Metabolism of Sesamin by CYPs and UGTs in Human Liver

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ABSTRACT

Sesamin is a major lignan in sesame, and its biological effects such as antioxidant effect, anti-carcinogenic effects, and suppression of hypertension have been extensively studied by many researchers. However, its metabolic pathways and metabolic enzymes in human bodies have not been identified. Recently we demonstrated that CYP2C9 was the most important cytochrome P450 isoform in human liver. Next, we focused on metabolism of sesamin mono-catechol by cytochrome P450 or UDP-glucuronosyltransferase (UGT). Further catecholization of sesamin mono-catechol by cytochrome P450 enhances its anti-oxidant activity, whereas glucuronidation by UGT strongly reduces anti-oxidant activity. In human liver microsomes, glucuronidation activity toward sesamin mono-catechol was much higher than the di-catecholization activity. In contrast, both activities were similar in rat liver microsomes. These results suggest a large species-based difference between humans and rats in sesamin metabolism. *In vitro* studies using 10 individual human liver microsomes suggested that UGT2B7 was responsible for glucuronidation of sesamin mono-catechol in human liver. In addition, we observed a significant methylation activity toward sesamin mono-catechol by catechol O-methyl transferase (COMT) in human liver cytosol. Based on these results, we concluded that CYP2C9, UGT2B7, and COMT played essential roles in the metabolism of sesamin in human liver.

Key words: sesamin, metabolism, cytochrome P450, UDP-glucuronosyltransferase, catechol O-methyl transferase

INTRODUCTION

Sesamin is a major lignan in sesame, and its biological effects such as antioxidant, cholesterol- and lipid-lowering, anticarcinogenic effects, and suppression of hypertension have been extensively studied by many researchers[(1-7)]. Among them, antioxidant effects appear to be attributed to its metabolites, because sesamin itself has little antioxidative properties.

Sesamin was converted to its mono- and dicatechol forms by P450s in rat liver to show high antioxidative activity[(1)]. In addition, recent studies suggested that sesamin monocatechol had the ability to induce enhancement of endothelium-dependent vasorelaxation[(8)] and neuronal differentiation[(9)]. 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. On the other hand, very little is known about the plasma concentrations of sesamin mono- and dicatechols in humans. Recently, Moazzami *et al*.[(10)] suggested the presence of glucuronide of sesamin monocatechol in human urine, but other metabolites such as dicatechol were not detected. Our recent studies demonstrated the conversion of sesamin to its monocatechol by P450s in human liver microsomes[(11)]. However, the dicatechol form was not detected. Our results are consistent with those of Moazzami *et al*.[(10)] and suggest a species-based difference in sesamin metabolism by P450 between humans and rats. To understand the biological significance of the sesamin metabolites, it is essential to reveal what controls the ratio of conversion of sesamin to its mono- or dicatechol or glucuronide forms. In addition, from the viewpoint of drug-sesamin interaction, it is important to identify the P450 and UGT isoforms that are responsible for sesamin metabolism. Our previous study clearly 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 inhibiton (MBI) of CYP2C9 by sesamin[(11)] as well as other MDP compounds.

To reveal overall metabolic pathways of sesamin in humans, we focused on the metabolism of sesamin that occurs after its monocatecholization. First, we identified the UGT isoform responsible for sesamin glucuronidation, and then we examined methylation of sesamin monocatechol by catechol O-methyl transferase (COMT). Finally we describe a species-based difference between humans and rats in sesamin metabolism[(12)].

MATERIALS AND METHODS

I. *Apparatus and Conditions*

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HPLC conditions are described below: 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, 40ºC; linear gradients of 10 - 90% methanol aqueous solution containing 0.05% trifluoroacetic acid (TFA) in 30 min, and 90 - 100% containing 0.05% TFA in 5 min for metabolites of sesamin and sesamin monocatechol. 3'-Azido-3'- deoxythymidine (AZT) metabolites by UGT were analyzed under the following conditions: column, YMC-pack ODS-AM (4.6 × 300 mm) (YMC Co., Tokyo, Japan); UV detection, 267 nm; flow late, 1.0 mL/min; column temperature, 40ºC; linear gradients of 10 - 65% methanol aqueous solution containing 0.05% trifluoroacetic acid (TFA) in 20 min.

II. *Materials and Reagents*

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 S-adenosyl methionine (SAM) were purchased from Sigma-Aldrich (St. Louis, MO). 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). All other chemicals were purchased from standard commercial sources of the highest quality available.

III. *Measurement of CYP, UGT, and COMT Activities*

In the dicatechol formation by P450, the reaction mixture containing 0.5 mg protein/mL of the liver microsomes, 1 - 50 μM sesamin monocatechol, 1 mM NADPH in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 10 - 30 min at 37ºC and the metabolite was analyzed by HPLC.

In UGT-dependent glucuronidation of sesamin monocatechol, the reaction mixture containing 0.5 mg protein/ml of the liver microsomes, 1 - 50 μM sesamin monocatechol, 2 mM UDP-GlcUA, 1mM $MgCl_2$ in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 15 - 30 min at 37ºC, and the metabolite was analyzed by HPLC. The aliquot of the reaction mixture was further incubated for 60 min at 37ºC in the presence of 0.1 mg/mL β-glucuronidase in 20 mM potassium phosphate buffer (pH 7.4) to confirm that the metabolite was the glucuronide.

In COMT-dependent methylation, the reaction mixture containing 0.5 mg protein/mL of the liver cytosol, 1 - 50 μM sesamin monocatechol, 200 μM SAM, 2 mM $MgCl_2$, 1 mM dithiothreitol, in 100 mM potassium phosphate buffer (pH 7.4) was incubated for 5 - 20 min at 37ºC, and then the metabolites were analyzed by HPLC.

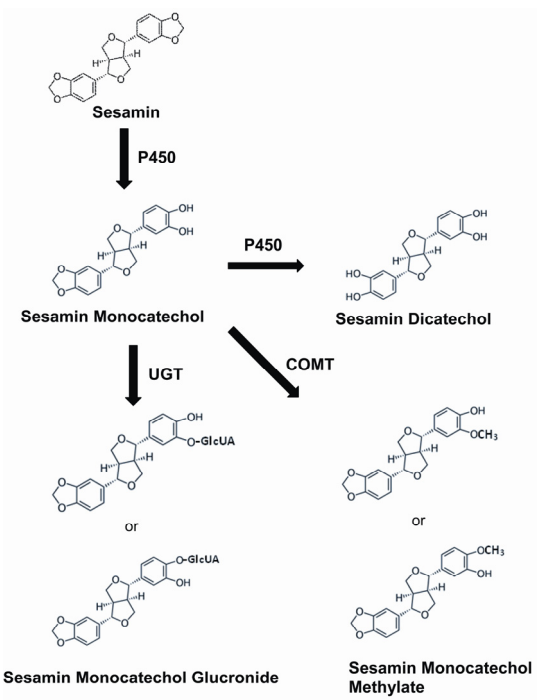
The kinetic studies for P450-dependent oxidation, UGT-dependent glucuronidation, and COMT-dependent methylation were performed using each of the microsomal and cytosolic fractions. The kinetic parameters, K_m and V_{max}, were calculated by the nonlinear regression analysis using KaleidaGraph (Synergy Software, Reading, PA). The equation was applied for Michaelis-Menten kinetics.

RESULTS AND DISCUSSION

I. *Metabolism of Sesamin in Hhuman Liver*

We demonstrated the successive metabolism of sesamin in human liver microsomes as shown in Figure 1 [12]. Sesamin was first metabolized to monocatechol metabolite in human liver microsomes in the presence of NADPH. When the monocatechol was used as a substrate, dicatechol (SC-2) was detected in the presence of NADPH. On the other hand, a glucuronide of SC-1 (SC-1-GlcUA) was detected in the presence of UDP-glucuronic acid in human liver microsomes, and two methylated metabolites of SC-1 (SC-1m) were detected in the presence of SAM in human liver cytosol[12]. Only one glucuronide was detected in human liver microsomes, while both glucuronide isomers were detected in rat liver microsomes.

Figure 1. Metabolic pathways of sesamin in human liver.



II. *Identification of CYP and UGT Isoforms Responsible for Sesamin Metabolism*

From the viewpoint of the safety taking of sesamin with the therapeutic drugs, it is important to identify the

P450 and UGT isoforms which are responsible for sesamin metabolism.

Our previous studies revealed that the oxidation reaction of sesamin was catalyzed by many of human drug-metabolizing P450s (CYP1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, and 3A4). Of these P450s, CYP2C9 was the enzyme responsible for metabolism of sesamin in human liver microsomes, and the secondary most important P450 was CYP1A2, whereas contribution of CYP1A2 was small[11]. Furthermore, we examined the metabolism of sesamin monocatechol (SC-1) by recombinant human UGTs expressed in baculovirus-infected insect cells. Among the 12 UGT isoforms (UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B15, and 2B17), UGT2B7 and UGT2B17 showed a glucuronidation activity towards SC-1. The V_{max}/K_m of UGT2B7 was approximately 3 times higher than that of UGT2B17. In addition, the expression level of UGT2B7 is significantly higher than UGT2B17 in human liver. These results suggest that UGT2B7 is the most important UGT isoform in the sesamin metabolism in human liver.

It is noted that inter-individual difference among 10 human liver microsomes is approximately 2-fold in both oxidation of sesamin by P450 and glucuronidation of SC-1 by UGT. These findings suggest a small inter-individual difference in sesamin metabolism.

III. *Mechanism-Based Inhibition of CYP2C9 by Sesamin*

Because sesamin is the substrate for most of the drug-metabolizing P450 isoforms as described above, any apparent competitive inhibition would be observed in the activities of those P450 isoforms. We examined the inhibitory effect of sesamin on CYP2C9-, 1A2-, and 3A4-dependent activities. Competitive inhibition by sesamin was observed in all three isoforms. The apparent K_i values of sesamin on CYP2C9, 1A2, and 3A4–dependent activities were 24, 75, and 4.2 μM, respectively. Thus, sesamin was found to be a potent inhibitor of CYP3A4.

From the viewpoint of the interaction with therapeutic drugs, we have to be more careful about the irreversible inhibition, which is called mechanism-based-inhibition (MBI), because the inhibitory effects are continued even after disappearance of inhibitory substance. Some reports have been published about MBI of P450s by methylenedioxyphenyl (MDP) compounds. There is a high possibility of the MBI of P450s by sesamin, because sesamin has two MDP groups. We examined the sesamin-dependent MBI of P450s, and demonstrated the MBI of CYP2C9 with the kinetic parameters k_{inact}; 0.13 /min, $K_{i\ app}$; 22 μM, although sesamin showed no MBI of CYP1A2. The $k_{inact}/K_{i\ app}$ value of sesamin (0.0059 /min/μM) was much lower than those of other methylenedioxyphenyl lignans[13]. However, this value is not so different from those of the well-known MBI inhibitors of CYP3A4 in grapefruit components such as bergamottin (0.002 - 0.071 /min/μM)[14,15].

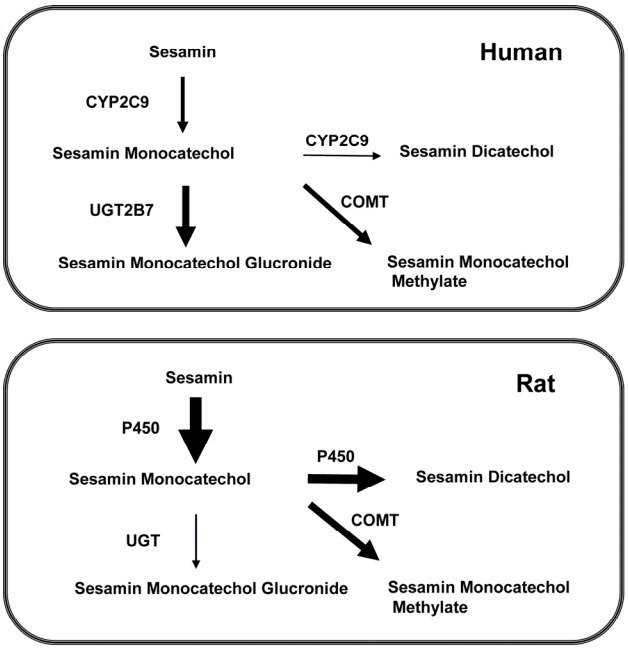


Figure 2. Comparison of metabolism of sesamin between human liver and rat liver. Thickness of arrows roughly represents the strength of the activity.

IV. *Spiecies-Based Differences in the Metabolism of Sesamin Between Humans and Rats*

Recently, we determined the kinetic parameters of the first and second reactions of sesamin metabolism using microsomal fraction and cytosolic fraction prepared from human liver or rat liver, and demonstrated remarkable species-based differences between humans and rats[12]. The V_{max}/K_m values of P450-dependent catecholization and UGT-dependent glucuronidation activities were quite different between humans and rats, whereas COMT-dependent methylation activity was at a similar level as shown in Figure 2. In rats, catecholisation by P450 was predominant over glucuronidation by UGT. These results appear to be consistent with the findings that monocatechol (SC-1) and dicatechol (SC-2) are the major metabolites in *in vivo* study using rats[8]. On the other hand, glucuronidation by UGT was predominant over catecholization by P450 in humans. Therefore, in the human body, catechol metabolites (SC-1 and SC-2) would exist at a low concentration, while the glucuronidation metabolite (SC-1-GlcUA) would be the major metabolite. Because glucuronide (SC-1-GlcUA) appears to have no bioactivity, it is possible that the biological activities of the catechol metabolites might not be expected as much in humans. However, Kawai *et al.*[16] proposed a novel mechanism for the function of quercetin in humans. Quercetin is mainly converted to its glucuronide form at the 3-position (Q3GA) by UGTs in the liver. Q3GA in the plasma could be taken up by macrophage cells probably by some transporters in the plasma membrane, and converted into its active aglycone form by β-glucuronidase. This finding could explain why

quercetin is effective to atherosclerosis in spite of the absence of quercetin aglycone in the serum. A similar mechanism might be applied to sesamin, because β-glucuronidase is expressed in most mammalian tissues.

CONCLUSIONS

We examined metabolism of sesamin by human drug-metabolizing enzymes, and revealed that CYP2C9, UGT2B7, and COMT were the most important enzymes in the metabolism of sesamin in the human liver. In addition, we compared sesamin metabolism between humans and rats and revealed the significant species-based difference. Inhibition studies clearly demonstrated the MBI of CYP2C9 by sesamin. Although we cannot conclude how serious the MBI is by sesamin at the present time, *in vivo* studies should be required to evaluate the interaction between sesamin and the therapeutic drugs metabolized by CYP2C9 such as diclophenac, phenytoin, indomethacin, and *S*-warfarin.

ACKNOWLEDGMENTS

This work was supported in part by Ministry of Education, Culture, Sports, Science and Technology grant.

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