Effects of Cigarette Smoking and Cytochrome P450 2D6 Genotype on Fluvoxamine Concentration in Plasma of Japanese Patients

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Fluvoxamine is a selective serotonin reuptake inhibitor widely used in the treatment of depression and other psychiatric diseases. The aim of this study was to assess the clinical impact of cigarette smoking on plasma fluvoxamine concentration in Japanese patients, and evaluate whether the cytochrome P450 (CYP) 1A2 and CYP2D6 genotypes have effects on that concentration. Thirty-two Japanese patients receiving fluvoxamine were enrolled. They were maintained on the same daily dose of fluvoxamine (mean±S.D., 109.4±66.2 mg/d) for at least 4 weeks to obtain the steady-state plasma concentration. The steady-state plasma concentration-to-dose (C/D) ratio of fluvoxamine in patients who smoked (n=6, 11.8±6.5 ng/ml/dose) was significantly lower than that in non-smoker patients (n=26, 22.8±11.2 ng/ml/dose). There was no significant difference for the C/D ratio of fluvoxamine in patients with CYP1A2 3860G/G, 3860G/A, and 3860A/A between non-smokers and smokers. Among non-smoker patients, the C/D ratios of fluvoxamine in those with one and two mutated alleles of CYP2D6 were 1.6- and 1.4-fold higher, respectively, than those with no mutated alleles, though the differences among those three genotype groups were not significant. Furthermore, stepwise multiple regression analysis revealed that cigarette smoking and daily dose had significant positive correlations with the plasma concentration of fluvoxamine. Our findings suggest that cigarette smoking has a significant impact on the steady-state plasma concentration of fluvoxamine in Japanese patients.

Key words fluvoxamine; cigarette smoking; cytochrome P450 2D6; cytochrome P450 1A2; genetic polymorphism

Fluvoxamine is a selective serotonin reuptake inhibitor widely used in the treatment of depression and other psychiatric diseases.1) The drug is extensively metabolized in the liver and the major metabolite in human urine is fluvoxamine acid, produced by oxidative demethylation.2) Previous in vitro results indicated that only cytochrome P450 (CYP) 2D6 catalyses the major metabolic pathway of fluvoxamine.3) The existence of genetic polymorphisms in CYP2D6 has been recognized to influence enzymatic activity, which has effects on the plasma concentrations of substrate drugs of CYP2D6.4,5) Several mutated allele of the CYP2D6 gene causing absent enzyme activity, e.g., CYP2D6*5 and CYP2D6*14, and decreased enzyme activity, e.g., CYP2D6*10, have been reported.5–8) Furthermore, human studies have shown that polymorphisms of CYP2D6 are associated with the poor metabolism of fluvoxamine,9,10) whereas other studies reported that the CYP2D6 genotype has no major impact on its steady-state plasma concentration.11,12) CYP1A2 is inducible by polycyclic hydrocarbons present in cigarette smoke and it is well established that cigarette smoking induces the metabolism of drugs catalyzed by CYP1A2, such as theophylline,13,14) caffeine,15) and imipramine.16) Several studies have shown that the disposition of fluvoxamine is affected by cigarette smoking in human, suggesting a potential role of CYP1A2 in the metabolism of fluvoxamine in addition to CYP2D6.9,17) However, in an in vitro examination, CYP1A2 was found to be not involved in the metabolism of fluvoxamine to fluvoxamine acid.3) Together, the role of CYP1A2 on the metabolism of fluvoxamine is still unclear in human. In addition, a single nucleotide polymorphism in the 5′-flanking region of the human CYP1A2 gene, CYP1A2*1C (3860G>A), was reported to be associated with decreased enzyme inducibility in Japanese smokers.18) Thus, the polymorphism of CYP1A2 in relation to cigarette smoking may have an effect on the metabolism of fluvoxamine.

The aim of this study was to assess the clinical impact of cigarette smoking on plasma fluvoxamine concentration in Japanese patients, and evaluate whether the CYP1A2 and CYP2D6 genotypes have effects on that concentration.

MATERIALS AND METHODS

Drugs Patients were treated with fluvoxamine maleate (Depromel®, Meiji Seika Kaisha Ltd., Tokyo, Japan). DNA Extractor WB Kit was purchased from Wako Pure Chemical Industries (Osaka, Japan). All other drugs and materials were obtained from commercial source.

Subjects Thirty-two Japanese patients (15 males, 17 females) receiving fluvoxamine were enrolled in this study. They were being treated at the outpatient clinic of Hamamatsu University School of Medicine Hospital, and each had normal liver and renal functions. Their ages ranged from 20 to 67 years (mean±S.D., 39.0±15.0 years) and body weights ranged from 32.5 to 87.2 kg (56.8±12.6 kg). Six were smokers (≥10 cigarettes/d). Daily doses of fluvoxamine ranged from 50 to 300 mg/d (109.4±66.2 mg/d), equivalent to 0.65

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to 7.50 mg/kg of body weight/d (2.09±1.62 mg/kg/d), according to clinical symptoms. Patients receiving drugs that could interact with the metabolism of fluvoxamine were excluded. The daily dose of fluvoxamine in each patient was not changed for at least 4 weeks prior to our analysis, in order to obtain the steady-state plasma concentration. Seven milliliters of venous blood was collected before the morning dose for determination of the CYP2D6 and CYP1A2 genotypes. Plasma samples were separated from remaining blood samples and stored at −80 °C until analysis. The study protocol was approved by the Ethics Committee of Hamamatsu University School of Medicine and written informed consent was obtained from each subject.

**Determination of Plasma Concentrations of Fluvoxamine** An internal standard solution of cloxovarone fumarate was added to 0.5 ml of plasma and the mixture was eluted with 4 ml of diethyl ether with shaking for 10 min. The diethyl ether phase was separated and dried under nitrogen. The residue obtained was dissolved in a 200-µl mixture solution of 0.1% acetic acid and methanol. After centrifugation for 5 min at 2500 rpm, 20 µl of supernatant was used for determination of fluvoxamine concentration by liquid chromatography-tandem mass spectrometry. A reverse phase column (SYMMETRY C8, 3.5 µm, 2.1×100 mm, Waters, Milford, MA, U.S.A.) was employed for liquid chromatography and elution was performed with a solution of 0.1% acetic acid in methanol at a rate of 0.2 ml/min. The conditions for mass spectrometry were as follow. Ionization was performed in electrospray ionization mode and the monitoring ion was [M+H] z 3860G for fluvoxamine and [M+H] z 3860G for clovoxamine, with positive ion monitoring. The detection limit was 0.5 ng/ml of fluvoxamine.

**Genotyping of CYP2D6 and CYP1A2** For genotype determination, DNA was isolated from peripheral leukocytes using a DNA Extractor WB Kit. CYP2D6 genotypes were determined as described previously. In brief, CYP2D6*1, *10, and *14 were determined by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, CYP2D6*2 by an allele-specific PCR method, and CYP2D6*5 by a long-PCR method. CYP1A2*1C was detected using PCR-RFLP.

**Statistical Analysis** Data are shown as the mean±S.D. The significance of differences between two groups was assessed using a Mann–Whitney U-test, while the significance among more than three groups was assessed with a Kruskal–Wallis test. Stepwise multiple linear regression analysis was performed to determine independent factors that have an influence on the plasma concentration of fluvoxamine with JMP (ver. 8, SAS Institute Inc., Cary, NC, U.S.A.). A p value of 0.05 or less was considered statistically significant.

**RESULTS**

The steady-state plasma concentration-to-dose (C/D) ratio of fluvoxamine in smoker patients (11.8±6.5 ng/ml/dose) was significantly lower than that in non-smoker patients (22.8±11.2 ng/ml/dose) (Fig. 1). There were no significant differences for the C/D ratio of fluvoxamine among non-smoker patients with CYP1A2 −3860G/G, −3860G/A, and −3860A/A (Table 1), or for the C/D ratio of fluvoxamine between smoker patients with CYP1A2 −3860G/G and −3860G/A (Table 1).

Six CYP2D6 genotypes were identified in 32 patients; CYP2D6*1/*1 (n=11), CYP2D6*1/*5 (n=3), CYP2D6*1/*10 (n=11), CYP2D6*5/*10 (n=2), CYP2D6*10/*10 (n=4), CYP2D6*10/*14 (n=1). We divided the patients into three groups by the number of mutated alleles (*5, *10, *14). Among the non-smoker patients, the C/D ratio of fluvoxamine in groups with one and two mutated alleles of CYP2D6 was 1.6- and 1.4-fold higher, respectively, as compared to that in the no mutated alleles group (16.9±7.2, 27.5±12.3, and 23.2±11.5 ng/ml/dose, respectively), though the differences were not significant (Fig. 2).

Results of our multiple linear regression analysis are summarized in Table 2. A daily dose of fluvoxamine and cigarette smoking affected the steady-state plasma concentration of fluvoxamine. In stepwise multiple regression analysis, significant positive correlations with the plasma concentration of fluvoxamine were found for cigarette smoking (standardized partial regression coefficient: β=0.229, p=0.0175) and daily dose of fluvoxamine (β=0.888, p<0.0001). The coefficient of determination (R²) was 0.753.

**DISCUSSION**

We evaluated the clinical impact of cigarette smoking and presence of the CYP1A2 and CYP2D6 genotypes on the steady-state plasma concentration of fluvoxamine in Japanese patients. Our results showed that the C/D ratio of fluvoxamine in smoker patients was significantly lower than that in...
non-smoker patients. Furthermore, significant positive correlations with the plasma concentration of fluvoxamine were found for cigarette smoking by multiple regression analysis. Similarly, it has been reported that smokers had a lower fluvoxamine concentration than non-smokers after a single oral dose (50 mg) in a study of healthy volunteers. Together, these results strongly suggest that smoking has a major impact on the steady-state plasma concentration of fluvoxamine in Japanese patients being treated with the drug. It is well established that smoking increases the activity of CYP1A2. Together, these results strongly suggest that smoking has a major impact on the steady-state plasma concentration of fluvoxamine in Japanese patients being treated with the drug. It is well established that smoking increases the activity of CYP1A2.13—16) In addition, Carrillo et al. have reported a significant correlation between the CYP1A2 activity (caffeine N-demethylation) and the oral clearance of fluvoxamine. Thus, there are possibility that the induction of CYP1A2 caused by smoking may contribute to the decreased plasma concentration of fluvoxamine in this study. It will be important to clarify the impact of the reduction of fluvoxamine concentrations by smoking on its clinical effects and adverse drug reactions. Fluvoxamine is a well-known to be a potent inhibitor of CYP1A2, 2C19 and 3A4. Recently, Sugahara et al. have recently reported that the concentration of alprazolam in non-smokers was increased by fluvoxamine, while that in smokers was unchanged. Thus, it is plausible that the extent of drug interactions by fluvoxamine may be affected by smoking.

In contrast, Gerstenberg et al. found no significant difference between smokers and nonsmokers in regard to the concentration of fluvoxamine given at a daily dose of 200 mg in Japanese patients.13) The dose-dependent effects of the CYP2D6 and adenosine triphosphate-binding cassette B1 (ABCB1, MDR1) genotypes on the steady-state plasma concentration of fluvoxamine was suggested in a recent study of psychiatric patients.10,22) It has been reported that the effect of the CYP2D6 genotype on plasma concentration was observed only at a low dose of fluvoxamine (50 mg/d). On the other hand, it has been reported that the plasma fluvoxamine concentration depends on 3435C/T genotype of ABCB1 only at a high dose (200 mg/d). Therefore, it is likely that these contrasting results may have resulted from the daily dose of fluvoxamine.

We detected the CYPIA2*1C allele at a frequency of 0.25, which is comparable to previous studies conducted in Japan. Nakajima et al. reported decreased inducibility of CYPIA2 by cigarette smoking in Japanese smokers with the CYPIA2*1C allele. Although this mutation was not shown to have an effect on plasma fluvoxamine concentration in smoker patients in our study, a larger study is required for assessing the impact of the CYPIA2 genotype on the pharmacokinetics of fluvoxamine.

In the present study, the plasma concentration of fluvoxamine was not significantly different among nonsmoking patients with no, one, and two mutated alleles of the CYP2D6 gene. This result agrees with previous studies of Japanese patients. Watanabe et al. reported that the effect of the CYP2D6 genotype on fluvoxamine metabolism in patients treated with a high dose (>100 mg/d) was less than the effect with a low dose (50 mg/d). In our study, patients were treated with fluvoxamine at a mean daily dose of 110 mg. Thus, saturation of the CYP2D6 metabolic pathway may decrease the impact of the CYP2D6 genotype on the concentration of fluvoxamine in plasma, and increase the effects of cigarette smoking and other factors. One of the limitations of this study is that the sample size was relatively small and we could not simultaneously evaluate the influence of the CYP2D6 genotype on the plasma fluvoxamine concentration in association with other factors including the dose of fluvoxamine and ABCB1 genotype.

In conclusion, our findings strongly suggest that cigarette smoking has a significant impact on the steady-state plasma concentration of fluvoxamine in Japanese patients. Our multiple linear regression analysis data also indicate that 75% of the variability in steady-state plasma concentrations of fluvoxamine can be explained by both daily dose and smoking. Additional studies are needed to clarify the factors that influence the pharmacokinetics and pharmacodynamics of fluvoxamine.

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REFERENCES