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PHARMACOGENETICS

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Effect of *CYP2D6* and *CYP2C9* genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions

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Abstract *Objectives*: CYP2D6 drug-metabolising enzyme has been shown to be involved in fluoxetine metabolism in vitro and in vivo. CYP2C9 has also been shown to influence the metabolism of fluoxetine in vitro; however, this relationship has not been studied in humans. The aim of the present study was to evaluate the influence of *CYP2D6* and *CYP2C9* genotypes on the plasma concentration of fluoxetine and norfluoxetine in psychiatric patients during steady-state conditions.
Methods: White European psychiatric patients ($n = 64$) receiving antidepressant monotherapy with fluoxetine were studied. *CYP2D6* and *CYP2C9* genotypes were determined by polymerase chain reaction-specific methods. The plasma concentrations of fluoxetine and its metabolite, norfluoxetine, were measured by high-performance liquid chromatography.
Results: The dose-corrected plasma concentrations of fluoxetine were related ($P < 0.01$, $r = -0.36$) to *CYP2D6* genotypes (number of active genes). The fluoxetine/norfluoxetine ratio also correlated ($P < 0.01$, $r = -0.39$) with the number of active *CYP2D6* genes. Among patients with two *CYP2D6* active genes, the dose-corrected plasma concentrations of fluoxetine and active moiety (fluoxetine plus norfluoxetine) were significantly ($P < 0.05$) higher in the *CYP2C9*1/*2* and *CYP2C9*1/*3* genotype groups than in *CYP2C9*1/*1*. However, dose-corrected (C/D) plasma concentrations of fluoxetine, active moiety and fluoxetine/norfluoxetine ratios were not highly different in the individuals with two mutated alleles as compared with those heterozygous for **2* or **3*.
Conclusion: The present results show that *CYP2D6* and potentially *CYP2C9* genotypes seem to influence fluoxetine plasma concentration during steady-state conditions in patients.

Keywords Selective serotonin reuptake inhibitors · CYP2C9 · CYP2D6

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Introduction

Fluoxetine is a widely used selective serotonin reuptake inhibitor (SSRI) antidepressant drug prescribed for a variety of psychopathological conditions, including mood and eating disorders, obsessive–compulsive disorders, and dysthymia [1]. Generally, fluoxetine is regarded as a safe antidepressant medication, although side effects, such as gastrointestinal symptoms, nervousness, anxiety, insomnia or tremor, may frequently occur [2]. Fluoxetine has also been reported to be involved in potentially dangerous pharmacokinetic interactions with warfarin [3, 4, 5], phenytoin [6, 7, 8], tricyclic antidepressants [9, 10, 11] or antipsychotic drugs [12, 13]. Two recent studies have shown that the concurrent use of SSRIs, including fluoxetine, and non-steroid anti-inflammatory drugs (NSAIDs), increased the risk of upper gastrointestinal bleeding [14, 15]. Therefore, these interactions are of major concern, due to the long elimination half-lives of fluoxetine and its major active metabolite, norfluoxetine. As a result, the clinical management of these potential drug interactions may not be easy.

In humans, fluoxetine is mainly metabolised to N-demethylfluoxetine (norfluoxetine), which seems to

have a similar pharmacological activity to the parent compound [1]. Other metabolic pathways include glucuronidation of both fluoxetine and norfluoxetine and the formation of multiple poorly characterised metabolites [16]. A great inter-individual variability exists in the plasma concentrations of fluoxetine and norfluoxetine after administration of the same dose of the drug, which may be partly related to the different activity of the drug-metabolising cytochrome P_{450} enzymes CYP2D6 and CYP2C9 [16, 17, 18, 19, 20].

CYP2D6 has been shown to determine the plasma concentration of several important drugs (such us antiarrhytmics, tricyclic antidepressants, SSRIs and antipsychotic drugs, inter alia) [21]. CYP2D6 has also been shown to be related to the metabolism of fluoxetine to norfluoxetine in vitro and among healthy volunteers after a single dose [17, 19, 22]. In patients, the plasma concentrations of fluoxetine and norfluoxetine have been shown to be related to *CYP2D6* genotype [23]. Furthermore, fluoxetine has been reported to be a potent inhibitor of the enzyme both in vitro and in vivo [24, 25, 26, 27]. Five to ten percent of Caucasians have a genetically determined decreased capacity of the CYP2D6 enzyme due to several inactivating alleles [21, 28]; thus, in those patients, the disposition of fluoxetine may be altered.

The polymorphic CYP2C9 enzyme is also involved in the disposition of such widely used drugs as warfarin, phenytoin, losartan, perazine and NSAIDs [29, 30]. In vitro, fluoxetine is metabolised by CYP2C9 and also inhibits the activity of the enzyme [18, 22, 31]. Thus far, no data about fluoxetine disposition in different *CYP2C9* genotypes in humans are available. We have recently described that the frequencies of *CYP2C9* allelic variants (**1*, **2*, **3*) in Spaniards are similar to those found among other white European populations [32]. To the best of our knowledge, no study has yet evaluated the effect of both *CYP2D6* and *CYP2C9* genotypes on the metabolism of fluoxetine among psychiatric patients.

The aim of the present study was to evaluate the influence of *CYP2D6* and *CYP2C9* genotypes on the plasma concentration of fluoxetine and norfluoxetine in psychiatric patients during steady-state conditions.

Materials and methods

White European psychiatric patients ($n = 64$) without any relevant organic disease were studied. They were outpatients visiting the Mental Health Center of Don Benito (Extremadura, Spain). Ten patients (15.6%) were classified as tobacco smokers, defined as smoking more than ten cigarettes per day. The patients were on continuous oral antidepressant monotherapy treatment with the same fluoxetine dose for at least 45 days (mean ± SD: 130 ± 104 days; range 45–365 days) before the study. The concomitant drugs that 40 of the patients were taking were not known as inhibitors or substrates of CYP2D6 or CYP2C9. The concomitant drugs included: acetylsalicylic acid, amiloride, enalapril, atenolol, benzodiazepines, hidroaldosterone, L-dopa, nimodipine, nitroglycerine, spironolactone, sulpiride, tiamazol, zolpiclone and zolpidem. The fluoxetine dose range was from 10 mg/day to 60 mg/day and the average dose was 25.2 ± 10.0 mg/day (mean ± SD). The mean age of the patients was 51 ± 15 years (range: 18–77 years). Patients gave their prior written consent to participate in the study. The study was performed according to the Helsinki Declaration and was approved by the ethics committee of the Extremadura University Hospital.

CYP2D6 and *CYP2C9* genotyping

Blood samples for *CYP2D6* and *CYP2C9* genotyping were available for 64 patients. *CYP2D6* genotype was determined using genomic DNA purified from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Quiagen, Hilden, Germany). The AmpliTaq Gold System (Perkin-Elmer Inc., Wellesley, USA) was used to amplify the *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*6* alleles by tetra-primer polymerase chain reaction (PCR), and the Expand Long Template PCR System (Roche Diagnostics, Basel, Switzerland) was used to detect the *CYP2D6*5* allele by multiplex PCR. Amplifications were performed with a PTC-100 thermocycler (MJ Research, Inc. Watertown, USA). Oligonucleotide sequences and PCR conditions for the detection of the *CYP2D6*3*, **4*, **5* and **6* alleles have been described previously [33, 34, 35]. Duplicated *CYP2D6* genes (ultrarapid metabolisers) were determined according to a previously published method [36]. PCR products were separated by electrophoresis in agarose gels and visualised by staining with ethidium bromide.

The *CYP2C9* genotypes were determined by a PCR-restriction fragment length polymorphism method, as described previously, allowing identification of the allelic variants *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* [32].

Plasma concentrations of fluoxetine and norfluoxetine

Blood sampling was performed before the morning fluoxetine dose, and the plasma samples were stored at −20°C until measurement. Plasma concentrations of fluoxetine and its metabolite, norfluoxetine, were measured by a validated previously published high-performance liquid chromatography method [37].

Data analysis

Kruskal-Wallis non-parametric test was used to compare the different groups, and Dunn's post-test was applied for multiple comparisons. Correlations of different variables were tested by non-parametric Spearman's test. Statistical calculations were carried out using GraphPad Prism 3.02 software (GraphPad Software Inc., http://www.graphpad.com); $P < 0.05$ was considered as significant.

Results

The *CYP2D6* and *CYP2C9* genotypes of the patients are given in Table 1 and Table 2. There was a great inter-individual variability in plasma concentrations of fluoxetine and norfluoxetine. The steady-state C/D plasma concentrations of fluoxetine showed a higher than 16-fold inter-individual variation (from 2.1 nmol/l/mg to 33.2 nmol/l/mg), while the C/D plasma concentrations of norfluoxetine varied from 2.5 nmol/l/mg to 43.5 nmol/l/mg, (17.4-fold variation). The C/D plasma concentrations of fluoxetine plus norfluoxetine (active moiety) also showed substantial inter-individual variability (4.6 nmol/l/mg to 74.2 nmol/l/mg, 16-fold variation).

Table 1 *CYP2D6* genotypes of the patients receiving fluoxetine antidepressant monotherapy ($n=64$). *EM* extensive metaboliser, *PM* poor metaboliser, *UM* ultrarapid metabolizer

CYP2D6 genotype	Proposed phenotype	Number of patients	Percentage of total	Active genes
**1/*1* (wild-type)	Homozygous EM	41	64.1	2
**1/*4*	Heterozygous EM	17	26.6	1
**1/*6*	Heterozygous EM	2	3.1	1
**4/*4*	PM	1	1.5	0
**1/*1xN or *1/*2xN*[†]	UM	3	4.7	>2

[†] $N \geq 2$

Table 2 *CYP2C9* genotypes of the patients receiving fluoxetine antidepressant monotherapy ($n=64$)

CYP2C9 genotype	Number of patients	Percentage of total
**1/*1* (Wild-type)	34	53.2
**1/*2*	14	21.9
**1/*3*	11	17.2
**2/*2*	2	3.1
**3/*3*	2	3.1
**2/*3*	1	1.5

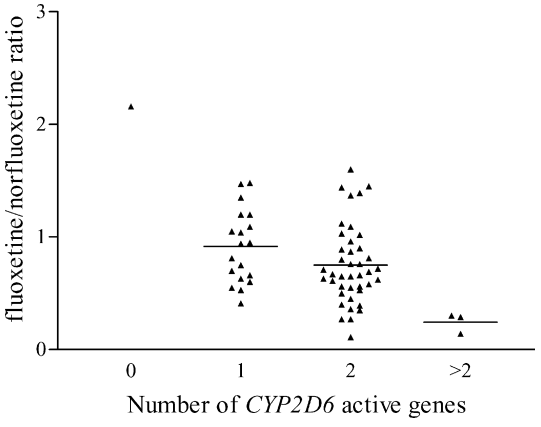

Fig. 1 The mean in fluoxetine/norfluoxetine ratio among patients with different number of *CYP2D6* active genes: zero active genes (2.16, $n=1$); one active gene (0.92 ± 0.33, $n=19$); two active genes (0.75 ± 0.35, $n=41$), >two active genes (0.24 ± 0.1, $n=3$)

The C/D plasma concentrations of fluoxetine were overall significantly ($P<0.01$, $r=-0.36$) related to the number of active *CYP2D6* genes. The mean ± SD C/D plasma concentrations of fluoxetine (nmol/l/mg) were 16.7 for the only patient with zero active genes, 17.2 ± 8.2 for patients with one active gene ($n=19$), 13.0 ± 7.6 with two active genes ($n=41$) and 7.3 ± 4.5 with more than two active genes ($n=3$). No significant correlation was found between the C/D plasma concentrations of norfluoxetine or active moiety and the number of active genes. The fluoxetine/norfluoxetine ratio was overall significantly correlated ($P<0.01$, $r=-0.39$) with the number of active *CYP2D6* genes (Fig. 1).

To eliminate the possible confounding effect of CYP2D6, the influence of *CYP2C9* was evaluated among patients with two *CYP2D6* active genes (*CYP2D6*1/*1* genotype) and statistical analyses were carried out among patients carrying *CYP2C9*1/*1*, *CYP2C9*1/*2*, or *CYP2C9*1/*3* genotypes ($n=38$). The C/D plasma concentrations of fluoxetine and the active moiety were higher ($P<0.05$) in the *CYP2C9*1/*2* or *CYP2C9*1/*3* genotype groups compared with the *CYP2C9*1/*1* genotype, while the C/D plasma concentrations of norfluoxetine were higher ($P<0.05$) in the *CYP2C9*1/*3* genotype in comparison with the homozygous *CYP2C9* wild-type patients (Table 3). Three patients with genotypes *CYP2C9*2/*2, *2/*3* and **3/*3* were found among patients with two *CYP2D6* active genes. The C/D plasma concentrations of fluoxetine of these patients were 21.6, 13.0 and 14.1 nmol/l/mg for *CYP2C9*2/*2, *2/*3* and **3/*3* genotypes, respectively. The corresponding C/D plasma concentrations of norfluoxetine were 14.8, 19.7 and 14.7 nmol/l/mg. The C/D plasma concentrations of active moiety were 36.4, 32.7 and 28.8 nmol/l/mg. The fluoxetine/norfluoxetine ratio was 1.45 for *CYP2C9*2/*2*, 0.66 for *CYP2C9*2/*3* and 0.96 for *CYP2C9*3/*3* genotype patients. Smoking habits had no significant effect on any of the parameters studied (results not shown).

Discussion

There has been an increasing recognition of the importance of CYP2C9 enzyme in the metabolism of several important drugs (such as anticoagulants, losartan, phenytoin, perazine and NSAIDs) [29]. To the best of our knowledge, this is the first study that shows the potential effect of *CYP2C9* genotypes on the fluoxetine plasma concentration among psychiatric patients during steady-state conditions; the present results also confirm the influence of *CYP2D6* genotypes.

The plasma concentrations of fluoxetine and norfluoxetine in the present patient population were within the range reported in the literature [20, 38], although a great inter-individual variability was found in both fluoxetine and norfluoxetine plasma concentrations as well as in the fluoxetine/norfluoxetine ratio.

The present study suggests the potential involvement of CYP2C9 in fluoxetine metabolism in psychiatric patients, which has only been reported previously for in vitro studies [18, 31]. *CYP2C9* genotypes seem to have influence on fluoxetine metabolism, since both fluoxetine and the active moiety plasma concentrations were higher in patients with *CYP2C9*1/*2* or *CYP2C9*1/*3* genotype than in those with *CYP2C9*1/*1* genotype. However, C/D plasma concentrations of fluoxetine, active moiety and fluoxetine/norfluoxetine ratios were not

Table 3 The dose-corrected (C/D) plasma concentrations (nmol/l/mg, mean ± SD) of fluoxetine, norfluoxetine, active moiety (fluoxetine plus norfluoxetine) and fluoxetine/norfluoxetine ratio among *CYP2D6* extensive metaboliser homozygote patients ($n = 38$) with different *CYP2C9* genotype groups

CYP2C9 genotypes	**1/*1* ($n = 19$)	**1/*2* ($n = 11$)	**1/*3* ($n = 8$)
C/D of fluoxetine[†]	9.9 ± 4.8	15.1 ± 9.3[#]	16.3 ± 9.8[‡]
C/D of norfluoxetine[†]	15.8 ± 7.0	20.4 ± 10.5	22.3 ± 12.4[#]
C/D of active moiety[†] (fluoxetine + norfluoxetine)	25.1 ± 10.1	35.5 ± 18.5[#]	38.6 ± 22.1[‡]
Fluoxetine/norfluoxetine ratio	0.73 ± 0.41	0.74 ± 0.34	0.72 ± 0.14

[†](Kruskal-Wallis test, overall $P < 0.05$)
[#]Significantly different ($P < 0.05$) from the wild-type (**1/*1*) group with Dunn's post test
[‡]Significantly different ($P < 0.01$) from the wild-type (**1/*1*) group with Dunn's post test

highly different in the individuals with two mutated alleles as compared with those heterozygous for **2* or **3*. Thus, data from these single individuals do not clearly support any strong influence of *CYP2C9* genotype. We acknowledge that the low number of patients with *CYP2C9*3/*3* genotype is a limitation of the present study to conclude the influence of *CYP2C9* genotype on fluoxetine plasma concentrations.

Nevertheless, CYP2C9 enzyme activity impairment may also result from concomitant medication, with an inhibitor or substrate of CYP2C9, and a consequence of this interaction could be an alteration of the disposition of fluoxetine and/or the concomitant drug. In light of the present results, the previously reported drug interactions during concomitant administration of fluoxetine with CYP2C9 substrates might be explained [2, 3, 4, 5, 6, 7, 8].

In the present study, fluoxetine/norfluoxetine ratio was correlated with *CYP2D6* genotypes and the plasma concentration of fluoxetine was influenced by the number of *CYP2D6* active genes. These results and a previous report [23] support that the CYP2D6 enzyme is involved in the metabolism of fluoxetine to norfluoxetine in patients under steady-state conditions. Since the activity of CYP2D6 is polymorphic [21], and, in addition, fluoxetine and norfluoxetine are potent inhibitors of CYP2D6 [17, 18, 19, 20, 22, 23, 24, 25, 26], interindividual variability of drug and metabolite plasma concentrations might occur during fluoxetine treatment.

Fluoxetine is administered as a racemic drug containing 50% of each of the S- and R-enantiomers of fluoxetine. It would appear that both CYP2D6 and CYP2C9 contribute to the formation of R-norfluoxetine, whereas only CYP2D6 is involved for the conversion to S-norfluoxetine [19, 22, 39]. In the present study, the stereospecific metabolism of fluoxetine enantiomers was not studied, but, rather, the overall effect of CYP2C9 and CYP2D6 enzymes under clinical conditions was evaluated. In a recent study, the plasma levels of individual enantiomers and the active moiety (sum of the concentrations of R-fluoxetine, S-fluoxetine and S-norfluoxetine) in responder patients did not differ significantly from those found in patients with an unsatisfactory therapeutic response [40]. It has also been reported that the concentrations of individual enantiomers and of the active moiety are similar in patients with or without adverse effects [40]. This finding suggests that fluoxetine enantiomers may not be clinically relevant in patients, although this question requires further studies.

In conclusion, *CYP2D6* and potentially *CYP2C9* genotypes may influence the plasma concentrations of fluoxetine. Since both CYP2C9 and CYP2D6 are involved in the metabolism of several commonly used drugs, interactions with fluoxetine may occur when these drugs are administered together.

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