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Lack of correlation between fluvoxamine clearance and CYP1A2 activity as measured by systemic caffeine clearance

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Abstract *Objective*: Evidence exists to suggest that fluvoxamine is metabolized by CYP1A2. The present study was undertaken in order to further elucidate the role of CYP1A2 in fluvoxamine disposition.
Methods: Twelve healthy non-smoking male volunteers participated in this cross-over study. Six subjects received first fluvoxamine 50 mg as a single oral dose and, some weeks later, caffeine 200 mg as a single oral dose. The other six subjects received the drugs in reverse order. Serum concentrations of fluvoxamine, caffeine and paraxanthine were measured and standard pharmacokinetic parameters were calculated.
Results: There were no significant correlations between caffeine clearance and fluvoxamine oral clearance ($r_s = -0.30$; $P = 0.43$) or between the paraxanthine/caffeine ratio in serum 6 h after caffeine intake and fluvoxamine oral clearance ($r_s = -0.18$; $P = 0.58$).
Conclusion: CYP1A2 does not appear to be of major importance in the metabolism of fluvoxamine.

Key words Caffeine · CYP1A2 · Fluvoxamine

Introduction

The selective serotonin reuptake inhibitors citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline display marked differences in their drug metabolism pattern. Fluoxetine and paroxetine are metabolized by and inhibit the polymorphic liver enzyme CYP2D6 [1, 2]. Citalopram is predominantly a substrate of the polymorphic CYP2C19 [3]. Fluvoxamine is metabolized by CYP2D6 [4, 5], but not by CYP2C19 [4], although it inhibit the activity of CYP2C19 [6]. Fluvoxamine is also a potent inhibitor of drugs catalysed by CYP1A2, such as caffeine [6], theophylline [7] and clozapine [8].

In a recent study, smokers were found to have lower serum concentrations of fluvoxamine than non-smokers after a single oral dose of fluvoxamine [9]. In another study in which both smokers and non-smokers were included, a significant positive correlation was found between caffeine N3-demethylation and fluvoxamine clearance [5]. However, both CYP1A1 and CYP1A2 are inducible by polycyclic aromatic hydrocarbons in cigarette smoke [10, 11]. The present study was performed in order to elucidate the role of CYP1A2 in fluvoxamine metabolism without the possible influence of CYP1A1 when both smokers and non-smokers are studied.

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Materials and methods

After giving their informed consent, 12 volunteers took part in the investigation, which was approved by the regional Ethics Committee at the University of Umeå. All subjects were healthy, as assessed by medical history, physical examination and routine blood chemistry tests. They were all extensive metabolizers of drugs catalysed by CYP2D6 and CYP2C19, tested by means of dextromethorphan and mephenytoin, respectively [4]. All the subjects were non-smoking males, and they had been entirely drug-free for at least 2 weeks prior to the study periods. Their age [mean with (SD)] was 23.6 (2.3) years and their body weight was 74.2 (8.0) kg.

The subjects were randomized into two groups. One group received a single oral dose of 50 mg fluvoxamine (Fevarin; enteric-coated fluvoxamine maleate, Solvay Duphar, Veesp, The Netherlands) on day 1 in the first study period, whereas the other group received a single oral dose of 200 mg caffeine (2 tablets of 100 mg Koffein ACO; ACO, Helsingborg, Sweden) the same day. On day 1 in the second study period 1–5 weeks later, the subjects received the other drug. Both drugs were ingested at 0800 hours after an overnight fast. No food was allowed for the next 8 h, except for a standardized lunch at 1200 hours. A standardized dinner was given at 1600 hours. Intake of food or beverages containing caffeine or other methylxanthines was not allowed from 30 h before the start of the study periods until the last blood sample in the same period had been obtained.

After intake of the study drugs, venous blood samples (10 ml) were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 32 h and for fluvoxamine also after 48 h. Serum was separated within 30 min and stored at −20 °C until analysis.

Drug assays

Serum concentrations of fluvoxamine were determined by a method described in detail elsewhere [9]. In brief, to 2 ml serum was added 5 ml 0.3 M Na_3PO_4 and 400 µl di-isopropylether. Imipramine was used as internal standard. After mixing for 20 min and centrifuging for 10 min, the organic layer was transferred to new tubes and analysed by high-performance liquid chromatography (HPLC). The mobile phase consisted of 65 ml methanol, 345 ml acetonitrile and 1.7 ml ammonia 25%. The separation was performed on a straight phase 150 × 4.6 mm Apex Silica 3-µm column (Jones Chromatography, Mid Glamorgan, UK) with a flow of 1.3 ml·min^{-1}. The ultraviolet detector was set at a wavelength of 254 nm. The limit of quantification was 0.5 nmol·l^{-1} and the method was linear at least up to 3000 nmol·l^{-1}.

Serum concentrations of caffeine were determined by an HPLC method based on a method for detection of caffeine in saliva [12], with minor modifications. In brief, solid-phase extraction cartridges (Isolute MF C18, International Sorbent Technology, Mid Glamorgan, UK) were conditioned with 2 × 1 ml methanol with 0.01 M sodium phosphate (pH 7.5). Thereafter, serum and the internal standard β-hydroxyethyltheophylline were mixed with 0.01 M sodium phosphate and applied to the columns. The columns were then washed with 2 × 1 ml 0.01 M sodium phosphate and 350 µl acetone. The compounds were eluted with 850 µl acetone, evaporated to dryness under nitrogen and redissolved in the mobile phase, consisting of 3 mM sodium acetate with 1.4% acetonitrile, 1% methanol and 1.6% tetrahydrofurane. The separation was performed on a Nucleosil ODS 5-µm 25-cm column (Jones Chromatography, Mid Glamorgan, UK) with a flow of 1.2 ml·min^{-1}. The ultraviolet detector was set at a wavelength of 273 nm. Mean recoveries after solid-phase extraction were 108% for caffeine, 95% for paraxanthine and 101% for β-hydroxyethyltheophylline. At concentrations of 15 µmol·l^{-1}, the intraassay and interassay coefficients of variation were 1.9% and 10.9%, respectively, for caffeine, and 5.1% and 12.1%, respectively, for paraxanthine. The limit of quantification was 0.5 µmol·l^{-1}, and the method was linear at least up to 250 µmol·l^{-1}.

Pharmacokinetic and statistical analyses

Peak concentrations (C_{max}) and time of peak concentrations (t_{max}) were derived directly from the measured values. Other pharmacokinetic parameters were calculated by use of the pharmacokinetic program package Siphar/Win, version 1.13 (Simed, Creteil, France). The parameter estimates describing the linear terminal slopes (λ_z) of the log concentrations of fluvoxamine and caffeine were calculated by means of the peeling procedure, using a non-compartmental model. Areas under the serum concentration-time curve (AUC) were calculated by use of the linear trapezoidal rule with extrapolation to infinity. Mean residence time (MRT) was calculated as AUMC/AUC, in which AUMC is the area under the concentration × time product versus time curve from zero to infinity. Oral clearance (CL/F, in which F is the oral bioavailability) was calculated as Dose/AUC. As an additional measure of CYP1A2 activity, the paraxanthine/caffeine ratio in serum 6 h after caffeine intake was calculated [13].

The study was designed to reveal correlation coefficients of 0.6 or higher between caffeine and fluvoxamine clearances or AUCs. Given this assumption, a total of 12 subjects is required with $\alpha = 0.05$ and $\beta = 0.20$ [14]. For statistical analysis, Spearman's rank correlation test was used. P values of less than 0.05 were regarded as statistically significant.

Results

Drug disposition data for caffeine and fluvoxamine and r_s values for the correlations between the pharmacokinetic parameters for caffeine and fluvoxamine are presented in Table 1. The lack of relationship between caffeine clearance and fluvoxamine clearance is illustrated in Fig. 1. The r_s value for the correlation between the paraxanthine/caffeine ratio in serum 6 h after caffeine intake and fluvoxamine clearance was −0.18 ($P = 0.58$, not significant). There was a significant positive correlation between caffeine clearance and the paraxanthine/caffeine ratio ($r_s = 0.80$, $P = 0.002$).

Discussion

The principal finding in the present study was that no relationship was revealed between fluvoxamine clearance and two commonly used measures of CYP1A2 activity, the systemic caffeine clearance and the paraxanthine/caffeine ratio in serum. These observations indicate, in contrast to the results from earlier studies [5, 9], that CYP1A2 is not of major importance in the metabolism of fluvoxamine.

The lack of significant correlations in the present study is probably not explained by methodological factors. The mean serum concentration of fluvoxamine after 48 h was 2.9 nmol·l^{-1}, and the observed maximum half-life was 14.3 h. It is therefore unlikely that fluvox-

Table 1 Pharmacokinetic parameters for caffeine and fluvoxamine, and r_s values for the correlation between them in 12 healthy non-smoking volunteers. There were no significant correlations. *AUC* area under the serum concentration-time curve, *CL/F* oral clearance, C_{max} peak concentration, t_{max} time of peak concentration, $t_{½}$ elimination half-life, *MRT* mean residence time

	Caffeine Median (range)	Fluvoxamine Median (range)	r_s value	P value
AUC[a]	169 (131–268)	729 (371–2004)	−0.30	0.43
CL/F (ml·min^{-1})	101 (64–131)	3599 (1303–7046)	−0.30	0.43
C_{max}[b]	23.5 (17.7–30.1)	44.0 (30.0–77.3)	0.47	0.12
t_{max} (h)	1 (1–2)	5 (3–8)	0.37	0.24
$t_{½}$ (h)	5.9 (2.7–8.0)	9.6 (7.3–14.3)	−0.12	0.71
MRT (h)	7.5 (4.6–10.8)	17.3 (10.8–13.7)	−0.21	0.51

[a] h·µmol^{-1}·l^{-1} for caffeine, h·nmol^{-1}·l^{-1} for fluvoxamine
[b] µmol·l^{-1} for caffeine, nmol·l^{-1} for fluvoxamine

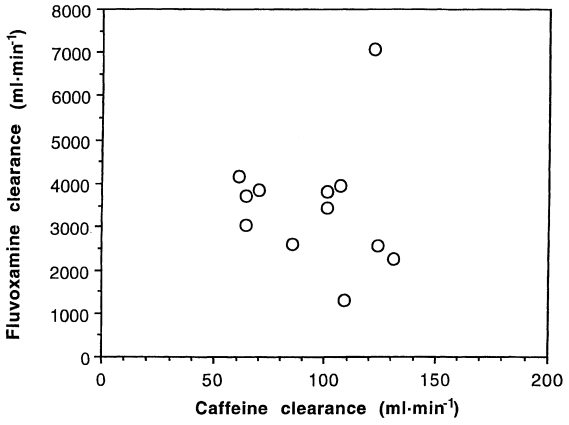

Fig. 1 Relationship between caffeine clearance and fluvoxamine clearance in 12 non-smoking healthy volunteers who were given caffeine and fluvoxamine in single oral doses on two different occasions. There was no significant correlation ($r_s = -0.30$, $P = 0.43$)

amine could inhibit CYP1A2 and diminish the metabolism of caffeine 1–5 weeks later, when caffeine was given. Moreover, the subjects received the drugs in randomized order and the mean caffeine AUCs in the group receiving caffeine before fluvoxamine and in the group receiving caffeine after fluvoxamine were comparable [186 (54) vs 196 (46) $h \cdot \mu mol \cdot l^{-1}$]. The inclusion of 12 subjects in the study makes the occurrence of type II errors unlikely, and the interindividual variability in fluvoxamine clearance (almost six-fold) should be sufficiently wide great to allow generalizations from the findings. However, the variability in caffeine clearance (two- to three-fold) was, as expected, less than in another study including both smokers and non-smokers [5]. Thus, it cannot be excluded that this relatively small variability might be a factor contributing to the lack of significant correlations in the present study.

The major pathway of the caffeine metabolism is the N3-demethylation, which leads to the formation of paraxanthine. This pathway accounts for approximately 80% of the elimination of caffeine [15], and CYP1A2 is clearly the most important enzyme involved in this route [16, 17]. Moreover, CYP1A2 is also involved in the other primary metabolic steps in caffeine degradation. Thus, CYP1A2 most probably accounts for more than 95% of the primary systemic caffeine clearance in the great majority of subjects [18] and systemic caffeine clearance is therefore held to be the gold standard for measurements of CYP1A2 activity [18]. Several different urinary metabolic ratios have been proposed as measures of CYP1A2 activity after caffeine intake [19]. However, the amount of caffeine recovered in urine is sparse [20] and depends on the urinary flow [21], and the correlation between several of the suggested ratios and systemic caffeine clearance is low [13]. Therefore, it has been concluded that the use of urinary metabolic ratios is an inaccurate probe for assessing the distribution of CYP1A2 activity [19]. An alternative method is measurement of the caffeine/paraxanthine plasma ratio, which has a correlation of 0.72–0.96 to systemic caffeine clearance [13]. Thus, both caffeine measures employed in the present study are valid markers of CYP1A2 activity, although CYP1A1 may also contribute to some extent, at least to caffeine N3-demetylation [22, 23].

CYP1A1 seems to be present in larger amounts in subjects who smoke than in non-smokers [10, 11], although the effect of polycyclic aromatic hydrocarbons on hepatic CYP1A1 induction have been very low in some studies [24, 25]. CYP1A1 also demonstrates considerable interindividual heterogeneity in its expression among non-smokers [26]. One possible reason for this heterogeneity might be the existence of a CYP1A1 polymorphism [27].

Although CYP1A1 and CYP1A2 are closely related, some evidence of substrate specificity and inhibitor specificity exists [28, 29]. Interestingly, in human liver microsomes, fluvoxamine has been found to inhibit CYP1A2 without inhibiting CYP1A1 [27]. As fluvoxamine is known to be a very potent inhibitor of CYP1A2 in vivo [6], CYP1A2 might be inhibited to such a degree that the metabolism of fluvoxamine via this enzyme is limited compared with the CYP1A1 pathway. A drug may well be an inhibitor of an enzyme without being metabolized by it, as exemplified by quinidine and CYP2D6 and fluvoxamine and CYP2C19 [4, 6].

The results from earlier studies indicated that CYP1A2 could be one of the major enzymes in fluvoxamine metabolism [5, 9]. In one of these studies [5], the significantly positive correlation between caffeine N3-demethylation and fluvoxamine clearance was based upon a sample of 14 subjects, of whom five were non-smoking CYP2D6 extensive metabolizers and five were smoking CYP2D6 extensive metabolizers. When these two groups are analysed separately, it appears that a possible positive within-group correlation is caused by one outlier among the non-smokers, and that the correlation among smokers seems to be negative and caused by an outlier also in this group. Thus, smoking (and thereby increased CYP1A1 as well as CYP1A2 activity) could clearly be a confounding factor causing the overall significant correlation [5]. In the other study [9], in which smokers were found to have higher fluvoxamine clearances than non-smokers, the effect of CYP1A2 induction cannot be clearly separated form the effect of CYP1A1 induction.

In conclusion, the present study indicates that CYP1A2 activity, as measured by systemic caffeine clearance and the paraxanthine/caffeine ratio in serum, does not significantly correlate with fluvoxamine disposition in non-smokers and therefore that the impact of CYP1A2 on fluvoxamine biotransformation in non-smoking subjects is low. Taken together with the results from earlier studies demonstrating that smoking increases fluvoxamine biotransformation, one hypothesis, although speculative, is that CYP1A1 might be as important as CYP1A2 in fluvoxamine metabolism. Further studies are needed to clarify the role of CYP1A1 relative to CYP1A2 in fluvoxamine metabolism.

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