

Effect of Cytochrome P450 (CYP) 2D6 Genetic Polymorphism on the Inhibitory Action of Antidepressants on CYP2D6-Mediated Dopamine Formation from *p*-Tyramine

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ABSTRACT - PURPOSE: The inhibitory effects of antidepressants, such as imipramine, desipramine, and fluvoxamine, on dopamine formation from *p*-tyramine catalyzed by cytochrome P450 (CYP) 2D6.2 (Arg296Cys, Ser486Thr) and CYP2D6.10 (Pro34Ser, Ser486Thr), were compared with those on dopamine formation catalyzed by CYP2D6.1 (wild type), to investigate the effect of a *CYP2D6* polymorphism on neuroactive amine metabolism in the brain. **METHODS:** Inhibition constants (K_i) of the antidepressants toward dopamine formation catalyzed by CYP2D6.1, CYP2D6.2, and CYP2D6.10, which were expressed in recombinant *Escherichia coli*, were compared. **RESULTS:** Imipramine and desipramine competitively or non-competitively inhibited dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10 with K_i values of 3.9–4.9, 5.9–9.6, and 26.7–37.5 μM , respectively. The maximal velocity (V_{max}) values for dopamine formation by all CYP2D6 variants gradually increased with increasing fluvoxamine concentrations up to 40–100 μM , indicating that fluvoxamine stimulated dopamine formation. **CONCLUSIONS:** These results suggest that the inhibition/stimulation of CYP2D6-mediated dopamine formation by these antidepressants would be affected by *CYP2D6* polymorphism in the brain.

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INTRODUCTION

Cytochrome P450s (P450s or CYPs) comprise a superfamily of enzymes that catalyze the oxidation of not only a wide variety of exogenous drugs and carcinogens, but also endogenous compounds, including steroid hormones and neuroactive amines (1,2). Although CYP2D6 accounts for only 2–9% of all P450s present in human livers (3,4), this enzyme metabolizes approximately 20% of all therapeutic agents, indicating that it is one of the most important P450s (5,6). CYP2D6 is expressed not only in the liver, but also in the brain, especially the midbrain (7). However, its physiological and pharmacological functions in the human brain are still unknown.

CYP2D6 is expressed polymorphically; to date, 113 allelic variants and a series of subvariants of *CYP2D6* gene have been reported. Moreover, this number continues to increase (8,9). Interestingly, *CYP2D6* polymorphism has been demonstrated to have some relationship with the behavior of an individual (10,11). For example, CYP2D6 poor metabolizers had a higher frequency of extreme responses than extensive metabolizers, and scored significantly lower on the Karolinska Psychasthenia scale (12,13). The *CYP2D6*2* allele is present in

Caucasian populations at frequencies of 27–32%, and contains two amino acid substitutions, Arg296Cys and Ser486Thr (14). Michaelis constants (K_m), maximal velocities (V_{max}), and V_{max}/K_m values (intrinsic clearance, CL_{int}) for 62–85% of all metabolic reactions mediated by CYP2D6.2 are comparable with those mediated by CYP2D6.1 (wild-type). However, in 31% of reactions mediated by CYP2D6.2, the K_m values are more than 2-fold higher than those mediated by CYP2D6.1, and 15 and 38% of the V_{max} and V_{max}/K_m values, respectively are less than one-half (9). The *CYP2D6*10* allele, including *CYP2D6*10A* and *CYP2D6*10B* variants, is widely observed in Japanese (31–38%) (13,14) and Chinese (51%) (15) populations, and involves 2 amino acid substitutions, Pro34Ser and Ser486Thr (16,17). *CYP2D6*10C* (*CYP2D6*36*) has a gene-conversion event at exon 9 derived from CYP2D7 and contains 13 more base substitutions than *CYP2D6*10B* (15). In metabolic reactions mediated by CYP2D6.10, 63% of the K_m values are more than 2-fold higher, and 74 and 93% of the V_{max} and

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V_{\max}/K_m values, respectively are less than one-half (9). We recently demonstrated that quinidine (a typical inhibitor of human CYP2D6) and quinine (an inhibitor of rat CYP2D subfamily, including rat brain CYP2D4, rather than human CYP2D6) inhibited CYP2D6.10-mediated dopamine formation from *p*-tyramine to a lesser extent than those mediated by CYP2D6.1 and CYP2D6.2 (18).

Tyramine is not only an exogenous compound found in fermented foods, such as cheese and wine, but is also endogenously present in the brain, especially in the basal ganglia and limbic system (19). Dopamine is a neurotransmitter and a precursor of noradrenaline and adrenaline. It is formed from *p*- and *m*-tyramine through ring-hydroxylation by CYP2D6, as well as from dihydroxyphenylalanine (L-DOPA) in the brain (5,19,20,21).

Imipramine and desipramine (a pharmacologically active *N*-demethylated metabolite biotransformed from imipramine by CYP1A2, CYP2C19, and CYP3A4) are older generation tricyclic antidepressants (22-24). These agents are metabolized to inactive 2-hydroxy compounds by CYP2D6 (24). To overcome the toxicity of older generation antidepressants, fluvoxamine, one of selective serotonin reuptake inhibitors (SSRIs), is widely used in the treatment of depression (22,23,25,26). Fluvoxamine is a weak inhibitor of CYP2D6, a moderate inhibitor of CYP2C19 and CYP3A4, and a potent inhibitor of CYP1A2; however, it is metabolized predominantly by CYP2D6 (27,28). There are only a few reports on the effect of these antidepressants on dopamine formation. Thus, in the present study, we studied the effects of CYP2D6 polymorphism on the inhibitory effects of imipramine, desipramine, and fluvoxamine on CYP2D6-mediated formation of endogenous dopamine from *p*-tyramine in the brain.

METHODS

Materials

CYP2D6.1, CYP2D6.2, and CYP2D6.10, which were expressed in recombinant *Escherichia coli*, (Bactosomes), were obtained from Cypex Ltd. (Dundee, UK). These recombinant P450s were coexpressed with NADPH-P450 reductase. *p*-Tyramine, imipramine hydrochloride, and fluvoxamine maleate were purchased from Tokyo Chemical Industry (Tokyo, Japan). Desipramine hydrochloride was obtained from Wako Pure Chemical Industry (Osaka, Japan). All other reagents and organic solvents used were of the highest purity commercially available.

Determination of dopamine formation activities

Dopamine formation from *p*-tyramine in the presence or absence of antidepressants was determined as described previously (18,20,29). The incubation mixture consisted of 10 nM CYP2D6 variant, 0.06–10 mM *p*-tyramine, 1 mM NADPH, 50 μ l of water or 0.01–1000 μ M antidepressants dissolved in water, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 500 μ l. After preincubation at 37°C for 3 min, the reaction was started by adding NADPH, and the mixture was incubated at 37°C for 10 min. Dopamine concentrations in the mixtures were measured by high performance liquid chromatography with an analytical column TSK-gel ODS-120T (5 μ m, 4.6 \times 250 mm, Tosoh, Tokyo, Japan). The fluorescence intensity was determined at excitation wavelength of 280 nm and emission wavelength of 340 nm (18,20,29). The linearity of the reaction with P450 concentrations and incubation times were confirmed for CYP2D6.1 and its variants in preliminary experiments. Otherwise described, the inhibitory effects of antidepressants on dopamine formation mediated by CYP2D6.1 and CYP2D6.2, were investigated at 0.06 mM (for imipramine and desipramine) and 0.1 mM (for fluvoxamine) substrate concentration. However, 1 mM substrate concentration was used for reactions mediated by CYP2D6.10. These substrate concentrations were near or lower than the K_m value reported previously (18,29).

DATA ANALYSIS

All data were analyzed using the average of duplicate or triplicate reactions, and K_m , V_{\max} , and inhibitory constants (K_i) and the standard deviations (S.D.) as indexes of the precision of the calculated parameters were calculated from Michaelis-Menten kinetics using nonlinear least squares regression by means of MULTI (30).

RESULTS

Imipramine and desipramine at 10 μ M concentration inhibited dopamine formation mediated by CYP2D6.1 and CYP2D6.2 by more than 40%, whereas CYP2D6.10 was inhibited only at 100 μ M concentration (Fig. 1).

Interestingly, fluvoxamine at 1, 10, and 100 μ M concentrations stimulated dopamine formation catalyzed by all variants of CYP2D6; the activities of CYP2D6.2 and CYP2D6.10 were stimulated by 2.5-fold at 10 μ M concentration.

Imipramine competitively inhibited dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10 with K_i values of 4.9, 9.6, and 26.7 μM , respectively. Desipramine competitively inhibited CYP2D6.1 and CYP2D6.2 activities with K_i values of 3.9 and 5.9 μM , respectively, but noncompetitively inhibited CYP2D6.10 with a K_i value of 37.5 μM (Fig. 2). Thus, the estimated K_i values of imipramine and desipramine against CYP2D6.10 were 5–10 times higher than that against CYP2D6.1.

Dopamine formation by CYP2D6 and its mutants in the presence of various concentrations of fluvoxamine was compared (Fig. 3). The K_m values for both CYP2D6.1 and CYP2D6.2 increased with the increasing fluvoxamine concentrations, whereas the value for CYP2D6.10 decreased at concentrations below 10 μM and then increased with increasing fluvoxamine concentrations. Although the V_{max} value for CYP2D6.1 gradually increased with the increasing fluvoxamine concentrations up to 100 μM , V_{max} values for CYP2D6.2 and CYP2D6.10 increased up to 40 μM , but decreased at 100 μM . Thus, V_{max} values for all CYP2D6 mutants increased at maximum by 2–3 times.

DISCUSSION

Human CYP2D6 is expressed in the brain, especially the midbrain, as well as in the liver (7). We previously demonstrated that dopamine formation from *p*-tyramine as well as progesterone

hydroxylation were affected by CYP2D6 polymorphism (18,29). In addition, we have previously compared the kinetic parameters of CYP2D6.1 and its variants for 41 metabolic reactions of 31 substrates (9). Parameters, such as K_m , V_{max} , and V_{max}/K_m , for more than 62% of the metabolic reactions mediated by CYP2D6.2 were comparable with those mediated by CYP2D6.1 (wild type); however, 31% of the K_m values were more than 2-fold higher, and 15 and 38% of the V_{max} and V_{max}/K_m values, respectively were less than one-half of those of CYP2D6.1.⁹ On the other hand, 63% of the K_m values in metabolic reactions mediated by CYP2D6.10 were more than 2-fold higher, and 74 and 93% of the V_{max} and V_{max}/K_m values, respectively were less than one-half.⁹ To determine the underlying reason, further investigation, for instance, using three-dimensional structural analysis, such as molecular docking simulation (31), should be conducted.

On the other hand, there are few reports on the effect of CYP2D6 polymorphism on the inhibition of CYP2D6-mediated reactions by various compounds, including antidepressants. We recently demonstrated that the K_i values of quinidine (a typical strong inhibitor of CYP2D6 *in vitro*, as listed in the guidance for drug interaction studies by US FDA (32), EMA (33), and Japanese PMDA (34)) and quinine (a potent inhibitor of the rat CYP2D subfamily, including rat brain CYP2D4 (35-38)) against CYP2D6.1 (wild-type) were lower than those against CYP2D6.10, which are frequently observed in Asian populations (13-15).

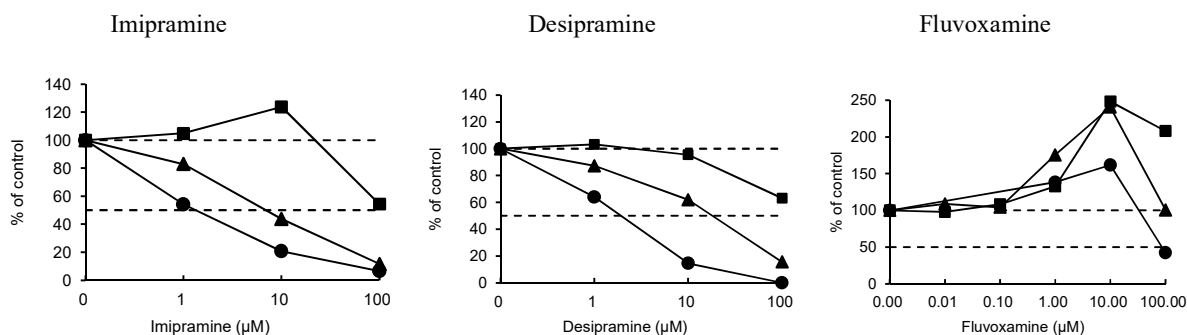
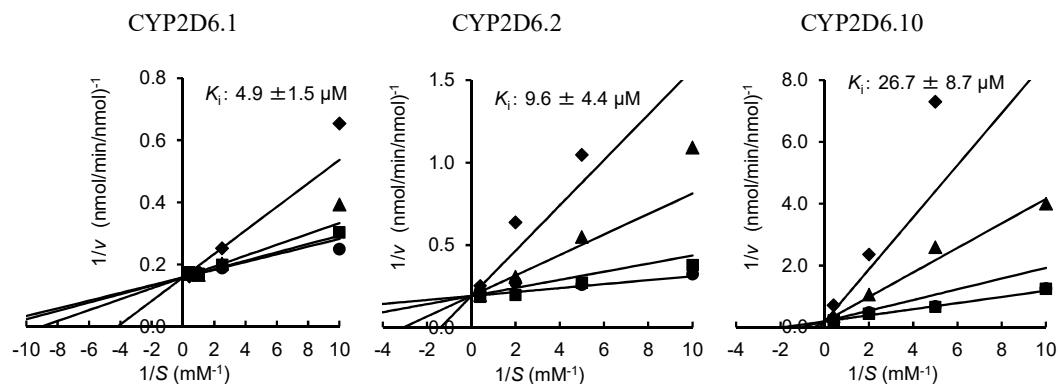


Figure 1. Effects of antidepressants on dopamine formation from *p*-tyramine mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10.

●: CYP2D6.1, ▲: CYP2D6.2, ■: CYP2D6.10. Substrate concentrations for CYP2D6.1 and CYP2D6.2 were 0.06 mM (for imipramine and desipramine) and 0.1 mM (for fluvoxamine), and 1 mM substrate concentration was used for reactions mediated by CYP2D6.10. Values are means of duplicate or triplicate determinations.

Imipramine



Desipramine

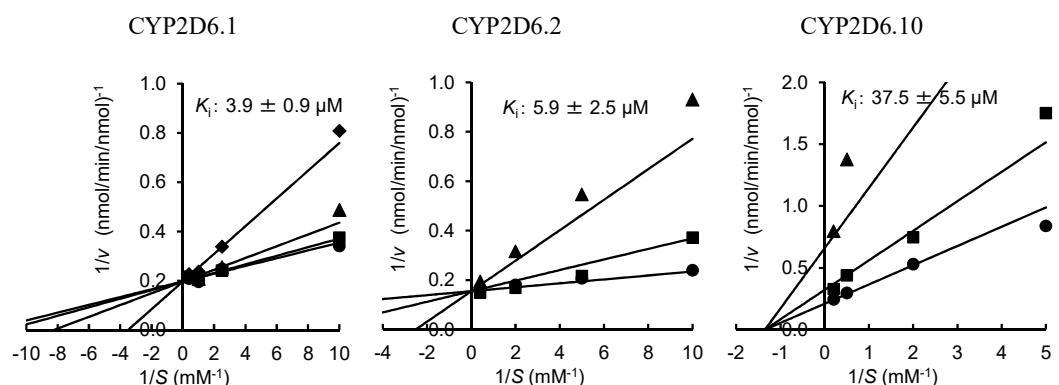


Figure 2. Inhibitory effects of imipramine and desipramine on dopamine formation from *p*-tyramine mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10.

Imipramine and desipramine concentrations for CYP2D6.1, ●: 0 μ M, ■: 0.4 μ M, ▲: 2 μ M, ◆: 10 μ M, for CYP2D6.2, ●: 0 μ M, ■: 10 μ M, ▲: 40 μ M, ◆: 100 μ M, for CYP2D6.10, ●: 0 μ M, ■: 20 μ M, ▲: 80 μ M, ◆: 200 μ M. K_i values are means \pm S.D. of the data set using a nonlinear kinetic analysis from mean values obtained in duplicate or triplicate at each substrate/inhibitor concentration.

In addition, we previously reported that psychotropic drugs, such as imipramine, desipramine, and fluoxetine, inhibited 21-hydroxylation of progesterone and allopregnanolone (a neuroactive steroid) mediated by CYP2D6 and CYP2D4 (39). In the present study, we found that imipramine and desipramine, which are tricyclic antidepressants (22-24), competitively or noncompetitively inhibited dopamine formation from *p*-tyramine. However, the K_i values for CYP2D6.10 were higher than those for CYP2D6.1 (Fig. 2). These results suggested that the inhibition of CYP2D6 by various psychotropic drugs, including these antidepressants, is affected by CYP2D6 polymorphism in the brain.

Interestingly, fluvoxamine, a typical SSRI, increased the K_m and V_{max} values for dopamine formation mediated by CYP2D6.1 and its mutants (Fig. 3). We have previously demonstrated that

fluoxetine, an SSRI, also increased the K_m and V_{max} values for CYP2D6-mediated 21-hydroxylation of progesterone, suggesting CYP2D6 activation (39). We have reported that steroid hormones, such as progesterone and testosterone, stimulate the metabolism of CYP3A4 substrates; however, selection of the enzyme source (liver microsomes or recombinant P450s) can affect enzyme activation (40). In addition, a number of investigators have demonstrated the activation of CYP3A4-mediated metabolic reactions, whereas the detailed mechanism is still unknown (39-41). The activation is reported not only of CYP3A4, but also of other enzymes, such as CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A7 (41). The activation of CYP2D6-mediated 21-hydroxylation of progesterone by fluoxetine was the first finding in relation to CYP2D6 (39,41).

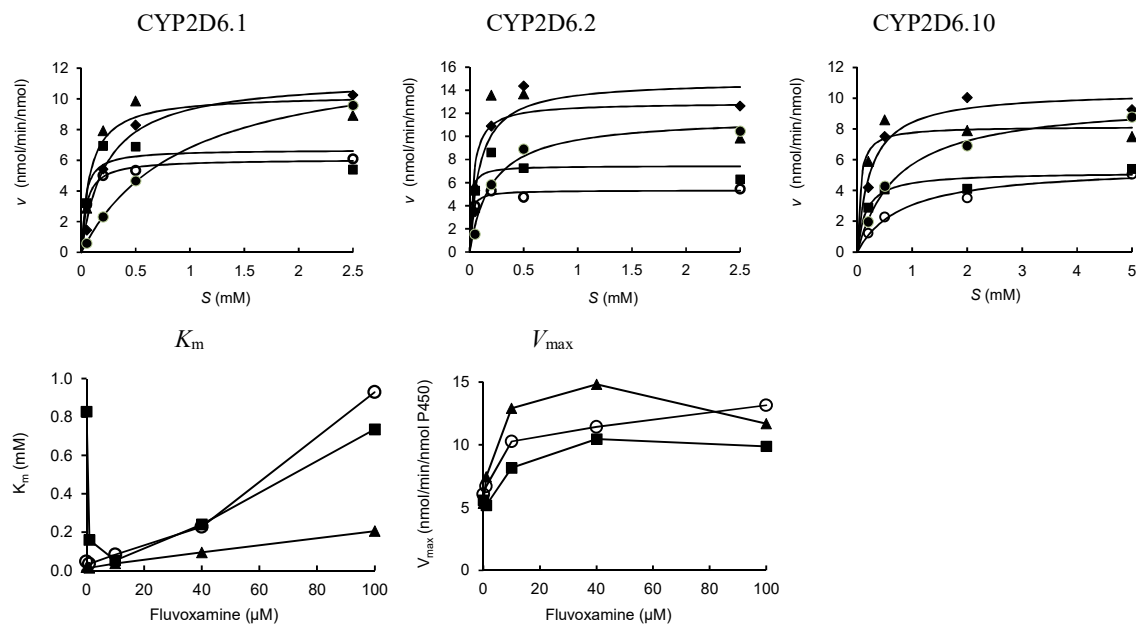


Figure 3. Stimulatory effects of fluvoxamine on dopamine formation from *p*-tyramine mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10.

Fluvoxamine concentration against CYP2D6.1, CYP2D6.2 and CYP2D6.10 activities, ○: 0 μM , ■: 1 μM , ▲: 10 μM , ◆: 40 μM , ●: 100 μM . For K_m and V_{max} , ○: CYP2D6.1, ▲: CYP2D6.2, ■: CYP2D6.10. Values are means of duplicate or triplicate determinations.

Apart from selective inhibition of serotonin reuptake, some SSRIs, such as fluvoxamine and fluoxetine that are metabolized by CYP2D6, exhibit novel physiological actions via the activation of dopamine formation and 21-hydroxylation of neurosteroids, including progesterone and allopregnanolone, mediated by brain CYP2D6 (42).

When the substrate concentration is lower than the K_m value, CL_{int} in the presence and absence of the inhibitor can be expressed by the following equation independent of the inhibition type, except in the case of uncompetitive inhibition (43,44):

$$CL_{\text{int}}(+ \text{Inhibitor})/CL_{\text{int}}(- \text{Inhibitor}) = 1/(1 + I_u/K_i)$$

where I_u is the unbound concentration of the inhibitor. According to the packing insert and interview form of imipramine hydrochloride (45), the mean observed plasma concentrations of imipramine and desipramine after repeated oral dosing of imipramine (75 mg once a day) to Japanese patients with depression were 70 (0.25 μM) and 29 ng/ml (0.10 μM), respectively. Maximum plasma concentrations after single oral dosing of imipramine (75 mg) to healthy subjects in Sweden were 34–137

ng/ml (0.12–0.49 μM) (46). Recent reports have demonstrated that most individuals optimally respond to imipramine, when the combined serum/plasma levels of imipramine and desipramine are between 175 and 300 ng/ml (0.6–1.1 μM) (47,48). It is difficult to measure the degree of brain drug-permeation in humans. However, it is reported that after an intravenous dose to rabbits, the brain concentrations of imipramine and desipramine were more than 10 times higher than the plasma concentrations (45). The maximum plasma concentration after a single oral dosing of 100 mg fluvoxamine maleate to Japanese healthy subjects was 43.77 ng/ml (0.14 μM), and the concentrations in the cerebrum and cerebellum at 1 hr after a single oral dosing of ^{14}C -labelled fluvoxamine (5 mg/kg) to rats (180–191 ng/g tissue, 0.57–0.60 μM) were 10–11% of the plasma concentration (27). Although plasma concentrations of these antidepressants have been measured, there are few reports about their distribution in the human brain. In addition, dopamine is formed from L-DOPA as well as from *p*-tyramine in the brain (5,20,21). To predict the pharmacological effect of antidepressants in the brain, further pharmacokinetic studies, such as

measuring their distribution in the brain, are required.

In conclusion, the present study suggests that CYP2D6 polymorphism affects the inhibitory potency of antidepressants on dopamine formation from *p*-tyramine in the brain and fluvoxamine, an SSRI, stimulates the metabolic activity of CYP2D6. However, further clinical studies are required to confirm the therapeutic relevance of our observations.

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