METABOLISM OF SESAMIN BY CYTOCHROME P450 IN
HUMAN LIVER MICROSONES*

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Abbreviations: P450 or CYP, cytochrome P450; MBI, mechanism-based inhibition; MDP, methylenediphenyl; DMSO, dimethylsulfoxide
Abstract

Metabolism of sesamin by cytochrom P450 (CYP) was examined using yeast expression system and human liver microsomes. *Saccharomyces cerevisiae* cells expressing each of human CYP isoforms (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, and 3A4) were cultivated with sesamin, and mono-catechol metabolite was observed in most of CYPs. Kinetic analysis using the microsomal fractions of the recombinant *Saccharomyces cerevisiae* cells revealed that CYP2C19 had the largest $k_{cat}/K_m$ value. Based on the kinetic data and average contents of the CYP isoforms in the human liver, the putative contribution of CYPs for sesamin metabolism was large in the order of CYP2C9, 1A2, 2C19, and 2D6. A good correlation was observed between sesamin catecolization activity and CYP2C9-specific activity in *in vitro* studies using ten individual human liver microsomes, strongly suggesting that CYP2C9 is the most important CYP isoform for sesamin catecolization in human liver. Inhibition studies using anti-each CYP-specific antibody confirmed that CYP2C9 was the most important, and the secondary most important CYP was CYP1A2. We also examined the inhibitory effect of sesamin for CYP isoform-specific activities, and found a mechanism-based inhibition of CYP2C9 by sesamin. In contrast, no mechanism-based inhibition by sesamin was observed in CYP1A2-specific activity. Our findings strongly suggest that further studies are needed to reveal the interaction between sesamin and therapeutic drugs mainly metabolized by CYP2C9.
Introduction

Sesamin is a major lignan in sesame, and its 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) have been extensively studied by many researchers. Thus, sesamin appears to be one of the most reliable food factors whose effects can be expected by individuals taking it as a supplement or remedy. Meanwhile, some food factors are known to affect the xenobiotic metabolism. For example, some flavonoids show potent inhibitions of CYPs, and interfere with the metabolism of therapeutic drugs. (Cermak and Wolffram, 2006). Furthermore, there have been some reports of mechanism-based inhibition (MBI) of CYPs by methylenedioxyphenyl (MDP) compounds (Nakajima et al., 1999; Murray, 2000; Chatterjee and Franklin, 2003; Usia et al., 2005). Usia et al. (2005) demonstrated that some lignans which contain MDP group have potent MBI of CYP3A4. As sesamin has two MDP groups, it might cause MBI of some CYPs. However, no reports on MBI of CYPs by sesamin have been published to our best knowledge. Thus, in this study, we examined whether sesamin could cause MBI or not.

Nakai et al. (2003) demonstrated that sesamin was metabolized to mono- and di-catechol metabolites in rat liver. They assumed that some CYPs were involved in sesamin catecholization, since MDP compounds were demethylated by CYP-dependent oxidation (Kumagai et al., 1991; Murray, 2000). Sesamin was also converted to some metabolites including mono- and di-catechol metabolites by in vitro fermentation of human feces or in vivo administration (Penalvo et al., 2005; Liu et al., 2006). Moreover, mono-catechol compound was also detected as a human urinary sesamin metabolite (Moazzami et al., 2007). Liu et al. (2006) proposed tentative metabolic pathways of sesamin. Among the 11 kinds of the metabolites, mono- and di-catechol metabolites,
which appear to be metabolized by CYPs, are known to have high antioxidative activities (Nakai et al., 2003; Miyake et al., 2005). It was reported that sesamin elevated CYP2B and CYP4A mRNA levels in rats (Tsuruoka et al., 2005; Ikeda et al., 2007), and CYP1A activity in rainbow trouts (Trattner et al., 2008). In addition, sesamin inhibited CYP4F2 to elevate tocopherol levels (Sontag and Parker, 2002). However, to the best of our knowledge, there is no report to identify CYP isoforms responsible for sesamin metabolism in humans or to estimate the drug-sesamin interaction.

To predict drug metabolism in human body, we established yeast expression system for human CYPs, and showed its usefulness for the prediction of drugs or environmental contaminants (Imaoka et al., 1996; Hayashi et al., 2000; Sakaki and Inouye, 2000; Inouye et al., 2002). In this study, the yeast expression system and human liver microsomes were used to identify a CYP isoform responsible for the metabolism of sesamin in human body. In addition, we describe the mechanism-based inhibition of CYP2C9 by sesamin.
Materials and methods

Materials.

Sesamin, sulfaphenazole, and (+)-N-3-benzylpirvanol were purchased from Sigma-Aldrich (St. Louis, MO). 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) and a 50 donor human liver microsome pool were purchased from BD Gentest (Woburn, MA). Polyclonal antibodies against each of human CYPs (1A2, 2C9, 2C19, and 2D6) were purchased from Nihon Nosan Kogyo (Yokohama, Japan). The recombinant \textit{S. cerevisiae} AH22 cells expressing each of human CYPs (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, and 3A4) with the vector pGYR were kindly provided by Sumitomo Chemical Co., Ltd. (Osaka, Japan). The vector pGYR is an episomal \textit{S. cerevisiae}/\textit{E. coli} shuttle vector which contains a 2 \(\mu\)m DNA ori, \textit{Leu2} gene as a marker, \textit{S. cerevisiae} NADPH-P450 reductase gene, pUC ori, \textit{Amp}’, and a glyceraldehydes-3-phosphate dehydrogenase (GAPDH) promoter and terminator derived from \textit{zygosaccharomyces rouxii}. The microsomes prepared from the recombinant \textit{S. cerevisiae} cells expressing human CYPs were purchased from Sumika Chemical Analysis Service (Osaka, Japan). Sesamin mono-catechol and di-catechol were obtained by using rat liver microsomes, and used them as authentic standards. All other chemicals were purchased from standard commercial sources of the highest quality available.

Metabolism of Sesamin by Whole Cell Fraction of Recombinant Yeast Cells.

The recombinant \textit{S. cerevisiae} cells expressing human CYPs were cultivated in synthetic minimal medium containing 2% glucose, 0.67% yeast nitrogen base without amino acids, and 20 mg/l L-histidine. When the cell density (O.D.660) of culture
reached 0.5, 20 mM sesamin in dimethylsulfoxide (DMSO) was added to the culture at a final concentration of 100 \( \mu \text{M} \). At 24 hr after addition of sesamin, the cultures were 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: column, YMC-pack ODS-AM (4.6 \( \times \) 300 mm) (YMC Co., Tokyo, Japan); UV detection, 280 nm; flow rate, 1.0 ml/min; column temperature, 40° C; liner gradients of 10-90% methanol aqueous solution per 30 min, and 90-100% per 5 min.

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

The metabolite of sesamin produced in the recombinant *S. cerevisiae* cells 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 \( \times \) 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 Sesamin Catecholization Using Microsomal Fraction of Recombinant Yeast Cells.**

The reaction mixture (200 \( \mu \text{l} \)) containing 0.5 mg protein/ml of recombinant yeast microsomes containing each of CYP1A2, 2C9, 2C19, and 2D6, 1 mM NADPH, and various concentrations of sesamin (2, 5, 10, 20, 50, and 100 \( \mu \text{M} \)) 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 10 min. The reaction mixture also contains 0.5 % DMSO, since 1 μl of sesamin DMSO solution was added as the substrate.

The 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.

**Metabolism of Sesamin, Phenacetin and Diclofenac in Human Liver Microsomes.**

The reaction mixture (200 μl) containing 0.5 mg protein/ml of each of human single donor liver microsomes, 1 mM NADPH, 5 μM sesamin or sesamin monocatechol or phenacetin or diclofenac in 100 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 15 min. Their metabolites were analyzed by HPLC as described above for sesamin, and according to the method described by Distlerath el al. (1985) for phenacetin. For diclofenac, the same methods used for sesamin were used except for the HPLC conditions; UV detection, 267 nm; liner gradients of 20-95% acetonitrile aqueous solution per 25 min containing 0.05% of trifluoloacetic acid.

**Inhibition of Sesamin Catecholization in Human Liver Microsomes by CYP Isoform-specific Inhibitors**

The reaction mixture (100 μl) containing 0.5 mg protein/ml of pooled human liver microsomes and 0.1 to 5 μM sulfaphenazole (CYP2C9 specific inhibitor) or (+)-N-3-benzyl nirvanol (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 sesamin and 1 mM NADPH were added, and incubation was continued for 15 min. The metabolite was analyzed as described above.
Inhibition of Sesamin Catecholization in Human Liver Microsomes by Polyclonal Antibodies against Human CYPs.

The pooled human liver microsomes (50 μg of protein) and polyclonal antibodies against each of the human CYPs (0-200 μg of protein) were incubated at 4°C for 1 hr, and the reaction mixture containing 1 mM NADPH and 5 or 50 μM sesamin 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 above.

Inhibition of the CYP isoform-specific Activity by Sesamin.

The reaction mixture containing 0.5 mg protein/ml of recombinant yeast microsomes containing each of CYP1A2, 2C9, and 3A4, 1 mM NADPH, and various concentrations of sesamin (0-50 μM) and phenacetin (5-50 μM) for CYP1A2 or diclofenac (5-50 μM) for CYP2C9 or testosterone (5-100 μM) for CYP3A4 in 100 mM potassium phosphate buffer (pH 7.4) was incubated at 37°C for 15 min. Their metabolites were analyzed as described above for sesamin, phenacetin, and diclofenac. For testosterone, the same methods used for sesamin were used except for the HPLC conditions; UV detection, 290 nm; linear gradients of 20-100% acetonitrile aqueous solution per 25 min. The types of inhibition were determined from Lineweaver-Burk plots, and the inhibitor constants (K_i) were calculated from the Dixon plot (Dixon, 1953).

Mechanism-based Inhibition of CYP.

The reaction mixture containing 0.5 mg protein/ml of recombinant yeast microsomes or 50 donor human liver microsome pool, 1mM NADPH and various concentrations of sesamin (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 CYP-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 ($k_{\text{obs}}$) 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 ($k_{\text{inact}}$) and the appeared inhibitor constants ($K_i$) were calculated from the double reciprocal plots of $k_{\text{obs}}$ versus sesamin concentration.

**Measurement of CYP Hemoproteins.**

The contents of CYP hemoprotein in the recombinant yeast cells were measured as described previously (Oeda et al., 1985). Yeast cells (100mL culture) were harvested during logarithmic growth phase, washed twice by suspension in 100mM potassium phosphate buffer pH 7.4, and then suspended in 5mL of the buffer. The concentration of CYP hemoprotein in the whole cell fraction or microsomal fraction was determined from the reduced CO-difference spectrum using a difference of the extinction coefficients at 450 and 490 nm of 91 mM$^{-1}$cm$^{-1}$ (Omura and Sato, 1964).
Results

Identification of the Metabolite of Seasamin.

Figure 1(A) shows the HPLC profiles of sesamin and its metabolite by the recombinant yeast cells expressing CYP2C19. One major metabolite M1 was observed at a retention time of 25.3 min, and eluted with authentic sesamin mono-catechol with co-chromatography (data not shown). The same metabolite (M1) was detected in microsomes of recombinant yeast cells expressing CYP2C19 (Fig. 1 (B)). To confirm the chemical structures of the metabolites, we collected the metabolites in the effluents from HPLC and subjected them to mass spectrometric analysis. Relative intensities (%) of major ion fragments of the authentic standard of sesamin and its metabolite were as follows. Sesamin: m/z 319 (M+H-2H2O), 4 %; m/z 337 (M+H-H2O), 100 %; m/z 355 ((M+H) 55 %. The metabolite M1: m/z 307 (M+H-2H2O), 4 %; m/z 325 (M+H-H2O), 100 %; m/z 343((M+H) 43 %. These results strongly suggest that M1 was mono-catechol metabolite of sesamin (Fig. 1). Other metabolites such as di-catechol metabolite were not detected.

Sesamin Catecholization by Human CYPs.

Table 1 shows the conversion ratio of sesamin catecholization by recombinant yeast cells expressing each of human CYPs. Only mono catechol was detected as a metabolite of sesamin for different CYP isoforms. It is noted that all of the human CYPs except for CYP2E1 showed activity. Specific activity per P450 molecule based on the normalized conversion ratio in Table 1 indicated that CYP1A1, 1A2, 2B6, 2C9, 2C19, and 2D6 had high activity. Of these CYPs, however, CYP1A1 and 2B6 are considered to be minor CYPs whose contents in human liver are lower than other 4 CYPs. Thus, we selected CYP1A2, 2C9, 2C19, and 2D6 to identify essential CYPs that are responsible for
Kinetic Parameters of CYP1A2, 2C9, 2C19 2D6 for Sesamin Catecholization.

Table 2 shows kinetic parameters determined by using microsomes of recombinant yeast cells expressing each of four CYPs, CYP1A2, 2C9, 2C19, and 2D6. The $k_{cat}/K_m$ value was high in order of CYP2C19, 2D6, 2C9, and 1A2, suggesting that CYP2C19 is the most efficient enzyme for mono-catecolization of sesamin among the 11 human CYPs examined in this study. However, the average content of CYP2C19 in human liver is only 1% from the previous reports (Shimada et al. 1994. Inoue et al. 1997) and our Western blots analysis of 50-donor pooled human liver microsomes confirmed their results (data not shown). To predict contribution of each CYP for sesamin metabolism in human body, we consider the value of $(k_{cat}/K_m) \times$ (the relative content (%) of each CYP in human liver). The relative contents are 1% for CYP2C19, 2% for CYP2D6, 13% for CYP1A2, and 20% for 2C9 (Shimada et al., 1994; Inoue et al., 1997). Table 2 demonstrates that the putative contribution ratios in human liver are high in the order of CYP2C9, CYP1A2, CYP2C19, and CYP2D6.

Correlation between Sesamin Catecholization Activity and Each CYP-specific Activity in Human Liver Microsomes.

To confirm the prediction that CYP2C9 is essential for the metabolism of sesamin in human liver, we examined the correlation between sesamin catecholization activity and each CYP-specific activity using human single donor liver microsomes. Figure 2 shows sesamin catecholization activity. Activity range was 2.5-fold in sesamin catecholization (Fig 2), and 6.3-fold in diclofenac 4’-hydroxylation among 10 human liver microsomes, respectively. These data suggest that the inter-individual difference of sesamin...
catecholization is not so large. As shown in Fig 3A, good correlation was observed between sesamin catecholization activity and diclofenac 4’-hydroxylation activity, which is known to be a CYP2C9-specific activity \((r = 0.94)\). On the other hand, phenacetin O-deethylation activity, which is known to be CYP1A2-specific reaction, showed a little correlation \((r = 0.33)\) with sesamin catecholization (Fig 3B). Both S-mephenytoin 4’-hydroxylation activity, and bufurarol 1’-hydroxylation activity, that are known to be a CYP2C19 and CYP2D6-specific reaction, respectively, showed almost no correlations with sesamin catecholization (data not shown). These results clearly demonstrate that CYP2C9 is a major CYP isoform of sesamin catecholization in human liver.

**Inhibition of Sesamin Catecholization in Human Liver Microsomes by Anti-CYP Antibody**

Figure 4 shows the effect of antibodies against human CYPs on sesamin catecholization in the pooled human liver microsomes at different sesamin concentrations, 5 μM (A) or 50 μM (B). At 5 μM of sesamin, catecholization was significantly inhibited by anti-CYP2C9 antibody. In contrast, at 50 μM of sesamin, catecholization was significantly inhibited by not only anti-CYP2C9 antibody but also anti-CYP1A2 antibody, indicating that the contribution of CYP1A2 is larger at 50 μM than that at 5 μM. These results appear to be consistent with the fact that the \(K_m\) value of CYP1A2 is significantly larger than that of CYP2C9 (Table 2). At both 5 and 50 μM, catecholization was slightly inhibited by anti-2C19 antibody, and was not inhibited by anti-2D6 antibody. It should be noted that the anti-CYP2C19 antibody has some cross-reactivity with CYP2C9 judging from Western-blot analysis using the recombinant yeast microsomes (data not shown). Thus, the effect of anti-CYP2C19
antibody in Fig.4 appears to be over-estimated because of its cross-reactivity with CYP2C9.

**Inhibition of Sesamin Catecholization in Human Liver Microsomes by CYP2C9- or CYP2C19-specific Inhibitor**

To confirm that CYP2C9 is the most essential CYP isoform but CYP2C19 is not, we examined the effect of chemical inhibitors for human CYP2C9 (sulfaphenazole) and CYP2C19 ((+)-N-3-benzylnirvanol). The $K_i$ values of sulfaphenazole and (+)-N-3-benzylnirvanol in the human liver microsomes were reported to be 0.12 μM (Miners et al., 1988) and 0.24 μM (Suzuki et al., 2002), respectively. As expected, sulfaphenazole showed a remarkable inhibition of sesamin catecholization activity in pooled human liver microsomes. The $K_i$ value was estimated to be $0.24 \pm 0.03$ μM regarding sulfaphenazole as a competitive inhibitor. In contrast, the activity was hardly reduced even in the presence of 5 μM of (+)-N-3-benzylnirvanol. These results strongly suggest that CYP2C9 is the most essential CYP isoform for sesamin monocatecholization in human liver, while the contribution of CYP2C19 is much lower than CYP2C9.

**Correlation between Sesamin Catecholization Activity and Each CYP-specific Activity in the Presence of Anti-CYP2C9 Antibody.**

To examine a contribution of other CYP isoforms, we further examined the correlation between sesamin catecholization activity and each CYP activity in the presence of anti-CYP2C9. As shown in Fig. 3C sesamin catecholization activity and phenacetin O-deethylation activity, which is known to be CYP1A2-specific reaction, showed a considerable correlation ($r = 0.86$). However, both S-mephenytoin
4’-hydroxylation activity and bufuralol 1’-hydroxylation activities showed little correlations with sesamin catecholization activity (data not shown). These results indicate that CYP1A2 is also involved in sesamin catecholization in human liver, though its contribution is smaller than CYP2C9.

Inhibition of the CYP isoform-specific Activity by Sesamin.

As sesamin is a substrate of most of CYP isoforms examined in this study (Table 1), it seems likely that a competitive inhibition is observed in drug metabolizing activities of those CYP isoforms. The effect of sesamin on CYP3A4 was also examined, because CYP3A4 metabolizes a large number of medicines, and is the most essential CYP isoform in drug metabolism. It is noted that most of severe drug-drug and drug-food factor interactions are related to CYP3A4. Thus, inhibitory effects of sesamin on each of CYP2C9-, 1A2-, and 3A4- dependent activities were examined in this study. As expected, competitive inhibition by sesamin was observed in these CYPs-dependent activities. The apparent $K_i$ values of sesamin were estimated to be 24, 75, and 4.2 μM, respectively. Thus, it was found that sesamin was a potent inhibitor of CYP3A4.

Mechanism-based inhibition of CYPs.

To examine MBI by sesamin, first we used the microsomal fraction of the recombinant yeast expressing each of CYP2C9, CYP1A2, and CYP3A4. Although CYP1A2 and CYP3A4 showed no MBI, CYP2C9 showed time- and concentration-dependent MBI (Fig. 5). The apparent $K_i$ and $k_{inact}$ values of sesamin in CYP2C9-dependent dicrofenac 4’-hydroxylation were estimated to be 1.6 μM and 0.22 min$^{-1}$, respectively. Next, a similar experiment was performed with human liver microsomes using dicrofenac as a substrate to confirm the sesamin-dependent MBI of
CYP2C9 in human liver. The apparent $K_i$ and $k_{inac}$ values were estimated to be 22 μM and 0.13 min$^{-1}$, respectively, for CYP2C9-specific dicrofenac 4'-hydroxylation in human liver microsomes (Table 3).
Discussion

To our best knowledge, this is the first report to identify the human CYP isoforms responsible for sesamin metabolism, although some previous studies have demonstrated that sesamin appears to be catecholized by some kinds of CYPs (Nakai et al., 2003; Penalvo et al., 2005; Liu et al., 2006). First, we used the recombinant yeast expression system, which had been used as a tool for prediction of drug metabolism in human body (Imaoka et al., 1996; Hayashi et al., 2000; Sakaki and Inouye, 2000; Inouye et al., 2002), to identify the human CYP isoforms responsible for sesamin metabolism. Surprisingly, most of human CYPs examined in this study showed sesamin catecholization (Table 1). In particular, four kinds of CYPs (CYP1A2, 2C9, 2C19, and 2D6) had high ability to catecholize sesamin, compared with other CYPs. The kinetic studies suggested that CYP2C19 had the highest turnover for sesamin catecholization among human CYPs (Table 2). However, based on their average contents in human liver, we predicted that the contribution in human liver is large in order of CYP2C9, 1A2, 2C19, and 2D6.

To examine our assumption, we performed experiments using individual human single donor liver microsomes (Figs.2 and 3) and a 50 donor human liver microsome pool (Fig.4). The experimental data strongly suggested that CYP2C9 was largely responsible for sesamin catecholization in human liver, whereas CYP1A2 also made a small contribution. A significant inhibitory effect of the CYP2C9-specific inhibitor, sulfaphenazole, on sesamin catecholization in the pooled human liver microsomes confirmed that CYP2C9 was the most essential CYP isoform. However, the fact that inter-individual difference in sesamin catecholization activity (activity range: 2.5-fold) was smaller than that of diclofenac 4’-hydroxylation activity (activity range: 6.3-fold) might be related with the contribution of CYP1A2. Contribution of CYP2C19 for sesamin catecholization in human liver appears to be small, though CYP2C19 has the
largest turnover (Table 1). No significant inhibitory effect of the CYP2C19-specific inhibitor, (+)-N-3-benzylnirvanol, on sesamin catecholization in the pooled human liver microsomes confirmed that the contribution CYP2C19 is small. The contribution of CYP2D6 is also quite small, although some kind of methylenediphenyl compounds such as methylenedioxyamphetamine (MDMA) is metabolized to catechol metabolites mainly by CYP2D6 (Tucker et al., 1994; Lin et al., 1997; Kreth et al., 2000). Both of CYP2C19 and CYP2D6 are known to have genetic polymorphisms. The frequency of CYP2C19 poor metabolizer (PM) in Japan and Korea is approximately 20 % and that of CYP2D6 PM in Caucasian is approximately 7~10 %. Thus, our conclusion that CYP2C19 and CYP2D6 are not essential for sesamin catecholization may predict a small inter-individual difference in sesamin metabolism.

One of the most important findings in this study is that sesamin is a mechanism-based inhibitor of CYP2C9. Figure 6 shows a putative reaction mechanism of sesamin catecholization proposed by Murray (2000). A part of the metabolite (I) produced by CYP2C9 could be converted spontaneously to form a reactive carbene compound (II) which probably covalently binds to a heme iron of CYP2C9. As MBI inactivates the enzyme irreversibly, it is a remarkably severe inhibition as compared with a reversible competitive inhibition. The $K_i$ and $k_{inact}$ values of sesamin were estimated to be 22 μM, and 0.13 min$^{-1}$ respectively, in human liver microsomes. Usia et al. (2005) demonstrated that some lignans which contain MDP groups inhibit CYP3A4 irreversibly ($K_i$, 0.082~0.37 μM; $k_{inact}$, 0.23~0.32 min$^{-1}$). Bergamottin in grapefruit juice is a well-known mechanism-based inhibitor of CYP3A4 with the $K_i$ value of 4.2 μM and the $k_{inact}$ value of 0.30 min$^{-1}$ (Zhou et al., 2004). Judging from $k_{inact}/K_i$ value, sesamin is a weak inhibitor as compared with these inhibitors (Table 3). However, CYP2C9 is a major CYP isoform to metabolize therapeutic drugs such as diclofenac, phenytoin, and
S-warfarin. These results suggest that a drug-sesamin interaction should be evaluated on these therapeutic drugs. On the other hand, sesamin did not irreversibly inhibit CYP1A2 which is the secondary most important CYP isoform for sesamin catecholization. Conversion from (I) to (II) or (III) is a spontaneous reaction (Fig. 6). Thus, it is possible that the reactive metabolite (II) is also produced in the substrate-binding pocket of CYP1A2, but the metabolite (II) may not rapidly attack a heme iron of CYP1A2. Otherwise, MI-complex with CYP1A2 might be much less stable than that with CYP2C9 on the basis of different stabilities of the MI-CYP complexes formed [Murray 2000]. The difference between CYP2C9 and CYP1A2 appears to be originated from the difference of the structures and microenvironment of their substrate-binding pockets.

Sesamin mono-catechol is known as a compound that has a potent antioxidative activity different from sesamin itself (Nakai et al., 2003). In addition, recent studies suggested that sesamin mono-catechol has the ability to induce enhancement of endothelium-dependent vasorelaxation (Nakano et al., 2006) and neuronal differentiation (Hamada et al., 2009). Thus, conversion from sesamin to its mono-catechol is considered to be a useful reaction to produce antioxidant or other bioactive compound in human body. Previous studies demonstrated that sesamin is metabolized to sesamin di-catechol via sesamin mono-catechol in rats (Nakai et al., 2003; Penalvo et al., 2005; Liu et al., 2006). It is noted that sesamin di-catechol is a more potent antioxidant than sesamin mono-catechol (Nakai et al., 2003; Miyake et al., 2005). Thus, the conversion from mono-catechol to di-catechol is pharmacologically important. We examined the conversion of mono-catechol into di-catechol by adding sesamin mono-catechol to human liver microsomes as a substrate. However, only a small amount of di-catechol was detected (data not shown). These results demonstrate that sesamin mono-catechol is not a good substrate of CYPs as compared with sesamin.
itself.

In this study, we examined the metabolism of sesamin and revealed CYP isoforms involved in sesamin metabolism by combination of the yeast expression system for human CYPs and human liver microsomes. In particular, whole cell system of the recombinant yeast cells is useful for screening of CYP isoforms involved in the metabolism, and production of the metabolite to identify its chemical structure. We found that CYP2C9 was the most important CYP isoform in sesamin catecholization in human liver, but also found that sesamin was a mechanism-based inhibitor of CYP2C9. At the present time, it is difficult to regard the MBI of CYP2C9 by sesamin as a serious problem, because no severe drug-sesamin interaction has been reported to our best knowledge. Pelnavo et al. (2005) reported that the peak plasma concentration of sesamin was only 0.1 μM after a single serving of sesame seeds containing 170mg of sesamin. Thus, much lower concentration of sesamin in vivo than the apparent inhibitor constant (K_{i,app}) shown in Table 3 might lead us to the conclusion that MBI of CYP2C9 by sesamin could be negligible. However, our findings suggest that in vivo studies would be required to reveal the interaction between sesamin and therapeutic drugs metabolized by CYP2C9.
References


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


Tsuruoka N, Kidokoro A, Matsumoto I, Abe K and Kiso Y (2005) Modulating effect of...
sesamin, a functional lignan in sesame seeds, on the transcription levels of lipid- and alcohol-metabolizing enzymes in rat liver: a DNA microarray study. *Biosci Biotechnol Biochem* **69**:179-188.


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Legends to Figures

Fig. 1  HPLC profiles of sesamin and its metabolite in the whole cell culture of the recombinant yeast cells expressing CYP2C19 at 24hr after addition of sesamin (A), and those in microsomes (B). Chemical structures of sesamin and its metabolite are shown.

Fig. 2  Sesamin catecholization activity in the each of human single donor liver microsomes. Each point represents the mean of at least duplicate determination.

Fig. 3  Correlation between sesamin catecholization activity and CYP2C9-specific activity (diclofenac 4'-hydroxylation activity) (A), and correlation between sesamin catecholization and CYP1A2-specific activity (phenacetin O-deethylation) in the absence (B) and presence (C) of anti-CYP2C9 antibody.

Fig. 4  Inhibition of sesamin catecholization of pooled human liver microsomes by each CYP-specific antibody (CYP1A2 (○), CYP2C9 (■), CYP2C19 (▲), CYP2D6 (×)) at different sesamin concentrations; 5 μM (A), 50 μM (B). Each point represents the mean of triplicate determination.

Fig. 5  Time- and concentration-dependent inactivation of CYP2C9-specific activity in recombinant CYP2C9 (A). Sesamin 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 sesamin concentrations (B).

Fig. 6  Putative mechanism of sesamin oxidation. This mechanism is based on the
carbene formation proposed by Murray (2000). The intermediate carbene (II) could generate an MI-complex with CYP.
Table 1  Sesamin catecholization by the recombinant *S. cerevisiae* cells expressing each of human CYP isozymes.

<table>
<thead>
<tr>
<th>CYP</th>
<th>Conversion ratio ( % )</th>
<th>P450 conc. (nM)</th>
<th>Normalized conversion ratio (%)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A1</td>
<td>0.040</td>
<td>2.6</td>
<td>0.015</td>
</tr>
<tr>
<td>1A2</td>
<td>0.56</td>
<td>10</td>
<td>0.054</td>
</tr>
<tr>
<td>2A6</td>
<td>0.53</td>
<td>64</td>
<td>0.008</td>
</tr>
<tr>
<td>2B6</td>
<td>0.30</td>
<td>17</td>
<td>0.017</td>
</tr>
<tr>
<td>2C8</td>
<td>0.068</td>
<td>99</td>
<td>0.001</td>
</tr>
<tr>
<td>2C9</td>
<td>3.4</td>
<td>104</td>
<td>0.033</td>
</tr>
<tr>
<td>2C18</td>
<td>0.56</td>
<td>170</td>
<td>0.003</td>
</tr>
<tr>
<td>2C19</td>
<td>5.9</td>
<td>88</td>
<td>0.067</td>
</tr>
<tr>
<td>2D6</td>
<td>1.1</td>
<td>35</td>
<td>0.031</td>
</tr>
<tr>
<td>2E1</td>
<td>0.00</td>
<td>88</td>
<td>0.000</td>
</tr>
<tr>
<td>3A4</td>
<td>0.16</td>
<td>51</td>
<td>0.003</td>
</tr>
</tbody>
</table>

^a This value is the conversion ratio per nM of P450.
Table 2. Kinetic parameters of human CYPs in the recombinant yeast microsomes for sesamin catecholization.\(^{a}\)

<table>
<thead>
<tr>
<th></th>
<th>(k_{\text{cat}}) ((\text{min}^{-1}))</th>
<th>(K_m) ((\mu\text{M}))</th>
<th>(k_{\text{cat}}/K_m)</th>
<th>Contribution ratio(^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>3.6 ± 1.5</td>
<td>15 ± 2.1</td>
<td>0.24 ± 0.14</td>
<td>38</td>
</tr>
<tr>
<td>2C9</td>
<td>2.2 ± 0.11</td>
<td>5.4 ± 0.92</td>
<td>0.41 ± 0.045</td>
<td>100</td>
</tr>
<tr>
<td>2C19</td>
<td>5.0 ± 1.8</td>
<td>2.3 ± 0.9</td>
<td>2.2 ± 0.083</td>
<td>27</td>
</tr>
<tr>
<td>2D6</td>
<td>2.8 ± 0.48</td>
<td>5.8 ± 0.68</td>
<td>0.48 ± 0.14</td>
<td>12</td>
</tr>
</tbody>
</table>

\(^{a}\) \(K_m\) and \(k_{\text{cat}}\) values represent mean ± SD from three separate experiments.

\(^{b}\) Putative contributing ratio is the value of \((k_{\text{cat}}/K_m)\times(\text{The relative content (\%) of each CYP isoform in human liver})\). The average contents of each CYP isoform in human liver are 1A2:13\%, 2C9:20\%, 2C19:1\%, 2D6:2\%, respectively (Shimada et al., 1994; Inoue et al., 1997)
Table 3  Mechanism-based inhibition of CYPs by dietary compounds in human liver microsomes.$^a$

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>CYP</th>
<th>$k_{\text{inact}}$ (min$^{-1}$)</th>
<th>$K_{\text{app}}$ (μM)</th>
<th>$k_{\text{inact}}/K_{\text{app}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sesamin</td>
<td>2C9</td>
<td>0.13± 0.01</td>
<td>22.0± 2.8</td>
<td>0.0059± 0.0004</td>
</tr>
<tr>
<td>Lignans$^a$</td>
<td>3A4</td>
<td>0.23 - 0.32</td>
<td>0.082 - 0.37</td>
<td>0.084 - 5.7</td>
</tr>
<tr>
<td>Bergamottin$^b$</td>
<td>3A4</td>
<td>0.30</td>
<td>4.2</td>
<td>0.071</td>
</tr>
</tbody>
</table>

$K_{\text{app}}$ and $k_{\text{inact}}$ value represent mean ± SD from three separate experiments.

$^a$ Usia et al., 2005

$^b$ Zhou et al., 2004
Fig. 1
Fig. 3

(A) Correlation between CYP2C9 activity and sesamein catecholization activity. 

(B) Correlation between CYP1A2 activity and sesamein catecholization activity.

(C) Effect of anti-CYP2C9 Ab on the correlation between CYP1A2 activity and sesamein catecholization activity.
Fig. 4
Fig. 5
Fig. 6

The diagram illustrates the metabolism of Sesamin by CYPs, leading to the formation of MI-complex. The reaction involves the initial conversion of Sesamin to (I) and then to (II) by losing water. Further hydration of (II) leads to (III), which can then be hydrated again to Sesamin mono-catechol.