vitro studies using 10 individual human liver microsomes (Fig. 4); and 3) the inhibitory effect of sesamin monocatechol on the UGT2B7-specific activity (Fig. 5). However, we could not evaluate the contribution of UGT1A5, 2B10, 2B11, 2B28, 2A1, 2A2, and 2A3 because these isoforms were not commercially available. Thus, the possibility that these UGT isoforms contribute to glucuronidation of sesamin monocatechol cannot be excluded. The correlation coefficient (0.8) between the glucuronidation activity toward sesamin monocatechol and the

![Fig. 4. A. glucuronidation activity toward sesamin monocatechol in each of the 10 human single-donor liver microsomes. B, the correlation between glucuronidation activity toward sesamin monocatechol and UGT2B7-specific activity (AZT glucuronidation activity). Each point represents the mean of at least duplicate determination.](image-url)
Sesamin monocatechol glucuronide appears to be a major metabolite of sesamin in humans. Because this metabolite has low levels of antioxidant activity, it is possible that sesamin monocatechol glucuronide is an inactive form of sesamin and further processing is necessary to produce a functional molecule. Kawai et al. (2008) recently proposed a novel mechanism for the function of quercetin in humans. When quercetin is taken up into the human body, quercetin is mainly converted to its glucuronide form at the 3 position (Q3GA) by UGTs in the liver. Q3GA in the plasma can be taken up by macrophage cells, probably by a transporter in the plasma membrane, and converted into its active aglycone form by β-glucuronidase. This finding could explain why quercetin is effective in atherosclerosis despite the absence of quercetin aglycone in the serum. β-Glucuronidase is expressed in most mammalian tissues, thus a similar mechanism might apply to sesamin. It is interesting to note that Kawai et al. (2008) also suggested that not only the aglycone form but also the methylated aglycone form of quercetin by COMT showed the inhibitory effects on the transcription of class A scavenger receptor gene in macrophage cells. In this study, we detected COMT-dependent methylation activity of sesamin monocatechol in the liver cytosol. Thus, we may hypothesize that most sesamin is converted to its glucuronide form in the liver and transported to the target tissues through the blood stream. Sesamin glucuronides can then enter the target cells and be subsequently converted to the aglycone form by β-glucuronidase. Finally, a part of the aglycone is methylated by COMT to produce the active molecule. If this hypothesis is correct, then the metabolizing enzymes of sesamin consisting of P450s, UGTs, β-glucuronidase, and COMT play essential roles in the biological effects of sesamin.

**References**


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