Differential Mechanisms for the Inhibition of Human Cytochrome P450 1A2 by Apigenin and Genistein

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RUNNING TITLE: INHIBITION OF CYP1A2 BY FLABONOIDS

KEYWORDS: CYP1A2; apigenin; genistein; inhibitory potency; docking simulation

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ABSTRACT: The inhibitory effects of flavonoids on the human cytochrome P450 1A2 (CYP1A2) were examined. Among flavonoids tested, galangin, kaempferol, chrysin and apigenin were potent inhibitors. Although apigenin belonging to flavones and genistein belonging to isoflavones are similar in the chemical structures, the inhibitory potencies for CYP1A2 were distinguished markedly between these two flavonoids. In computer docking simulation, apigenin interacted with the same mode of co-crystallized α-naphthoflavone in the active site of CYP1A2, and then the B ring of apigenin was placed close to the heme iron of the enzyme with a single orientation. In contrast, the docked genistein conformation showed two different binding modes, and the A ring of genistein was oriented to the heme iron of CYP1A2. Furthermore, the binding free energy of apigenin was lower than that of genistein. These results demonstrate a possible mechanism that causes the differential inhibitory potencies of apigenin and genistein for CYP1A2.

INTRODUCTION

Human cytochrome P450 (CYP) enzymes are a family of proteins involved in the detoxification of xenobiotic compounds and the biosynthesis of endogenous compounds [1]. Among CYP enzymes, CYP1A2 contributes to the hepatic metabolism of various drugs including theophylline and flutamide [2]. This CYP isozyme also activates heterocyclic amines and polycyclic aromatic hydrocarbons to carcinogens [3, 4]. Thus, it is of interest to elucidate the mechanism of CYP1A2 inhibition by clinically used drugs and dietary components.

Flavonoids are a group of compounds found widely in vegetables, fruits, soy products and plant-derived beverages such as wine and tea. Their basic chemical structure consists of a benzene ring (A ring) that is linked by a heterocyclic pyrane or pyrone ring (C ring) and a phenyl ring (B ring), and these compounds are classified into several subclasses including flavones, flavonols, flavanones, flavanonols and isoflavones. It has been reported that flavonoids have the ability to act as potent inhibitors for a variety of CYP enzymes. In particular, the ability of flavonoids to inhibit CYP1A2 has been extensively examined [5-7]. For instance, flavones such as chrysin and apigenin are potent inhibitors of CYP1A2 [5, 6]. However, the detailed mechanisms for the inhibition of CYP1A2 by flavonoids remain to be clarified.

In the present study, the inhibitory effects of 16 flavonoids on CYP1A2 were examined. Among flavonoids tested, the A ring and the C ring of apigenin are the same as those of genistein, as shown in Figure 1. In addition, apigenin and genistein have the same B ring with a hydroxyl group at the 4'-position, even though it is substituted at the 2- and 3-position. Interestingly, the inhibitory potency of genistein

belonging to isoflavones for CYP1A2 was found to be much lower than that of apigenin belonging to flavones. It should be noted that although these two flavonoids are very similar in the chemical structures, the inhibitory potencies for CYP1A2 are distinguished markedly between apigenin and genistein. We therefore attempted to elucidate mechanisms for the inhibition of CYP1A2 by apigenin and genistein, based on docking studies using the crystal structure of CYP1A2 complexed with α-naphthoflavone (ANF) [8], which has been used to examine the mechanism of action on CYP enzymes [9].

MATERIALS AND METHODS

Chemicals

Recombinant human CYP1A2 expressed in *E. coli* was purchased from Cypex Ltd. (Dundee, UK). Flavonoids were obtained from the following sources: kaempferol, morin, taxifolin (racemate), daidzein and genistein (Sigma, St. Louis, Mo, USA); galangin, chrysin, apigenin, naringenin and (-)-epicatechin (Aldrich, Milwaukee, WI, USA); quercetin (Wako Pure Chemicals, Tokyo, Japan); fisetin and quercitrin (Tokyo Kasei, Tokyo, Japan). Rutin, genistin and (+)-catechin were donated by Dr. J. Kinjo (Faculty of Pharmaceutical Sciences, Fukuoka University, Fukuoka, Japan). All other chemicals were of reagent grade.

Enzyme Assays

CYP1A2 activity was determined using a commercially available kit, P450-Glo Assay (Promega, Madison, WI, USA). The reaction mixture consisted of 0.1 mM

luciferin-6'-methyl ether (substrate), NADPH-generating system (1.3 mM NADP, 3.3 mM glucose-6-phosphate, 20 mU glucose-6-phosphate dehydrogenase and 3.3 mM $MgCl_2$), 0.5 pmol CYP1A2 and 100 mM potassium phosphate buffer (pH 7.4) in final volume of 50 μl. The reaction mixture was incubated at 37 ˚C for 20 min and then 50 μl of luciferin detection reagent was added to stop the reaction. After 20 min incubation at room temperature, luminescence was determined using a luminometer, Mini Lumat LB9506 (Berthold Technologies, Germany).

Inhibition by flavonoids

Flavonoids were dissolved in methanol and then added to the reaction mixture. The final concentration of methanol did not exceed 2 % (v/v), and this concentration did not affect the enzyme reaction. The final concentration of each flavonoid was 5 μM

Docking studies

Automated docking was performed on PowerMac G5 computer using AutoDock 4.0 program [10]. The equilibrium structures of inhibitors apigenin and genistein for the docking studies were constructed by the B3LYP/6-31G* level optimization using Gaussian03 program [11]. In docking, all rotatable dihedrals in the inhibitors were allowed to rotate freely. The 1.95 Å resolution crystal structure of CYP1A2 complexed with inhibitor ANF (PDB code: 2hi4) was used as the model for the macromolecule in the docking studies. The water molecules except for Wat733 and ANF were removed from the coordinates. The model was prepared using UCSF Chimera package [12]. The polar hydrogens were added the protein, and Kollman united-atom partial charge were assigned using AutoDockTools. The charge of Fe

atom was corrected into +3.0 manually. The grid maps were constructed using AutoGrid program. The dimensions of the grid were 50 Å x 50 Å x 50 Å grid points separated by 0.375 Å. The grid center was positioned at coordinates 2.487, 17.834 and 20.155. The hybrid genetic algorithm with local search (GALS) was used to search the docking possibilities. For each initial structure of the inhibitors, 200 GALS docking runs were executed. After docking, the resulting structures for each run were clustered using an RMSD (root-mean-square deviation) cutoff of 2.0 Å. The clusters were ranked according to the binding energies of their representative structure within each cluster.

RESULTS

Inhibition of CYP1A2 by flavonoids

The inhibitory effects of 16 flavonoids at a concentration of 5 μM on CYP1A2 were examined (Figure 2). Among flavonoids tested, galangin, kaempferol, chrysin and apigenin were the potent inhibitor of the enzyme. Other flavonoids including isoflavones exhibited lower inhibitory potencies. Apigenin belonging to flavones and genistein belonging to isoflavones are very similar in the chemical structure (see Figure 1). However, the inhibitory potency of genistein for CYP1A2 was markedly lower than that of apigenin.

AutoDock simulation of apigenin and genistein for CYP1A2

To elucidate the mechanisms for the inhibition of CYP1A2 by apigenin and genistein, docking studies by AutoDock were conducted. In the docking simulations

of apigenin and genistein for CYP1A2, a water molecule (Wat733) was also included because it plays a significant role in the construction of their binding modes. The AutoDock simulation of apigenin for CYP1A2 produced one solution cluster having the binding free energy of -9.04 kcal mol^{-1}. As shown in Figure 3, apigenin interacted with the same mode of the co-crystallized ANF in the active site of CYP1A2, and the B ring was placed close to the heme iron with a single orientation. The distance between 4'-hydroxy oxygen and heme iron was 4.20 Å. The hydrogen bond was formed between the carbonyl oxygen of apigenin and Wat733. A similar binding mode was observed for chrysin having the binding free energy of -8.91 kcal mol^{-1}, which is one of the most potent inhibitors of CYP1A2 (Figure 3).

The AutoDock simulation of genistein for CYP1A2 produced two solution clusters (43% in type 1 and 57% in type 2). These two solutions had the binding free energies of -8.50 kcal mol^{-1} in type 1 and -8.52 kcal mol^{-1} in type 2. In the both binding modes, the A ring of genistein was oriented to the heme iron of CYP1A2 (Figure 4). The two binding modes were also observed for daidzein having the binding free energies of -8.05 kcal mol^{-1} in type 1 and -7.96 kcal mol^{-1} in type 2 (data not shown).

DISCUSSION

Many studies have shown potent inhibition of CYP isozymes by dietary flavonoids [6, 13-15]. Among flavonoids examined in this study, galangin, kaempferol, chrysin and apigenin were confirmed to be potent inhibitors of CYP1A2, as reported previously [5, 6]. It is well-known that the inhibitory effect of flavonoids on CYP isozymes is governed by the number of hydroxyl group [5]. In the present study, a similar result

was observed for the inhibitory effects of flavonoids on CYP1A2. For instance, the inhibition of CYP1A2 by flavonols (galangin, kaempferol and quercetin) decreased with an increase in the number of hydroxyl group. However, the present study has also found that the inhibitory potency of apigenin for CYP1A2 is markedly higher than that of genistein, even though apigenin and genistein have the A and B ring with the same number of hydroxyl group at the same position (see Figure 1). Thus, the differential inhibitory potencies of apigenin and genistein for CYP1A2 can not be explained due to only the number of hydroxyl group. In an attempt to elucidate this mechanism, docking studies by AutoDock were further conducted.

In the X-ray crystal structure of CYP1A2-ANF complex [8], the substrate was bound in the hydrophobic cavity located above the distal surface of the heme prosthetic group. As the result, the π facial interaction between the aromatic ring of ANF and the side chain of Phe226 contributed to a tight binding affinity. The hydrogen-bond network of the carbonyl group of ANF to the carbonyl oxygen of Gly316 *via* Wat733 also provided an additional attractive interaction.

In the CYP1A2-apigenin complex by docking simulation, the B ring was closely associated with the heme iron of CYP1A2 with a single orientation and the face-to-face interaction between the A ring and the phenyl ring of Phe226 exhibited the contribution to the binding affinity. Furthermore, the stabilization by the hydrogen bond between the carbonyl group of apigenin and Wat733 was retained. These observations demonstrate that apigenin potently inhibits CYP1A2 in a binding mode similar to ANF, which is a potent inhibitor of CYP1A2 [16]. Recently, apigenin has been reported to inhibit CYP1A2 in a competitive manner [17], and to be hydroxylated to luteolin at the 3'-position of the B ring by CYP1A2 [18]. In addition to the docked apigenin

conformation, these experimental data suggest that apigenin potently inhibits CYP1A2 by acting as a substrate inhibitor.

On the other hand, the docked genistein conformation showed two different binding modes of type 1 and 2. In the two binding modes of genistein, the A ring was oriented to the heme iron of CYP1A2. This may be supported from the fact that in the case of genistein, the A ring is also hydroxylated at its 6- or 8-position by CYP1A2 [19, 20]. Furthermore, the hydrogen-bond network *via* Wat733 was not observed, suggesting that genistein weakly binds to CYP1A2 in a different way other than apigenin. Thus, it is reasonable to assume that unlike apigenin, genistein has little ability to inhibit the binding of substrate to the active site of CYP1A2.

AutoDock simulation program has been employed to determine the binding mode, binding free energy and inhibition constant (*K*i) of monoamine oxidase inhibitors [21]. In the present study, the binding free energies of flavones (apigenin and chrysin) for CYP1A2 were found to be lower than those of isoflavones (genistein and daidzein). These findings suggest that the inhibitory potencies of flavones and isoflavones for CYP1A2 are dependent, at least in part, on their binding free energies. We are currently investigating the detailed correlations among the binding modes, free energies and inhibition constants of a variety of flavones and isoflavones.

In conclusion, the present docking studies demonstrate a possible mechanism that causes the differential inhibitory potencies of apigenin and genistein for CYP1A2, and provide a new insight into the mechanism for the inhibition of CYP enzymes by flavonoids.

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Figure legends

FIGURE 1. Chemical structures of apigenin and genistein.

FIGURE 2. Inhibitory effects of various flavonoids on CYP1A2. The concentration of flavonoids was 5 μM. Each bar represents the mean ± SD of three experiments.

FIGURE 3. Binding modes of apigenin and chrysin in the active site of CYP1A2.

FIGURE 4. Binding modes in two types (type 1 and type 2) of genistein in the active site of CYP1A2.