COMPARISON OF METABOLISM OF SESAMIN AND EPISESAMIN
BY DRUG METABOLIZING ENZYMES IN HUMAN LIVER

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Abbreviations: P450, cytochrome P450; UGT, UDP-glucuronosyltransferase; Glc-UA, glucuronic acid; COMT, catechol O-methyl transferase; MDP, Methylenedioxyphenyl; MBI, mechanism-based inhibition; HPLC, high-performance liquid chromatography; DMSO, dimethylsulfoxide; PPARα, peroxisome proliferator-activated receptor α
Abstract

Sesamin and episesamin are two epimeric lignans that are found in refined sesame oil. Commercially available sesamin supplements contain both sesamin and episesamin at an approximate 1 to 1 ratio. Our previous study clarified the sequential metabolism of sesamin by cytochrome P450 (CYP) and UDP-glucuronosyltransferase in human liver. In addition, we revealed that sesamin caused a mechanism-based inhibition (MBI) of CYP2C9, the cytochrome P450 enzyme responsible for sesamin monocatecholization. In the present study, we compared the metabolism and the MBI of episesamin with those of sesamin. Episesamin was first metabolized to the two epimers of monocatechol, S- and R-monocatechols in human liver microsomes. The cytochrome P450 enzymes responsible for S- and R-monocatechol formation were CYP2C9 and CYP1A2, respectively. The contribution of CYP2C9 was much larger than CYP1A2 in sesamin metabolism, whereas the contribution of CYP2C9 was almost equal to that of CYP1A2 in episesamin metabolism. Docking of episesamin to the active site of CYP1A2 explained the stereoselectivity in CYP1A2-dependent episesamin monocatecholization. Similar to sesamin, the episesamin S- and R-monocatechols were further metabolized to dicatechol, glucuronide, and methylate metabolites in human liver; however, the contribution of each reaction was significantly different between sesamin and episesamin. Interestingly, the liver microsomes from CYP2C19 ultra-rapid metabolizers showed a significant amount of episesamin dicatechol. In this study, we have revealed significantly different metabolism by cytochrome P450, UDP-glucuronosyltransferase, and catechol O-methyl transferase for sesamin and episesamin, resulting in different biological effects.
Introduction

Sesamin and episesamin are major lignans in refined sesame oil and they are epimers of each other. Episesamin is rarely found in unrefined sesame oil, but is generated from sesamin in equivalent amounts during acid-clay bleaching the oil (Li et al., 2005), therefore, commercially available sesamin supplements contain both sesamin and episesamin at about 1:1 ratio. Thus, effects of sesamin supplements depend on both sesamin and episesamin, which are known to have biological effects such as antioxidant effect (Ikeda et al., 2003; Nakai et al., 2003), anticarcinogenic effects (Hirose et al., 1992; Miyahara et al., 2000), and suppression of hypertension (Miyawaki et al., 2009).

Recently, the biological effects were compared between sesamin and episesamin (Kushiro et al., 2002; Ide et al., 2009). Kushiro et al. demonstrated that episesamin was much stronger at increasing the activity and gene expression of the enzymes involved in fatty acid oxidation in the liver of rats fed 0.2% of sesamin or episesamin for 15 days. Furthermore, Ide et al. (2009) compared the impact of sesame lignan containing sesamin and episesamin on hepatic gene expression of rats by using DNA microarray. They demonstrated that the changes in the gene expression were generally greater with episesamin than with sesamin, and speculated that the difference between episesamin and sesamin is caused by the difference in their rates of metabolism. Actually, the concentration of episesamin in each tissue and serum in rats taken sesame lignan (a mixture of almost same amount of sesamin and episesamin) was higher than that of sesamin, whereas no significant difference existed in lymphatic absorption between sesamin and episesamin (Umeda-Sawada et al., 1999). From previous study (Shimizu et al., 1991), it had been probed that not only the metabolic rates but also the inhibitory effects for Δ5 desaturase in
rats are different between sesamin and episesamin. They demonstrated that sesamin has stronger inhibitory effect than episesamin for Δ5 desaturase using rat liver microsomes. Based on these findings, it is possible that the biological effects of episesamin are significantly different from those of sesamin in humans as well as rats.

Several reports on the metabolism of sesamin have recently been published (Nakai et al., 2003; Penalvo et al., 2005; Liu et al., 2006; Moazzami et al., 2007). Nakai et al. (2003) demonstrated that sesamin was firstly metabolized to mono- and di-catechol metabolites in rat liver. They assumed that some P450s were involved in sesamin catecholization, since MDP (Methylenedioxyphenyl) compounds were demethylated by P450-dependent oxidation (Kumagai et al., 1991; Murray, 2000). Furthermore, just recently, Tomimori et al., (2012) demonstrated that episesamin was metabolized to two epimers of monocatechol and one dicatechol by P450s in human and rat liver microsomes. Thus, P450s would be essential for the metabolism of both sesamin and episesamin. Recently, we demonstrated that sesamin was catecholized in human liver microsomes predominantly by CYP2C9, and sesamin was a mechanism based inhibitor of CYP2C9 (Yasuda et al., 2010). Furthermore, we also demonstrated the species-specific differences in sesamin metabolism between humans and rats (Yasuda et al., 2011).

In this paper, we revealed P450 species responsible for episesamin metabolism and compared the sequential metabolism of episesamin by drug metabolizing enzymes with that of sesamin in human and rat liver.
Materials and methods

Materials.

Sesamin was purchased from Sigma-Aldrich (St. Louis, MO) and episesamin was purchased from Nagara Science Co., Ltd (Gifu, Japan). NADPH was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). Human single donor liver microsomes (HG43, HH47, HH18, HH74, HH77, HG95, HH715, HH581, HG3, and HH741), a 50 donor human liver microsomes pool, human liver cytosol pool, male Sprague-Dawley male rat liver microsomes and cytosol, and recombinant human UGTs (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, 2B17) expressed in baculovirus-infected insect cells were purchased from BD Gentest (Woburn, MA). *Saccharomyces cerevisiae* AH22 cells expressing human P450s (CYP2C9, 1A2, 2C19, 3A4, 2E1, and 2D6) with the vector pGYR were kindly provided by Sumitomo Chemical Co., Ltd. (Osaka, Japan). Polyclonal antibodies against each of human P450s (1A2 and 2C9) were purchased from Nihon Nosan Kogyo (Yokohama, Japan). Sesamin monocatechol, episesamin S- and R-monocatechols, sesamin dicatechol, and episesamin dicatechol were kindly provided by Suntory Wellness Ltd. (Tokyo, Japan) (Urata et al., 2008; Tomimori et al., 2012). All other chemicals were purchased from standard commercial sources of the highest quality available.

Metabolism of Episesamin, Phenacetin and Diclofenac in Human Liver Microsomes.

To measure enzyme activities and determine kinetic parameters, time and protein linearity studies were performed initially. The reaction mixture (200 μl) containing 0.5 mg protein/ml of human liver microsomes, 1 mM NADPH, 5 μM episesamin or phenacetin or diclofenac in 100 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 15
min. The reaction mixture was extracted with four volumes of chloroform/methanol (3:1. v/v). The organic phase was recovered and dried in a vacuum evaporator centrifuge (Sakuma Seisakusyo, Tokyo, Japan). The resultant residue was solubilized with methanol and applied to HPLC under the following conditions for episesamin: column, YMC-pack ODS-AM (4.6 × 300 mm) (YMC Co., Tokyo, Japan); UV detection, 280 nm; flow late, 1.0 ml/min; column temperature, 40°C; linear gradients of 10-90% methanol aqueous solution per 30 min, and 90-100% per 5 min. For diclofenac and phenacetin, we used the same methods as those for episesamin except for the following condition; UV detection, 267 nm; linear gradients of 20-95% acetonitrile aqueous solution per 25 min, containing 0.05% of trifluoroacetic acid for diclofenac; UV detection, 245 nm; linear gradients of 10-35% methanol aqueous solution per 15 min, containing 0.05% of trifluoroacetic acid.

Episesamin monocatechol had two possible epimers, R-catechol and S-catechol, as shown in Fig. 1. Thus, the metabolites, which was recovered from the HPLC conditions as described above, was further analyzed by HPLC using a chiral β-cyclodextrin column under the following conditions: column, SUMICHIRAL OA-7000 (4.6 × 250 mm) (Sumika Chemical Analysis Service, Ltd); UV detection, 280 nm; flow rate, 0.7 mL/min; column temperature, 25°C; mobile phase, methanol/water (75:25, v/v) containing 0.01 % TFA.

**LC-Mass Spectrometric Analysis of a Metabolite of Episesamin.**

The metabolite of episesamin produced in the human liver microsomes was isolated by HPLC, and subjected to mass spectrometric analysis, using a Finnigan LCQ ADVANTAGE MIX (ThermoFisher SCIENTIFIC, Waltham, MA, USA) with atmospheric pressure chemical ionization, positive mode. The conditions of LC were described below: column;
reverse phase ODS column (2 mm × 150 mm, Develosil ODS-HG-3, Nomura Chemical Co. Ltd., Aichi, Japan); mobile phase, acetonitrile : methanol : water = 3 : 4 : 3; flow rate, 0.2 ml/min; UV detection, 280 nm.

**Kinetic Analysis of Episesamin Catecholization Using Human Liver Microsomes or Microsomal Fraction of Recombinant Yeast Cells.**

The reaction mixture (200 μl) containing 0.5 mg protein/ml of human liver microsomes or recombinant yeast microsomes containing CYP2C9, CYP1A2 or CYP2C19, 1 mM NADPH, and various concentrations of episesamin (2, 5, 10, 25, 50 and 75 μM) in 100 mM potassium phosphate buffer (pH 7.4). The reaction was started by the addition of 1 mM NADPH, and continued at 37°C for 15 min. The reaction mixture also contains 0.5 % DMSO, since 1 μl of sesamin DMSO solution was added as the substrate. As a control, the same reaction was performed using the microsomes prepared from AH22 cells harboring the control vector.

HPLC calibration curve of authentic standards of sesamin, episesamin, and their catechol were used to determine their concentrations. In contrast, we have no authentic standards of monocatechol-glucuronide and monocatechol-methylated metabolites. We assumed that UV absorption at around 280 nm of monocatechol-glucuronide and monocatechol-methylated metabolites is not so different from those of corresponding monocatechols. Kinetic parameters, $K_m$ and $k_{cat}$, were calculated by the nonlinear regression analysis using KaleidaGraph (Synergy Software, Reading, PA). The equation was applied for Michaelis-Menten kinetics.
**Inhibition of Episesamin Catecholization in Human Liver Microsomes by Polyclonal Antibodies against Human P450s.**

The pooled human liver microsomes (50 μg of protein) and polyclonal antibodies against human CYP2C9 or CYP1A2 (0-200 μg of protein) were incubated at 4°C for 1 hr, and the reaction mixture containing 1 mM NADPH and 5 μM episesamin in 100 mM potassium phosphate buffer (pH 7.4) was added at a final volume of 200 μl, and then incubated at 37°C for 15 min. The metabolite was analyzed as described under *Metabolism of Episesamin, Phenacetin and Diclofenac in Human Liver Microsomes.*

**Inhibition of Episesamin Catecholization in Human Liver Microsomes by P450 Enzyme-Specific Inhibitors.**

The reaction mixture (100 μl) containing 0.5 mg protein/ml of pooled human liver microsomes and 1 to 5 μM sulfaphenazole (CYP2C9 specific inhibitor) or (+)-N-3-benzylirvanol (CYP2C19 specific inhibitor) in 100 mM potassium phosphate buffer (pH 7.4) were pre-incubated at 37°C for 5 min. After the pre-incubation, 5 μM episesamin and 1 mM NADPH were added, and incubation was continued for 15 min. The metabolite was analyzed as described under *Metabolism of Episesamin, Phenacetin and Diclofenac in Human Liver Microsomes.*

**Mechanism-based Inhibition of P450.**

The reaction mixture containing 0.5 mg protein/ml of recombinant yeast microsomes, 1mM NADPH and various concentrations of episesamin (0-50 μM) in 100 mM potassium phosphate buffer (pH 7.4) was pre-incubated at 37°C for 0, 5, and 10 min. After the pre-
incubation, substrates for each P450-specific activity were added at the final concentration of 100 μM and incubation was continued for 15 min under the same conditions. Their metabolites were analyzed as described above. Kinetic parameters of inactivation process were calculated according to the method of Waley (Waley, 1980; Waley, 1985). The observed rate constant of inactivation (k_{obs}) was calculated from the initial slopes of the linear regression line of the ‘residual activity’ versus ‘pre-incubation time’ profile plotted on a semilogarithmic scale. The inactivation rate constant at infinite concentration of inhibitor (k_{inact}) and the appeared inhibitor constants (K_i) were calculated from the double reciprocal plots of k_{obs} versus episesamin concentration, according to the following equation (eq.1). [I] indicates the concentration of inhibitor, episesamin.

\[ k_{obs} = \frac{k_{inact} [I]}{K_i + [I]} \]  

(1)

**Preparation of Each of Two Epimers of Episesamin Monocatechol.**

The mixture of 0.25 mg/ml of rat liver microsomal fraction, 1 mM of NADPH, and 30 μM of episesamin in 100 mM phosphate buffer was incubated for 10 min at 37°C. The metabolites were extracted and isolated by HPLC using a ODS column for the isolation of the mixture of M1 and M2 and chiral β-cyclodextrin column for the separation of M1 and M2 from the mixture. Details of the methods were described under Metabolism of Episesamin, Phenacetin and Diclofenac in Human Liver Microsomes. Separated M1 and M2 were recovered, dried, and then dissolved in DMSO to be used as substrates as follows.

**Metabolism of Episesamin Monocatechol by P450 or UGT or COMT in the Liver Microsomes or Liver Cytosol.**
Each of P450-dependent catecholization, UGT-dependent glucuronidation, and COMT-dependent-methylation of M1 and M2 were analyzed by the same methods in our previous study (Yasuda et al., 2011). Briefly, the conditions of each reaction were described as follows. In P450-dependent catecholization, the reaction mixture containing 0.5 mg protein/ml of the human liver microsomes or 0.25 mg protein/ml of the rat liver microsomes, 1-30 μM episesamin monocatechol, 1 mM NADPH in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10 to 30 min at 37°C. In UGT-dependent glucuronidation, the reaction mixture containing 0.5 mg protein/ml of the human or rat liver microsomes, 1 to 30 μM episesamin monocatechol, 2 mM UDP-GlcUA, and 1 mM MgCl₂ in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10-30 min at 37°C. In COMT-dependent methylation, the reaction mixture containing 0.25 mg protein/ml of the human or rat liver cytosol, 1 to 30 μM episesamin monocatechol, 200 μM SAM, 2 mM MgCl₂, and 1 mM dithiothreitol in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10-30 min at 37°C. Each metabolite was analyzed by HPLC.

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

The reaction mixture containing 0.1 mg protein/ml of the recombinant human UGT (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, or 2B17) microsomes 1 to 30 μM episesamin monocatechol, 2 mM UDP-GlcUA, and 1 mM MgCl₂ in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10 to 30 min at 37°C. The metabolites were analyzed by HPLC as described under **Metabolism of Episesamin, Phenacetin and Diclofenac in Human Liver Microsomes.**
Metabolism of 3’-Azido-3’-Deoxythymidine (AZT) in Human Liver Microsomes.

The AZT glucuronidation activity in pooled human liver microsomes were analyzed as described in our previous study (Yasuda et al., 2011). Briefly, the reaction mixture containing 0.5 mg protein/ml human liver microsomes, 1 mM AZT, 2 mM UDP-GlcUA, and 1 mM MgCl₂ in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 15 min at 37°C. Each metabolite was analyzed by HPLC.

Substrate Docking

Docking was performed using Surflex Dock program in SYBYL 8.1.1. The coordinate file 2HI4.pdb was used for CYP1A2 as protein structure. The Surflex Dock program uses an empirical scoring function to rank putative protein-ligand interactions by flexible docking of small molecules to protein structures.
Results

Identification of the Metabolites of Episesamin.

Figure 1A shows the HPLC profiles of sesamin (a) or episesamin (b) and their metabolites in human liver microsomes. As described previously (Yasuda et al., 2010), sesamin was converted to one metabolite; sesamin monocatechol. Meanwhile, episesamin was converted to two metabolites designated as M1 and M2 (Fig. 1A-b). Further HPLC analysis using the chiral column clearly separated both metabolites (Fig. 1A-c). These results are consistent with those reported by Tomimori et al. (2012). Co-chromatography of M1 and M2 with authentic standard of each epimers of episesamin monocatechol strongly suggests that M1 and M2 were S- and R-monocatechol, respectively (Figure 1B). To confirm the chemical structures of the metabolites, we collected the metabolites containing M1 and M2 in the effluents from HPLC and subjected them to mass spectrometric analysis. Relative intensities (%) of major ion fragments of the authentic standard of episesamin and its metabolites were as follows. Episesamin: m/z 319 (M+H-2H2O), 6%; m/z 337 (M+H-H2O), 100%; m/z 355 ((M+H) 42%; M1: m/z 307 (M+H-2H2O), 10%; m/z 325 (M+H-H2O), 100%; m/z 343 (M+H) 40%; M2: m/z 307 (M+H-2H2O), 10%; m/z 325 (M+H-H2O), 100%; m/z 343 (M+H) 36%.

Episesamin Metabolism by Recombinant Human P450s.

In our previous study (Yasuda et al., 2010), we revealed that some P450-enzyme had the sesamin catecholization activity and their activity was high in order of CYP2C19, 2D6, 2C9, and 1A2, though CYP2C9 was the most important in human liver. In this study, we examined the episesamin catecholization by P450 enzymes including these P450s.
Although no metabolites were observed in CYP2D6, CYP3A4, and CYP2E1 as well as the control (supplemental Fig. 1A), the metabolites were detected in CYP1A2, 2C9, and 2C19 (Fig. 2). Interestingly, each of the three P450 enzymes showed different metabolic patterns. CYP1A2 showed only one metabolite, M2 (Fig. 2a), while the major metabolite of CYP2C9 was M1. The ratio of M1 to M2 in CYP2C9 was approximately 5 to 1 (Fig. 2b). On the other hand, the ratio of M1 to M2 in CYP2C19 was approximately 1 to 1 (Fig. 2c). Surprisingly, another polar metabolite designated as M3, which was not observed in both CYP1A2 and CYP2C9, was observed in CYP2C19 (Fig. 2c). Cochromatography of M3 with the authentic episesamin dicatechol strongly suggested that M3 was episesamin dicatechol. It is noted that episesamin dicatechol was observed in the presence of much amount of episesamin. The ratio of sesamin dicatechol to monocatechol in 20 μM sesamin was reduced to 8.6 % of that in 2 μM sesamin, whereas the ratio of episesamin dicatechol in 20 μM episesamin was retained at 46 % of that in 2 μM episesamin (Supplemental Fig. 2). These results suggest that the mechanism of dicatechol formation by CYP2C19 could be different between sesamin and episesamin. It is possible that at least in part, episesamin is converted to dicatechol via monocatechol without a release of monocatechol from the substrate-binding pocket of CYP2C19.

**Correlation between Episesamin Catecholization Activity and Each P450-specific Activity in Human Liver Microsomes.**

Different metabolic patterns of episesamin by the three P450 enzymes in Figure 2 suggest inter-individual differences in episesamin metabolism. Comparison of metabolic patterns of episesamin among 10 human single donor liver microsomes confirmed that this
hypothesis is correct. Figure 3A shows HPLC profiles of episesamin metabolites by three donors, HH741, HH581, and HG43. The metabolite M1 is predominant in HH741, while M2 is predominant in HH581 and HG43. It is notable that only HG43 showed a remarkable peak of the metabolite M3 among 10 human single donor liver microsomes.

Although M1 and M2 were detected in all donors, the ratios of M1 to M2 were different among 10 donors as shown in Figure 3B. To determine the P450 enzymes responsible for M1 and M2 production, we examined the correlation between each epimer production and each P450-specific activity among 10 donors. As shown in figure 4A, a high correlation was observed between M1 production and diclofenac 4’-hydroxylation activity, which is known to be a CYP2C9-specific activity (R^2 = 0.83, p < 0.01). On the other hand, significant correlation was not observed between M2 production and diclofenac 4’-hydroxylation activity (R^2 = 0.19, p = 0.2), as shown in Figure 4B. However, a high correlation was observed between M2 production and phenacetin O-deethylation activity, which is known to be a CYP1A2-specific activity (R^2 = 0.52, p = 0.018). It is noted that HH581 has much higher CYP1A2-specific activity than any other donors. When HH581 was omitted from the 10 donors, the marginally significant correlation was observed (R^2 = 0.39, p = 0.074). However, no correlation was observed between M1 or M2 formation and S-mephenytoin 4’-hydroxylation activity, which is known to be the CYP2C19-specific activity. Based on these results, the P450 enzymes responsible for M1 and M2 production were considered to be CYP2C9 and CYP1A2, respectively.

To our surprise, significantly large amount of episesamin dicatechol was produced in HG43 (Fig. 3A-c), whereas no metabolite was detected in the heat-inactivated HG43 microsomes (supplemental Fig.1B). The CYP2C19-specific activity of this donor was about
10 times higher than the other donors, though other P450 enzyme-specific activities were not so different. These results are consistent with those using recombinant CYP2C19 shown in Fig. 2c, and suggest that dicatehol production should be considered in the people who have high CYP2C19-dependent activity.

**Inhibition of Episesamin Catecholization in Human Liver Microsomes by Anti-P450 Antibody or CYP2C9-Specific Inhibitor.**

To confirm that CYP2C9 and CYP1A2 are responsible for M1 and M2 formation, respectively, we examined the effects of antibodies against CYP1A2 and CYP2C9 on episesamin catecholization in the pooled human liver microsomes (Fig. 5). As expected, M1 production was significantly inhibited by anti-CYP2C9 antibody, but was not inhibited by anti-CYP1A2 at all (Fig. 5A). On the other hand, M2 production was significantly inhibited by anti-CYP1A2 antibody, and also inhibited by anti-CYP2C9 antibody (Fig. 5B). The activity was not inhibited by addition of control antibody. These results are consistent with those in Fig. 4B suggesting a small contribution of CYP2C9 to M2 production. Fig. 5C shows inhibitory effects of both antibodies on the total production in M1 and M2, and indicates that CYP2C9 is the most important P450 enzyme for production of episesamin monocatechol, but CYP1A2 bears comparison with CYP2C9.

**Inhibition of Episesamin Catecholization in Human Liver Microsomes by CYP2C9- or CYP2C19-Specific Inhibitor**

To confirm that CYP2C9 and CYP1A2 are most essential P450 enzymes for episesamin catecholization but CYP2C19 is not, we examined the effect of chemical inhibitors for
human CYP2C9 (sulfaphenazole) (Miners et al., 1988) and CYP2C19 ((+)-N-3-benzylirvanol) (Suzuki et al., 2002). Sulfaphenazole showed a significant inhibitory effect of episesmain monocatecholization activity in human liver microsomes, whereas (+)-N-3-benzylirvanol did not; the activity in the presence of 5 µM of sulfaphenazole was about 50% of that in absence of inhibitor, whereas the activity didn’t change in the presence of 5 µM of (+)-N-3-benzylirvanol. These results strongly suggest only a small contribution of CYP2C19 for the episamin monocatechol production in human liver microsomes, although CYP2C19 may be essential for the dicatechol production.

Comparison of the Kinetic Parameters between Episesamin and Sesamin Monocatecholization in Recombinant Human CYP2C9 and CYP1A2.

As described in the section above, both CYP2C9 and CYP1A2 were important for the episesamin metabolism. On the other hand, for sesamin metabolism, the contribution of CYP2C9 was much larger than that of CYP1A2 (Yasuda et al., 2010). Table 1 shows the comparison of kinetic parameters of CYP2C9 and CYP1A2 between sesamin and episesamin. The \( k_{\text{cat}} \) value of CYP2C9 for episesamin was a half of that for sesamin, while the \( K_m \) value was 3-times larger. On the other hand, the \( k_{\text{cat}} / K_m \) value of CYP1A2 for episesamin was not so different from that for sesamin. Thus, episesamin appears to be significantly poorer substrate of CYP2C9 than sesamin, while CYP1A2 equally catalyzes catecholization for both sesamin and episesamin.

Docking of Episesamin into CYP1A2.

To interpret the stereoselective catecholization of episesamin by CYP1A2, we
performed a docking study of episesamin. We obtained 47 diverse conformations of episesamin docked in CYP1A2, from which we selected nine conformations of episesamin whose acetal moiety was located close enough to iron atom-less than 6Å- and faced to the iron. All of these nine conformations have \( R \)-1,3-benzodioxole toward to heme iron (Fig. 6). \( S \)-1,3-benzodioxole seems to be not accommodated into the cavity with proper conformation since it would cause steric crash with Ala317. On the other hand Ala317 guides \( R \)-1,3-benzodioxole toward to heme iron atom, so that \( R \)-1,3-benzodioxole is able to be oxidized. This result is consistent with the fact that major metabolite of episesamin by CYP1A2 was \( R \)-monocatechol as shown in Fig. 1.

**Mechanism-based Inhibition of P450s.**

Our previous study demonstrated that sesamin caused MBI of CYP2C9 (Yasuda et al., 2010), which was the most responsible enzyme for sesamin metabolism. As described above, for the metabolism of episesamin, both CYP2C9 and CYP1A2 play a key role almost equally. To examine MBI of each P450 enzyme by episesamin, we used the microsomal fraction of the recombinant yeast expressing each of CYP2C9, CYP1A2, and CYP3A4. Although CYP1A2 and CYP3A4 showed no MBI (\( \Delta \) (Log activity % of control) per min were almost the same between 0 and 50 \( \mu \)M of episesamin), CYP2C9 showed time- and concentration-dependent MBI (Fig. 7). It is noted that these results were same with those of sesamin. Table 2 shows that the comparison of MBI parameters between sesamin and episesamin for the recombinant CYP2C9. The apparent \( K_i \) and \( k_{\text{inact}} \) values of episesamin in CYP2C9-dependent dicrofenac 4’-hydroxylation were estimated to be 6.8 \( \mu \)M and 0.17 min\(^{-1}\), respectively. The \( k_{\text{inact}} / K_i \) value of episesamin was 18% of that of
Comparison of Sequential Metabolism of Sesamin and Episesamin in Rat or Human Liver Microsomes.

In our previous study, we clarified the metabolism of sesamin monocatechol, which is the first metabolite of sesamin, and found that oxidation by P450, glucuronidation by UGT, and methylation by COMT played essential roles for its metabolism in human liver. To examine whether episesamin monocatechol is metabolized like sesamin monocatechol or not, each epimer of episesamin monocatechol, M1 or M2 was metabolized in human liver microsomes in the presence of NADPH or UDP-GlcUA or in human liver cytosols in the presence of SAM. Each coenzyme-dependent metabolites were detected (data not shown), and metabolic enzymes of episesamin monocatechol were similar to those for sesamin monocatechol (Yasuda et al., 2011). These results strongly suggest that oxidation, glucuronidation, and methylation of episesamin monocatechol would occur simultaneously in human liver.

To compare the sequential metabolism of sesamin and episesamin in human liver, we performed kinetic analysis of P450-dependent metabolism of episesamin, and then P450- or UGT- or COMT-dependent metabolism of episesamin monocatechol (Yasuda et al., 2011). In human liver microsomes, the $V_{\text{max}}/K_{m}$ value of episesamin was about 30% of that of sesamin, indicating that episesamin was a poorer substrate than sesamin in human liver. Comparing the metabolism of sesamin monocatechol and episesamin monocatechol, the $V_{\text{max}}/K_{m}$ value of glucuronidation reaction were significantly different, though those values
of oxidation and methylation were not so different. It should be noted that glucuronidation by UGT was predominant over catecholization by P450 in the metabolism of sesamin monocatechol. Because P450 and UGT reactions occur in microsomes and the COMT reaction occurs in cytosol, we cannot compare the kinetic parameter of COMT with that of P450 or UGT. However, it is obvious that the metabolism of sesamin and episesamin was significantly different; UGT was the most important for the sesamin metabolism as the second-step reaction, whereas COMT would be the most important and the contribution of UGT was not so large for the episesamin metabolism.

We further performed the same analysis using rat liver microsomes and cytosol. The \( \frac{V_{\text{max}}}{K_{m}} \) value of episesamin monocatecholization was 58% of that value of sesamin monocatecholization. Thus, episesamin was somewhat poorer substrate compared to sesamin in rat liver microsomes. Focused on the second step metabolism, it is noted that the oxidation and glucuronidation activities toward M1 were significantly different from those toward M2 in rat microsomes. M1 was predominantly metabolized by P450 and UGT over M2 in rat liver, while M1 and M2 were equally metabolized by P450 and UGT in human liver. Episesamin was metabolized to M1 and M2 at approximate 1 to 1 ratio in rat liver microsomes (data not shown) similarly to human liver microsomes. Based on the relative \( \frac{V_{\text{max}}}{K_{m}} \) of each reaction in Table 3, metabolic profiles of sesamin and episesamin in human or rat liver were summarized in Fig. 8. Roughly speaking, these results demonstrated that the difference of the sequential metabolism between sesamin and episesamin in rat liver was smaller than that in human liver.

**Identification of P450 Enzymes Responsible for Oxidation of Episesamin Monocatechol.**
As described above, episesamin was metabolized to dicatechol via monocatechol in human recombinant CYP2C19 and single donor of human liver microsomes which has high CYP2C19 activity. These results indicated that CYP2C19 has higher oxidation activity toward episesamin-monocatechol, compared to other P450 enzymes. To confirm this assumption, we examined the metabolism of monocatechol by some recombinant human P450 enzymes. As expected, both of episesamin-monocatechol, M1 and M2 were metabolized to eisesamin-dicatechol by CYP2C19. Our previous study demonstrated that CYP2C9 catalyzed oxidation of sesamin-moncatechol to produce its dicatechol. The CYP2C19-dependent oxidation activities toward sesamin monocatechol and episesamin moncatechol were not so different (\(K_m=5.1 \mu M\) and \(k_{cat} =14 \text{ min}^{-1}\) for the former, and \(K_m=7.3 \mu M\) and \(k_{cat} =15 \text{ min}^{-1}\) for the latter). CYP2C9 and CYP1A2 also converted episesamin moncatechol to its dicatechol, but their activities were much smaller than that of CYP2C19. The oxidation activities of CYP2C19, CYP2C9, and CYP1A2 in the presence of 20 \(\mu M\) episesamin moncatechol were 9.4, 0.19, and 0.38 nmol/min/nmol P450, respectively. Judging from their average contents in human liver are 2C19:1%, 2C9:20%, CYP1A2:13% (Shimada et al., 1994; Inoue et al., 1997), the P450 enzyme responsible for oxidation of episesamin moncatechol is considered to be CYP2C19. On the other hand, the oxidation activities of CYP2C19 and CYP2C9 in the presence of 20 \(\mu M\) sesamin moncatechol were 10.4 and 0.83 nmol/min/nmol P450, respectively. Based on their average contents in human liver, CYP2C9 appears to be responsible for oxidation of sesamin moncatechol to produce its dicatechol.

*Identification of UGT Enzyme Responsible for the Metabolism of Episesamin*
Monocatechol.

Our previous study demonstrated that the most important UGT enzyme for the sesamin monocatechol was UGT2B7. To identify the UGT enzyme responsible for the episesmain metabolism, we examined the metabolism of episesamin monocatechol by recombinant human UGTs expressed in baculovirus-infected insect cells. Among the 12 UGT enzymes, only UGT2B7 showed a significant glucuronidation activity. Further study demonstrated that correlation was not so good between episesamin monocatechol glucuronidation activity and AZT glucuronidation activity, which is known to be UGT2B7-specific activity ($R^2 = 0.28$, $p = 0.17$) as shown in supplemental Fig. 3. However, it is obvious that UGT2B7 plays an important role in the glucuronidation of episesamin monocatechol, as was the case of sesamin monocatechol.
Discussion

Sesamin and episesamin are epimers of each other, and commercially available sesamin supplements contain both epimers. There are some reports that, when taken at the same amount, the body concentrations and the extent of the gene expression of the hepatic enzymes involved in fatty acid β-oxidation were different between sesamin and episesamin in rats (Umeda-Sawada et al., 1999; Kushiro et al., 2002; Ide et al., 2009). Recently, Tomimori et al. (2012) reported that episesamin was metabolized to catechol metabolites by P450s like sesmin. However, to the best of our knowledge, there are no reports that compare sesamin and episesamin metabolism in detail. In this study, we compared the sequential metabolism of episesamin with that of sesamin in human liver.

As shown in Fig. 6, the tertiary structures of sesamin and episesamin are quite different. Thus, we predicted that the enzymes responsible for their metabolisms of each would be significantly different between sesamin and episesamin; unexpectedly, the major enzymes involved in the metabolism of episesamin in human liver microsomes were CYP2C9 and CYP1A2, as was the case for sesamin, yet the contribution ratio of CYP2C9 to CYP1A2 was quite different between the epimers. The contribution of CYP2C9 was much larger than CYP1A2 in sesamin metabolism, whereas the contribution of CYP2C9 was almost equal to that of CYP1A2 in episesamin metabolism. Interestingly, CYP1A2 and CYP2C9 produced different epimers of episesamin monocatechol, S- and R-monocatechols (Fig. 1B). CYP1A2 catalyzed R-monocatechol formation stereoselectively (Fig. 2a), and the docking study accords with this result (Fig. 6). The ratio of the two epimers was significantly different among 10 individual single human liver microsomes, probably due to the different expression levels of CYP2C9 and CYP1A2 in each liver microsomes.
The $k_{\text{cat}}/K_m$ value of the CYP2C9-dependent episesamin catecholization was 16% of that for sesamin, suggesting that episesamin is a significantly poorer substrate for CYP2C9 than sesamin. The fact that we have failed to perform the molecular docking of episesamin using the “auto-docking” method (data not shown) suggests that episesamin is an inferior substrate for CYP2C9. A clearly different binding mode of the substrate-binding pocket of CYP2C9 between episesamin and sesamin may affect both the $k_{\text{cat}}$ and $K_m$ values. However, the $k_{\text{cat}}/K_m$ value of the CYP1A2-dependent episesamin catecholization was almost the same as that of sesamin.

Because episesamin contains two MDP groups, it is likely that episesamin displays an MBI of CYP2C9, and episesamin, indeed, exhibited an MBI of CYP2C9. After the first monooxygenation by CYP2C9, a part of the metabolite could be spontaneously converted to form a reactive carbene compound that probably covalently binds to the heme iron of CYP2C9, as described in our previous report (Yasuda et al, 2010). The $k_{\text{inact}}/K_i$ value of episesamin for CYP2C9 and the $k_{\text{cat}}/K_m$ value of CYP2C9-dependent episesamin catecholization were 18% and 16% of those of sesamin, respectively. Furthermore, the $k_{\text{obs}}$ value of each monocatechol was about a half of that of sesamin and episesmain, respectively, and catecholization activity by CYP2C9 towards each monocatechol was also about a half of that of sesamin and episesamin, respectively (supplemental Table. 2). These results strongly suggest that the inactivation of CYP2C9 by the carbene formation occurs proportionally to the CYP2C9-dependent monooxygenation at the same ratio for sesamin, episesamin, and their monocatechols.

We further examined the metabolism of episesamin monocatechol and found that, as is the case for sesamin monocatechol, the P450-dependent oxidation, UGT-dependent
glucuronidation, and COMT-dependent methylation were important. However, the contributions of each reaction for sesamin and episesamin were significantly different. Although the UGT-dependent glucuronidation was predominant in sesamin metabolism, the contribution of the UGT-dependent glucuronide was not so large in episesamin metabolism (Fig. 8). Similar to sesamin monocatechol, the UGT enzyme responsible for episesamin monocatechol glucuronidation was UGT2B7, but the episesamin monocatechol glucuronidation activity and the UGT2B7-specific activity were not well correlated. These results might suggest the contribution of the other UGT enzymes which are not commercially available, such as UGT1A5, 2B10, 2B11, 2B28, 2A1, 2A2, and 2A3. As shown in Fig. 8, the contribution of COMT would become significant in episesamin metabolism. The P450-dependent oxidation of sesamin monocatechol and episesamin monocatechol to yield their dicatechols in human liver microsomes was much weaker than that in rat microsomes (Fig. 8). However, we obtained rather important results in the dicatechol production of episesamin (Figs 2 and 3). HG43 is derived from a CYP2C19 ultra-rapid metabolizer who has a CYP2C19*17 allele. (Sim et al., 2006) reported that some nuclear proteins specifically bind to the 5'-flanking region of this allele to induce gene transcription. Thus, the heterozygotes (*1/*17) would be ultra-rapid metabolizers (Wang et al., 2009). The frequency of the CYP2C19*17 allele is high (18%) in Ethiopians and Swedes (Sim et al., 2006), 0.64% in Chinese (Wang et al., 2009), and 1.3% in Japanese (Sugimoto et al., 2008). The antioxidant effects of episesamin in ultra-rapid metabolizers might be larger than normal metabolizers due to a high ability of dicatechol formation (Nakai et al., 2003; Miyake et al., 2005). However, CYP2C19 poor metabolizers, whose frequency is approximately 20% in Japanese and Koreans, appear to have a low ability to
form episesamin dicatehol. It is notable that CYP2C19 might catalyze a two-step catecholization from episesamin to its dicatehol without a release of the first product episesamin monocatechol. However, such reaction was only observed in the CYP2C19-dependent episesamin metabolism and not in sesamin metabolism. Further studies are needed to reveal the substrate recognition and reaction mechanism of the CYP2C19-dependent metabolism of the conversion of episesamin to its dicatehol.

In our previous study, we revealed the species-based differences of sesamin metabolism between humans and rats. As shown in Fig. 8 and Table 3, the remarkable species-based differences between humans and rats were also observed in the episesamin metabolism. In rats, P450-dependent oxidation was predominant over UGT-dependent glucuronidation in the metabolism of both sesamin and episesamin. Comparing the monocatecholization between sesamin and episesamin in rat liver microsomes, the $V_{\text{max}}/K_{\text{m}}$ value of episesamin monocatecholization was 58% of that of sesamin monocatecholization. Thus, episesamin was also a somewhat poorer substrate than sesamin in rat liver microsomes. In vivo rat studies have demonstrated the differences between sesamin and episesamin: the serum level of episesamin was higher than that of sesamin in rats (Umeda-Sawada et al., 1999), and episesamin was much stronger in increasing the activity and gene expression of the enzymes involved in fatty acid oxidation in rats taken the almost same amount of sesamin and episesamin (Kushiro et al., 2002; Ide et al., 2009). These results suggest that episesamin is more resistant to metabolism and that the resulting higher serum level of episesamin induces fatty acid oxidation. The difference of the $V_{\text{max}}/K_{\text{m}}$ values of monocatecholization between sesamin and episesamin in this study appears to be consistent with the in vivo rat studies. It is notable that the $V_{\text{max}}/K_{\text{m}}$ value of episesamin
monocatecholization in human liver microsomes was 29% of that of sesamin, and this difference was greater than in rat liver microsomes. Furthermore, the differences of monocatechol metabolism between sesamin and episesamin in rat liver were smaller than that in human liver. Judging from the in vivo rat studies, the efficacy of episesamin might be stronger than sesamin in humans.

In conclusion, we have revealed that sesamin and episesamin have significantly different metabolic patterns by P450, UGT, and COMT, and this difference might affect their biological effects in human body. It is noteworthy that the commercially available sesamin supplements contains equal amount of sesamin and episesamin. Although the separation of sesamin and episesamin would not be easy at the industrial level, we propose that they should be isolated from each other to maximize their usefulness.
Acknowledgement

We would like to express our gratitude to Suntory Wellness Ltd. (Tokyo, Japan) for providing us authentic standards of mono- and di-catechols of sesamin and episesamin.

Authorship Contributions

Participated in research design: Yasuda, Ikushiro, Yamamoto, and Sakaki

Conducted experiments: Yasuda, and Ohta

Contributed new reagents or analytical tools: Wakayama, Itoh, Kamakura, and Ohta

Performed data analysis: Yasuda, Ikushiro, Itoh, Munetsuna, and Ohta

Wrote or contributed to the writing of the manuscript: Yasuda, Yamamoto, and Sakaki
References


Liu Z, Saarinen NM and Thompson LU (2006) Sesamin is one of the major precursors of mammalian lignans in sesame seed (Sesamum indicum) as observed in vitro and in rats. J Nutr 136:906-912.


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**Legends to Figures**

Fig. 1  HPLC profiles of sesamin (A-a) and episesamin (A-b) metabolite in human liver microsomes which were incubated for 30 min in the presence of NADPH. The metabolites of episesamin were separated two metabolites (M1 and M2) by HPLC analysis using a chiral $\beta$-cyclodextrin column as indicated in the square (A-c).

Fig. 2  HPLC profiles of episesamin metabolites in recombinant human CYP1A2 (a), CYP2C9 (b), and CYP2C19 (c). HPLC profiles of M1 and M2 using a chiral $\beta$-cyclodextrin column were shown in the square.

Fig. 3  HPLC profiles of episesamin metabolites in three individual single donor human liver microsomes, HH741 (A-a), HH581 (A-b), and HG43 (A-c), and each of M1 and M2 production activity in 10 individual donors single human liver microsomes (B).

Fig. 4 Correlation between episesamin catecholization activity (M1) and CYP2C9-specific (diclofenac 4’-hydroxylation) activity (A), correlation between episesamin catecholization activity (M2) and CYP2C9-specific (diclofenac 4’-hydroxylation) activity (B), and correlation between episesamin catecholization activity (M2) and CYP1A2-specific (phenacetin O-deethylation) activity (C). Correlation in 9 individual donors without HH581 was shown in the square of (C).

Fig. 5  Inhibition of episesamin catecholization for M1 (A), M2 (B), and both (C) in pooled human liver microsomes by each P450-specific antibody (CYP1A2 (○), and CYP2C9 (▲)).
Each point represents the mean of triplicate determination.

Fig. 6 The docking model of human CYP1A2 and episesamin

Fig. 7 Time- and concentration-dependent inactivation of CYP2C9-specific activity in recombinant human CYP2C9 (A) by episesamin. Episesamin concentrations are shown under the plots. Each point represents the mean of at least duplicated determination. Double-reciprocal plots of the relationships between inactive rate constants ($k_{obs}$) and episesamin concentrations (B).

Fig. 8 Comparison of the metabolism of sesamin and episesamin between human and rat liver. Thickness of arrows roughly represents the strength of the activity on the basis for the relative $V_{max}/K_m$ values shown in Table 3.
Table 1  Comparison of kinetic parameters between episesamin and sesamin monocatecholization in recombinant human CYP2C9 and CYP1A2. Each value represents the mean ± SD from three separate experiments.

<table>
<thead>
<tr>
<th></th>
<th>Episesmain</th>
<th>Sesamin</th>
<th>Episesamin / Sesamin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CYP2C9</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{cat}$ (min⁻¹)</td>
<td>1.5 ± 0.1</td>
<td>3.1 ± 0.2</td>
<td>0.48</td>
</tr>
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<td>$K_m$ (μM)</td>
<td>15.8 ± 3.3</td>
<td>5.4 ± 0.9</td>
<td>2.9</td>
</tr>
<tr>
<td>$k_{cat}/K_m$</td>
<td>0.094</td>
<td>0.57</td>
<td>0.16</td>
</tr>
<tr>
<td><strong>CYP1A2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{cat}$ (min⁻¹)</td>
<td>5.3 ± 0.2</td>
<td>5.0 ± 1.3</td>
<td>1.1</td>
</tr>
<tr>
<td>$K_m$ (μM)</td>
<td>11.9 ± 0.2</td>
<td>15.0 ± 2.1</td>
<td>0.79</td>
</tr>
<tr>
<td>$k_{cat}/K_m$</td>
<td>0.45</td>
<td>0.33</td>
<td>1.4</td>
</tr>
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</table>
Table 2  Comparison of MBI parameters between episesamin and sesamin for recombinant human CYP2C9. Each value represents the mean ± SD from three separate experiments. The values for sesamin were obtained in our previous study (Yasuda et al., 2010).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Episesamin</th>
<th>Sesamin</th>
<th>Episesamin / Sesamin</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{inact}}$ (min$^{-1}$)</td>
<td>0.17 ± 0.02</td>
<td>0.22 ± 0.03</td>
<td>0.77</td>
</tr>
<tr>
<td>$K_{\text{i app}}$ (μM)</td>
<td>6.8 ± 1.2</td>
<td>1.6 ± 0.9</td>
<td>4.3</td>
</tr>
<tr>
<td>$k_{\text{inact}} / K_{\text{i app}}$</td>
<td>0.025</td>
<td>0.14</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 3 Comparison of kinetic parameters between episesamin and sesamin monocatecholization in human and rat liver microsomes. Each value represents the mean ± SD from three separate experiments.

<table>
<thead>
<tr>
<th>Species</th>
<th>Substrate</th>
<th>Reaction</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>0.81 ± 0.07</td>
<td>7.7 ± 1.7</td>
<td>0.11</td>
<td>1</td>
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<tr>
<td></td>
<td></td>
<td>Glu (UGT)</td>
<td>6.0 ± 0.9</td>
<td>18.0 ± 5.3</td>
<td>0.33</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met (COMT)</td>
<td>1.3 ± 0.1</td>
<td>5.6 ± 0.53</td>
<td>0.23</td>
<td>2.2</td>
</tr>
<tr>
<td></td>
<td>Episesamin</td>
<td>Cat (P450)</td>
<td>0.40 ± 0.04</td>
<td>13.0 ± 3.6</td>
<td>0.031</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
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<td>Glu (UGT)</td>
<td>0.55 ± 0.09</td>
<td>9.9 ± 2.9</td>
<td>0.056</td>
<td>0.53</td>
</tr>
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<td></td>
<td></td>
<td>Met (COMT)</td>
<td>0.59 ± 0.06</td>
<td>2.4 ± 0.7</td>
<td>0.25</td>
<td>2.3</td>
</tr>
<tr>
<td>Rat</td>
<td>Sesamin</td>
<td>Cat (P450)</td>
<td>4.8 ± 0.2</td>
<td>7.1 ± 1.8</td>
<td>0.68</td>
<td>6.4</td>
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<tr>
<td></td>
<td></td>
<td>Glu (UGT)</td>
<td>3.7 ± 0.1</td>
<td>5.3 ± 1.4</td>
<td>0.70</td>
<td>6.6</td>
</tr>
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<td></td>
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<td>Met (COMT)</td>
<td>1.1 ± 0.2</td>
<td>20.3 ± 6.8</td>
<td>0.054</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>Episesamin</td>
<td>Cat (P450)</td>
<td>6.3 ± 0.7</td>
<td>16.0 ± 3.9</td>
<td>0.39</td>
<td>3.7</td>
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<td></td>
<td></td>
<td>Glu (UGT)</td>
<td>4.4 ± 0.5</td>
<td>16.8 ± 3.6</td>
<td>0.26</td>
<td>2.5</td>
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<tr>
<td></td>
<td></td>
<td>Met (COMT)</td>
<td>3.2 ± 0.1</td>
<td>7.7 ± 0.4</td>
<td>0.42</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
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<td>Cat (P450)</td>
<td>0.85 ± 0.14</td>
<td>8.7 ± 2.9</td>
<td>0.098</td>
<td>0.93</td>
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<tr>
<td></td>
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<td>Glu (UGT)</td>
<td>0.55 ± 0.09</td>
<td>9.9 ± 2.9</td>
<td>0.056</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Met (COMT)</td>
<td>2.4 ± 0.2</td>
<td>5.5 ± 0.9</td>
<td>0.44</td>
<td>4.1</td>
</tr>
</tbody>
</table>

a. Cat: catecholization, Glu: glucuronidation, Met: methylation
Fig. 1
Fig. 4
Fig. 7
Fig. 8