PHARMACOKINETICS OF FLUVOXAMINE IN RELATION TO CYP2C19 PHENOTYPE AND GENOTYPE

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SUMMARY

Objective: To evaluate the pharmacokinetics of fluvoxamine (FLV) in poor metabolizers (PMs) versus extensive metabolizers (EMs) of cytochrome P450 (CYP)2C19.

Methods: This was a prospective, open-label study conducted at the Clinical Research Unit School of Pharmacy. Fifty-seven healthy, nonsmoking volunteers aged 21-40 years participated. Subjects abstained from caffeinated products 12 hours prior to and during each testing period. To assess CYP2C19 activity, blood samples were collected from each subject prior to and two hours after a single dose of omeprazole 20 mg. Once PMs were identified, a sample population of EMs were selected for comparison between the two groups regarding FLV disposition. A single 100 mg FLV dose was given to EMs and PMs; blood samples for FLV analysis were obtained prior to drug administration and 0.5, 1, 2, 3 ,4 6, 8, 12 and 24 hours later. A

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blood sample one day prior to FLV administration was also obtained for CYP2C19 and CYP2D6.

Results: Four PMs were identified with the omeprazole phenotype probe and had a mean ± SD hydroxylation index of 1.335 ± 0.271. Nine EMs were selected based upon a hydroxylation index between 0.100 and 0.400 (mean 0.193 ± 0.079). FLV pharmacokinetic parameters (AUC, elimination half-life, C_{max} and T_{max}) did not significantly differ between the two groups. Genotype analysis for CYP2C19 revealed a mutant allele for the *2 which confirmed phenotype detection of PM status. Genotype analysis for CYP2D6*3 and *4 alleles showed that all PMs of CYP2C19 were EMs of CYP2D6.

Conclusions: FLV disposition and dosing is unlikely to be affected by CYP2C19 polymorphism.

KEY WORDS

pharmacokinetics, fluvoxamine, phenotype, genotype, CYP2C19

INTRODUCTION

Fluvoxamine (FLV) is a selective serotonin reuptake inhibitor (SSRI) used for the treatment of obsessive-compulsive disorder and depression (Europe and Japan) /1/. FLV, like other SSRIs (e.g. paroxetine), displays marked intersubject pharmacokinetic variability. FLV is eliminated mainly via *O*-demethylation to various metabolites that lack significant pharmacological activity /2/. FLV metabolism is linked to the cytochrome P450 (CYP) isozymes; it has been reported to be a substrate for CYP1A2 and CYP2D6 /3/. FLV has been reported to have modest CYP inhibitory action on CYP2D6 and potent inhibition of CYP1A2 /4,5/. Recently, FLV has been shown to be an inhibitor of CYP2C9 and 2C19 /6-8/. Drugs which are also substrates of CYP2C9 and CYP2C19 could be significantly influenced by FLV. For example, tolbutamide and diazepam metabolism has been reported to be significantly altered upon FLV co-administration /8,9/. Like CYP2D6, CYP2C19 displays polymorphic distribution, with two populations which can be described as extensive metabolizers (EMs) and poor metabolizers (PMs) /10/. The pharmacokinetics of sertraline,

another SSRI antidepressant, has been reported to significantly differ in relation to the CYP2C19 genetic polymorphism: PMs showed significantly lower drug clearance than EMs /11/. FLV disposition in relationship to CYP2D6 and CYP1A2 phenotype and genotype has been evaluated /12,13/. FLV pharmacokinetics has been evaluated only with the CYP2C19 phenotype but not genotype in a small number of subjects /14/. The aim of this study was to investigate the relationship between FLV disposition and polymorphic CYP2C19 activity.

METHODS

Subjects

Healthy women and men (n = 57) volunteered for participation in this study. The study population included 14 Asians, nine African-Americans and 34 Caucasians. All subjects were non-smokers and screened by medical history for any medical illnesses and/or medications that could influence the results of the study. Each subject gave written informed consent; the University Institutional Review Board approved the protocol of this study.

Design

The study was conducted in two phases. In the first phase, each subject was phenotyped for CYP2C19 activity using a test dose of omeprazole 20 mg /15,16/. All subjects abstained from caffeine-containing beverages for 12 hours prior to the test and during the testing period. A 10 ml blood sample was obtained prior to drug administration and a second blood sample was collected two hours later. All blood samples were immediately placed in an ice bath. Serum was immediately separated from the blood sample by centrifugation for 20 minutes at 3,000 rpm and frozen at -80°C until assay. Omeprazole and the 5′-hydroxyomeprazole metabolite were assayed by liquid chromatography /15,16/.

Omeprazole hydroxylation activity was expressed as $\log_{10}$ [omeprazole/5′-hydroxyomeprazole] with the two hour sample. PMs were classified as those subjects with a log omeprazole hydroxylation (OM/OM-OH) index antimode of >0.8. Four PMs were identified

(three women and one man; three Asians and one Caucasian) with a mean log OM/OM-OH index of 1.335 ± 0.271. Nine EMs (five women and four men; three Asians and six Caucasians) were selected for comparison based upon OM/OM-OH ratios between 0.100 and 0.400. This range was selected to exclude any potential ultrarapid hydroxylators and to separate the EMs from the PMs. The EMs had a mean log omeprazole hydroxylation index of 0.193 ± 0.079. Age and weight did not differ significantly between the two groups, as shown in Table 1.

A 20 ml whole blood sample was obtained from the PMs for genotype analysis of CYP2C19 and CYP2D6. Genotyping procedures for identification of CYP2C19 and CYP2D6 mutant (mt) alleles were performed by polymerase chain reaction (PCR) amplification with primers specific for the CYP2C19*2 (m1) and CYP2C19*3 (m2) mutations and for CYP2D6*3 and *4 alleles /16,17/. PCR products were digested with *Sma* I and were analyzed by gel electrophoresis.

All PMs and selected EMs of CYP2C19 underwent a complete physical examination, laboratory tests and ECG, which were all within normal limits. A single dose of FLV 100 mg was administered to each subject. Blood samples (7 ml) were collected prior to drug administration and at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours later. FLV was assayed by high-performance liquid chromatography with fluorescence detection. The lower limit of detection was 10 ng/ml. The interassay and intra-assay precision at 25 ng/ml was ≤3% and <10%, respectively /18/.

Pharmacokinetic and statistical analysis

Pharmacokinetic parameters were calculated by noncompartmental analysis using the WinNONLIN computer program (Cary, NC). Peak serum concentration (C_{max}) and time to reach peak serum concentrations (T_{max}) were derived directly from observed data. Area under the serum concentration time curve (AUC) was determined by the use of the linear trapezoidal rule to 24 hours and log transformed for analysis. The linear terminal slope (λ) of the log-concentration of FLV was calculated by the least square regression method. The elimination half-life values were calculated as $0.693/\lambda$. Data analysis comparing EMs vs PMs was conducted with the two-tailed unpaired Student's t-test. A significant difference was defined as $p < 0.05$.

TABLE 1

Summary of the pharmacokinetic parameters of fluvoxamine (mean ± SD) in extensive metabolizers (EMs) vs poor metabolizers (PMs) of CYP2C19

	Age (yr)	Weight (kg)	AUC (ng/ml·hr)	$t_{½}$ (h)	C_{max} (ng/ml)	T_{max} (h)
EMs	22.8 ± 0.9	61.8 ± 13.3	508.8 ± 193.2	15.1 ± 7.0	38.2 ± 12.1	5.3 ± 1.7
PMs	24.5 ± 2.4	52.6 ± 6.0	554.6 ± 367.1	15.6 ± 7.2	36.6 ± 13.6	6.3 ± 4.0
p-value	0.08	0.09	0.34	0.40	0.38	0.22

AUC = area under the serum concentration time curve; $t_{½}$ = elimination half-life; C_{max} = peak serum concentration; T_{max} = time to reach peak serum concentration.

RESULTS

Subjects' age, weight and disposition of FLV in PMs and EMs are presented in Table 1. Significant differences between the two groups were not found despite the wide interindividual variability in FLV pharmacokinetics. The FLV serum concentrations over time also displayed similar profiles for both PMs and EMs (Fig. 1). FLV was well tolerated in all subjects. One PM subject showed mild sedation in the 2-4 hour time period and two EMs reported incidents of headache which resolved uneventfully and without use of PRN medication. The 2C19 genotype results of the four PMs identified through the omeprazole phenotype had a mutant allele noted for the C2C19*2 but not the CYP 2C19*3 allele. Identification of a CYP2C19 mutant *2 allele indicates an inactive enzyme, confirming PM status. Genotype analysis for CYP2D6*3 and *4 alleles revealed all subjects as wild-type, which indicates an active enzyme and CYP2D6 EM status /19/.

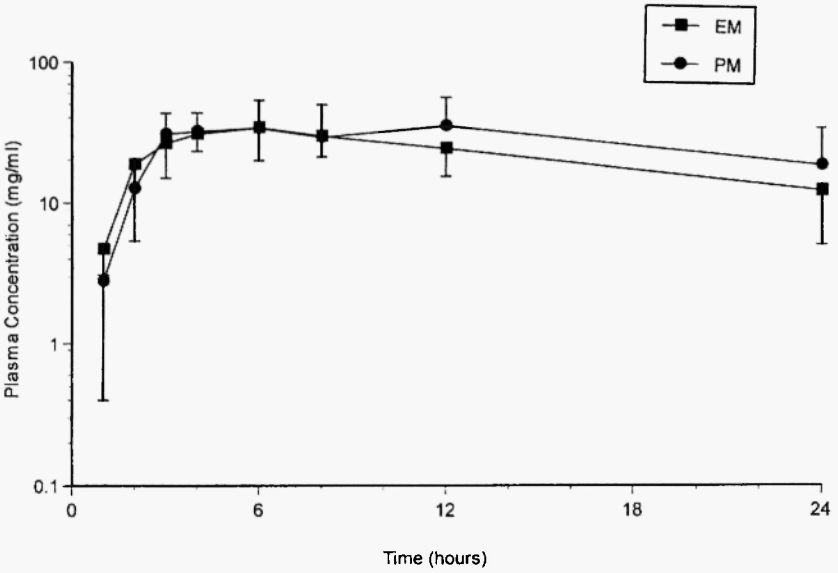

Fig. 1: Fluvoxamine plasma concentrations over time in EMs versus PMs of CYP2C19 when given a single dose of 100 mg.

DISCUSSION

FLV disposition shown in Table 1 in this study population resembled the pharmacokinetic data of previous FLV studies /1/. Further calculations of FLV pharmacokinetic parameters (e.g. K_{el}) were deemed unnecessary since determinations would not provide further significant findings regarding FLV disposition.

Subjects were classified as either EMs or PMs of CYP2C19 based on phenotyping results with omeprazole. This is an established method for detecting polymorphic groups that include different ethnic populations /15,16,20/. The mean log omeprazole hydroxylation index of 1.335 clearly separates the identified PMs from the EMs who had a mean hydroxylation index of 0.193.

Genotype analysis of the four PMs for CYP2C19*2 revealed mutant alleles, confirming the phenotype results of detecting PMs. The CYP2C19*3 results were wild-type alleles, indicating enzyme activity. The detection of mutant CYP2C19*2 alleles versus mutant *3 alleles in PMs is more prevalent among certain ethnic groups /19/. The polymorphic distribution of CYP2C19 indicates that Asian populations have the most prevalence of PMs compared to other ethnic groups /21/. An example of CYP2C19 genetic polymorphism influencing drug disposition has been reported in Chinese subjects treated with diazepam /22/. Subjects with mutant genes in either *2 or *3 alleles had significantly longer elimination half-lives of diazepam and desmethyldiazepam than subjects with wild-type alleles.

In comparing FLV disposition between PMs and EMs of CYP2C19, significant differences between the two groups were not found. Therefore, these results would suggest that FLV dosing should not differ between these two groups. Results of this study demonstrate FLV pharmacokinetics should not differ among Asians compared to other ethnic populations based upon CYP2C19 polymorphism. This finding with CYP2C19 coupled with the low incidence of PMs among Asians for CYP2D6 would also support this drug dosing recommendation.

Genotype results in this study confirmed that the PMs of CYP2C19 were also EMs of CYP2D6, thus excluding any polymorphic complications from CYP2D6. Other investigators have shown that FLV disposition is influenced by CYP2D6 polymorphism /13,14/. Only one study compared FLV pharmacokinetics in PMs and EMs of

both CYP2C19 and CYP2D6 using only phenotype detection /14/. Their findings indicated that FLV disposition differed among CYP2D6 PMs and EMs but not with CYP2C19 PMs and EMs. The results of the present study support those previous findings with CYP2C19 with the added confirmation of genotype analysis. The number of EMs and PMs in this study were similar to those of Spigset *et al.* /14/. Only non-smokers were allowed to participate in this study, thereby controlling for any impact of smoking upon FLV pharmacokinetics through CYP1A2 induction.

This study was limited by single dose administration of FLV and may not translate to patients treated clinically with FLV during chronic therapy. FLV pharmacokinetics may also display non-linear disposition /1/, which cannot be evaluated with single dose drug administration. The last blood sampling time period for this study was collected at 24 hours post-dose. This time period may have been inadequate to determine precisely fluvoamine's terminal elimination half-life. An additional sample for fluvoxamine pharmacokinetics at 48 hours was used in another study /14/; however, no significant differences in the drug's pharmacokinetics were found. Based upon this information, it was decided that the 48-hour blood sample would not be obtained. The number of subjects in this study was small; however, these findings were comparable to other previous studies that also utilized similar small subject populations /13,14/. Genotype analysis was conducted in only the CYP2C19 PMs and not the EMs and was used only as a confirmation of PM status and would not provide any additional information regarding EM activity. Furthermore, genotyping only the PMs of CYP2C19 for CYP2D6*3 and *4 alleles was conducted to screen for any potential PMs of CYP2D6. Other CYP2D6 alleles, such as the *5 (PM status) or *2xn alleles (ultra-rapid EM status) were not analyzed /19/. The incidence of CYP2D6 PMs for the *5 allele is lower than that for the *3 and *4 alleles /19/. The CYP2D6*10 mutant allele has also been identified as an enzyme with decreased activity especially noted to occur among Asians /23/. This study did not choose to analyze for the CYP2D6*10 allele based upon the lack of significant differences observed in the FLV serum concentrations between the two CYP2C19 groups. In a Japanese study with haloperidol, an antipsychotic drug that is partially influnced by CYP2D6, it was found that patients with the mutant CYP2D6*10 allele had 27% higher serum drug concentration than the

patients with the wild-type allele /23/. If differences in FLV disposition were found, this study would have included this analysis for the CYP2D6*10 allele. However, since FLV disposition did not significantly differ based upon the CYP2C19 PMs versus EMs, it was decided that additional genotype analysis of CYP2D6 (with the possible exception of CYP2D6*10) would be unlikely to yield any further significant findings.

The small numbers of subjects in this study is a limitation in interpreting these findings. However, other previous studies that employed genotyping and phenotyping have reported similar number of subjects: PMs (n = 4-6) vs EMs (n = 9-10) /7,12-14/. The small proportion of subjects who are PMs illustrates the difficulty in finding a larger group unless large numbers of subjects are screened in some routine manner with adequate laboratory and financial support. To detect a 30% change in drug clearance with a statistical power of $\beta = 0.80$ and $\alpha = 0.05$, which is typical of a drug interaction study, would require 14-16 subjects /24/. When these principles are applied to the present study, the number of subjects needed would be 16 in the PM and 16 in the EM group. To find 16 subjects who are CYP2C19 PMs could take a prolonged period, which may not provide any additional significant findings. However, when consistent findings occur even with these small numbers of subjects, an overall scope of FLV disposition emerges. To date, no other studies have shown differences in fluvoamine disposition, and a larger study with fluvoxamine may be needed to fully characterize the influence of CYP2C19 status (PMs vs EMs) upon its pharmacokinetics.

In conclusion, the results from this study indicate that CYP2C19 polymorphism is unlikely to be a significant factor in FLV pharmacokinetics. Phenotypic and genotypic studies with a larger number of subjects and under steady-state conditions may provide further information regarding FLV disposition and CYP polymorphism.

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