

A fluvoxamine-caffeine interaction study

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The selective serotonin reuptake inhibitor fluvoxamine is a very potent inhibitor of the liver enzyme CYP1A2, which is the major P450 catalysing the biotransformation of caffeine. Thus, a pharmacokinetic study was undertaken with the purpose of documenting a drug-drug interaction between fluvoxamine and caffeine.

The study was carried out as a randomized, *in vivo*, cross-over study including eight healthy volunteers. In Period A of the study, each subject took 200 mg caffeine orally, and in Period B, the subjects took fluvoxamine 50 mg per day for 4 days and 100 mg per day for 8 days. On day 8 in Period B, the subjects again ingested 200 mg caffeine. After caffeine intake, blood and urine were sampled at regular intervals. Caffeine and its three primary demethylated metabolites, paraxanthine, theobromine and theophylline in plasma and the same four compounds plus 11 more metabolites in urine were assayed by HPLC.

During fluvoxamine, the median of the total clearance of caffeine decreased from 107 ml min⁻¹ to 21 ml min⁻¹ and the half-life increased from 5 to 31 h. The N3-demethylation clearance of caffeine to paraxanthine decreased from 46 to 9 ml min⁻¹; the N1- and N7-demethylation clearances decreased from 21 to 9 ml min⁻¹ and from 14 to 6 ml min⁻¹, respectively.

The results confirm that CYP1A2 is the main enzyme catalysing the biotransformation of caffeine, in particular the N3-demethylation and partly the N1- and N7-demethylation. The results indicate that intake of caffeine during fluvoxamine treatment may lead to caffeine intoxication. Finally, our study provides additional evidence that fluvoxamine can be used to probe CYP1A2 in drug metabolism.

Keywords: fluvoxamine, caffeine, CYP1A2, interaction

Introduction

Fluvoxamine is an antidepressant that belongs to the class of selective serotonin reuptake inhibitors (SSRIs). Fluvoxamine is eliminated predominantly by oxidation in the liver (Benfield & Ward, 1986), however, the P450s responsible for the oxidation of fluvoxamine have not yet been identified. Fluvoxamine is a very potent inhibitor of the N-demethylation of imipramine both in human liver microsome preparation (Skjelbo & Broesen, 1992) and *in vivo* (Spina *et al.*, 1992). Cytochrome P4501A2 (CYP1A2) is an important enzyme catalysing the conversion of imipramine to the active metabolite desipramine (Lemoine *et al.*, 1993). These observations led to further investigations confirming that fluvoxamine indeed is a very potent inhibitor of

CYP1A2 (Broesen *et al.*, 1993), and among the SSRIs, fluvoxamine is the only one with this property.

Caffeine (1,3,7-trimethylxanthine) is a natural constituent of coffee, tea and coca-cola. Caffeine metabolism in humans is very complex and at least 14 metabolites have been identified (Fig. 1). The main route of elimination is N3-demethylation to paraxanthine (17X), and this route accounts for approximately 80% of the elimination of caffeine (Lelo *et al.*, 1986). Caffeine and its metabolites are eliminated both by demethylation or hydroxylation and by renal excretion.

CYP1A2 is major enzyme catalysing the formation of 17X (Butler *et al.*, 1989; Sesardic *et al.*, 1990; Berthou *et al.*, 1991), but only partially involved in the formation of 37X and 13X (Grant *et al.*, 1987; Berthou *et al.*, 1991; Gu *et al.*, 1992) (Fig. 1). At least three P450s, CYP1A2, CYP2E1 and CYP3A4 (Gu *et al.*, 1992), contribute to the formation of the minor metabolite

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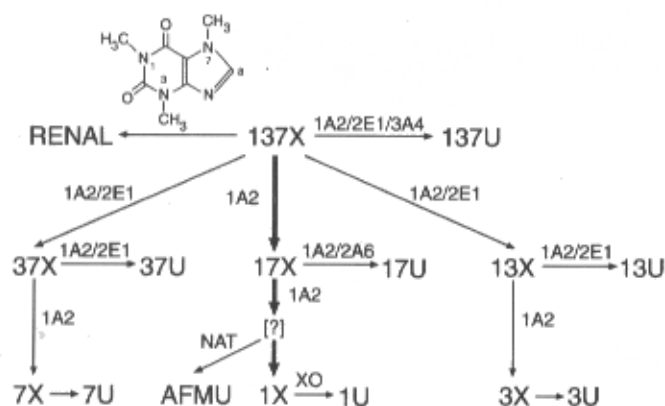


Fig. 1. The pattern of biotransformation of caffeine in humans. The thick arrows indicate the major pathway of the caffeine metabolism. Symbols beside the arrows indicate enzymes. Abbreviations: 137X, caffeine (1,3,7-trimethylxanthine); 137U, 1,3,7-trimethyluric acid; 17X, paraxanthine (1,7-dimethylxanthine); 13X, theophylline (1,3-dimethylxanthine); 37X, theobromine (3,7-dimethylxanthine); 17U, 1,7-dimethyluric acid; 13U, 1,3-dimethyluric acid; 37U, 3,7-dimethyluric acid; 1X, 1-methylxanthine; 1U, 1-methyluric acid; 3X, 3-methylxanthine; 3U, 3-methyluric acid; 7X, 7-methylxanthine; 7U, 7-methyluric acid; AFMU, 5-Acetylamino-6-formylamino-3-methyluracil; 1A2, CYP1A2 (cytochrome P4501A2); 2E1, CYP2E1 (cytochrome P4502E1); 3A4, CYP3A4 (cytochrome P4503A4); NAT, N-acetyltransferase; XO, xanthine oxidase.

137U (Fig. 1). The demethylation of 17X to 1X is also catalysed by CYP1A2 (Campbell *et al.*, 1987a; Grant *et al.*, 1987). Thus, CYP1A2 is a very important enzyme for the biotransformation of caffeine, and therefore caffeine is used as a model drug for the quantitative measurement of CYP1A2 *in vivo* (Kalow & Tang, 1991). At least four different urinary caffeine metabolic ratios have been proposed: (AFMU + 1X + 1U)/17U (Campbell *et al.*, 1987b), (17X + 17U)/137X (Kadlubar *et al.*, 1990), 17X/137X (Butler *et al.*, 1992) and (AFMU + 1X + 1U + 17X + 17U)/137X (modified from Carrillo & Benitez (1994)). However, the excretion of both 137X and 17X depends on the urinary flow, and besides, the 137X recovery in urine is very low (Tang Liu *et al.*, 1983). Alternative methods are the ^{13}C -[N3-methyl] caffeine breath test (Kotake *et al.*, 1982; Lambert *et al.*, 1990) and measurement of the 17X/137X plasma ratio (Fuhr & Rost, 1994). However, due to the complexity of caffeine metabolism and the contribution of other P450s, each of the employed methods has its shortcomings (Kalow & Tang, 1993; Fuhr & Rost, 1994).

The main purpose of the present study was to confirm an expected pharmacokinetic interaction between fluvoxamine and caffeine due to a very potent inhibition of the CYP1A2 catalysed metabolism of caffeine. A second

purpose of the study was to further substantiate a role of fluvoxamine for the quantitative assessment of CYP1A2 in the biotransformation of drugs.

Materials and methods

The study was carried out as an open, randomized, cross-over study including eight volunteers, all men with a median age of 27 years (range 21–33 years). All were extensive metabolizers of the model drugs sparteine and mephenytoin and all were non-smokers. They had no known heart, liver, or kidney disease according to a clinical investigation, clinical chemical/hematological screening and ECG-test. They neither consumed alcohol nor drugs on a regular basis at the time of the study. The volunteers consented to participate in the study on the basis of verbal and written information, and the study was approved by the Regional Ethics Committee and by the Danish National Board of Health.

Study procedure

The volunteers were randomized into two groups. One group started with caffeine alone (Period A), and the other group started with fluvoxamine pre-treatment for 1 week and then caffeine on day 8 (Period B). After 2 weeks without medication, the two groups changed periods. In Period A, the volunteers abstained from all methylxanthine-containing beverages, foods and medications for 2 days prior to and until the last blood sample was drawn. After intake of a tablet of 200 mg caffeine (Nycomed DAK, Denmark), blood samples were drawn at 0.5, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 14 and 24 h. After methylxanthine abstinence for 2 days, no caffeine or metabolites were expected in plasma; consequently, no sample at time zero was collected. Urine was collected from 0 to 12 h and from 12 to 24 h, and in addition, a 10 ml spot urine was delivered after 5 h. In Period B, the volunteers abstained from all methylxanthine-containing beverages, foods and medications for 1 day prior to the administration of fluvoxamine and until the last blood sample was drawn. During the first 4 days, the volunteers took a tablet of 50 mg fluvoxamine (Solvay Duphar BV, Netherlands) at 8 a.m., and during the following 8 days they took 100 mg fluvoxamine each day. On day 8, the volunteers took 200 mg caffeine, and the blood samples were drawn at 0.5, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 14, 24, 36, 48, 72 and 120 h. Urine was collected in four periods: 0–12, 12–24, 24–36 and 36–48 h and also, a 10 ml spot urine was delivered after 5 and 24 h. With an expected half-life of 4–5 h, a caffeine dose will be excreted in the urine within 24 h. The expected interaction between caffeine and fluvoxamine would lead to longer half-life. There-

fore, the urine was collected for 48 h in period B. In both periods, the blood samples were drawn via a heparinized intravenous catheter (Venflon®, Viggo Spectramed, Sweden) from 0–14 h and thereafter by vein puncture. The blood was drawn in EDTA containing tubes (Terumo, Belgium). After centrifugation, the plasma was separated and frozen at -20°C until analysis. The sampling of 10 ml urine was prepared with 100 μl 2N HCl in order to conserve AFMU and frozen at -20°C until analysis. The urine volumes of the 12 h urine samples were recorded and 10 ml urine from each sample was kept with 100 μl 2N HCl at -20°C until analysis.

Analytic methods

Plasma and urine were analysed for caffeine and metabolites by modifications of a previously published HPLC assay (Vistisen *et al.*, 1992). Genuine caffeine and metabolites were purchased from Sigma (St Louis, MO) except 17U which was purchased from Fluka (Büchs, Switzerland) and AFMU which was a kind gift from Dr M. Arnaud (Nestlé, Switzerland). Organic solvents were of at least analytical grade and purchased from Merck (Darmstadt, Germany). Standards were prepared in plasma or urine from the subjects after 3 days of xanthine-free diet.

Briefly, 200 μl of plasma or urine was extracted with 6 ml chloroform/isopropanol after addition of 120 mg ammonium sulphate. The organic phase was dried at 40°C under nitrogen. After reconstitution of the residue in 200 μl eluent A, 25 μl was injected on the HPLC system consisting of the following Merck-Hitachi Instruments (San Jose, California): 655A-40 auto-sampler (cooled to 3°C), L-6200 intelligent pump, L-6000 pump, T-6300 column thermostat operated at 30°C , D-6000 interphase unit and a L-4000 UV detector operated at 280 nm. The analytic column, a Beckman Ultrasphere ODS (5 μm , 25 cm) (Beckman Instruments Inc., California USA), was eluted at 1 ml min^{-1} using a gradient profile. For plasma samples, eluent A was 0.05 acetic acid, and eluent B was 30% methanol in 0.05 acetic acid (v/v). The gradient profile was 25% B from 0 to 10 min, a linear ramp to 100% B at 22 min continued for 1 min and followed by a return to 25% B for 8 min. For urine samples, eluent A was 6.5% methanol in 0.05% acetic acid pH 3.2, and eluent B was 22% methanol in 0.05% acetic acid pH 7.0 (v/v). The gradient profile was 100% A from 0 to 13 min, a linear ramp to 20% B at 28 min, a second ramp to 100% B at 31 min continued for 3 min, and followed by a return to 100% A for 9 min.

All plasma or urine samples from each subject were analysed in one run and in duplicate. Quantitation was done from peak areas using external standardization.

Calibration graphs were linear in the relevant range. For 137X, 37X, 13X and 17X in plasma samples, the limit of detection was 0.1 μM and the intra-assay and inter-assay coefficients of variation were 3–5% and 6–8%, respectively. For caffeine and its 14 metabolites in urine samples, the limit of detection was 0.2–0.5 μM . The intra-assay coefficients of variation were 3–5% for AFMU, 7X, 1U, 3X, 1X, 13U, 37X, 17U and 137U, 6–8% for 7U, 37U, 17X, 13X and 137X and 10% for 3U. The inter-assay coefficients of variation were 8–13%.

Pharmacokinetic analysis

The following pharmacokinetic parameters were calculated without (Period A) and with (Period B) concomitant intake of fluvoxamine.

The total caffeine clearance:

$$Cl_{137X} = \text{Dose}/AUC_{137X(\text{total})} \quad (\text{eq. 1})$$

$AUC_{137X(\text{total})}$ is the area under the plasma concentration time curve calculated by the trapezoidal rule with extrapolation from the last measurable concentration to infinity. Complete absorption of caffeine from the intestine was assumed (Blanchard & Sawers, 1983).

The partial caffeine clearance via N3-demethylation (17X formation), $Cl_{137X \rightarrow 17X}$ was calculated as:

$$Cl_{137X \rightarrow 17X} = (17X + 17U + 1X + 1U + \text{AFMU})/AUC_{137X,0-t} \quad (\text{eq. 2})$$

The numerator is the amount of 17X and subsequent metabolites recovered in urine from 0–24 h in Period A and from 0–48 h in Period B. The $AUC_{137X,0-t}$ is the corresponding AUC of caffeine. The two other partial demethylation clearances were calculated similarly. Thus, the partial demethylation clearance via N1-demethylation (37X formation), $Cl_{137X \rightarrow 37X}$ was calculated as:

$$Cl_{137X \rightarrow 37X} = (37X + 37U + 7X + 7U)/AUC_{137X,0-t} \quad (\text{eq. 3})$$

and the partial clearance via N7-demethylation (13X formation), $Cl_{137X \rightarrow 13X}$ was calculated as:

$$Cl_{137X \rightarrow 13X} = (13X + 13U + 3X + 3U)/AUC_{137X,0-t} \quad (\text{eq. 4})$$

The conversion of 17X to 7X and of 37X to 3X and of 13X to 1X is considered to be negligible.

The partial clearance via 8-hydroxylation (137U formation), $Cl_{137X \rightarrow 137U}$ was calculated as:

$$Cl_{137X \rightarrow 137U} = 137U/AUC_{137X,0-t} \quad (\text{eq. 5})$$

The renal clearance of caffeine, Cl_{renal} was calculated as:

$$Cl_{\text{renal}} = 137X/AUC_{137X,0-t} \quad (\text{eq. 6})$$

The partial clearance of 17X via N7-demethylation (1X formation), $Cl_{17X \rightarrow 1X}$ was calculated as:

$$Cl_{17X \rightarrow 1X} = (1X + 1U + AFMU)/AUC_{17X,0-t} \quad (\text{eq. 7})$$

where $AUC_{17X,0-t}$ is the AUC of 17X from 0–24 h (Period A) or 0–48 h (Period B). Similarly, the partial clearance of 17X via 8-hydroxylation (17U formation), $Cl_{17X \rightarrow 17U}$ was calculated as:

$$Cl_{17X \rightarrow 17U} = 17U/AUC_{17X,0-t} \quad (\text{eq. 8})$$

The partial demethylation and hydroxylation clearances of 37X and 13X were calculated similarly. Thus, the partial clearance of 37X via N3-demethylation (7X formation), $Cl_{37X \rightarrow 7X}$ was calculated as:

$$Cl_{37X \rightarrow 7X} = (7X + 7U)/AUC_{37X,0-t} \quad (\text{eq. 9})$$

The partial clearance of 37X via 8-hydroxylation (37U formation), $Cl_{37X \rightarrow 37U}$ was calculated as:

$$Cl_{37X \rightarrow 37U} = 37U/AUC_{37X,0-t} \quad (\text{eq. 10})$$

The partial clearance of 13X via N1-demethylation (3X formation), $Cl_{13X \rightarrow 3X}$ was calculated as:

$$Cl_{13X \rightarrow 3X} = (3X + 3U)/AUC_{13X,0-t} \quad (\text{eq. 11})$$

The partial clearance of 13X via 8-hydroxylation (13U formation), $Cl_{13X \rightarrow 13U}$ was calculated as:

$$Cl_{13X \rightarrow 13U} = 13U/AUC_{13X,0-t} \quad (\text{eq. 12})$$

Eqs 2–5 and 7–12 are based on the assumption that the rate of metabolite formation equals the rate of appearance in urine.

The renal clearance of the 3 demethylated metabolites, paraxanthine, $Cl_{\text{renal},17X}$; theobromine, $Cl_{\text{renal},37X}$ and theophylline, $Cl_{\text{renal},13X}$ was calculated as:

$$Cl_{\text{renal},17X} = 17X/AUC_{17X,0-t} \quad (\text{eq. 13})$$

$$Cl_{\text{renal},37X} = 37X/AUC_{37X,0-t} \quad (\text{eq. 14})$$

$$Cl_{\text{renal},13X} = 13X/AUC_{13X,0-t} \quad (\text{eq. 15})$$

The elimination half-life of 137X was calculated as:

$$T_{1/2} = \ln 2/\lambda \quad (\text{eq. 16})$$

where λ is the terminal log linear slope determined by least squares regression analysis.

The volume of distribution, V_d was calculated as:

$$\begin{aligned} V_d &= Cl_{137X}/\lambda \\ &= \text{Dose}/AUC_{137X}\lambda \end{aligned} \quad (\text{eq. 17})$$

In the 10 ml spot urine after 5 h in both Periods and after 24 h in Period B, the following metabolic caffeine

ratios for assessment of N3-demethylation were determined:

$$(AFMU + 1X + 1U)/17U \quad (\text{eq. 18}) \quad (\text{Campbell et al., 1987b})$$

$$17X/137X \quad (\text{eq. 19}) \quad (\text{Kadlubar et al., 1990})$$

$$(17X + 17U)/137X \quad (\text{eq. 20}) \quad (\text{Butler et al., 1992})$$

$$(AFMU + 1X + 1U + 17X + 17U)/137X \quad (\text{eq. 21}) \quad (\text{Carrillo & Benitez, 1994})$$

In plasma, a caffeine metabolic ratio was calculated as 17X/137X after 6 h.

All of the pharmacokinetic parameters are reported as median and range, and the median difference [95% confidence interval] were calculated between Period A (without intake of fluvoxamine) and Period B (with concomitant intake of fluvoxamine). A 95% confidence interval for the median difference, not including zero, was considered statistically significant. The caffeine metabolic ratios were compared with the Cl_{137X} (eq. 1) and $Cl_{137X \rightarrow 17X}$ (eq. 2) by the Spearman's rank correlation test. A p value less than 0.05 was considered statistically significant. The statistical analysis was carried out by use of the MEDSTAT program package, version 2.1 (Astra Group, Albertslund, Denmark, 1988).

Results

The total, partial and renal clearances of caffeine (calculated according to eqs 1–6) are listed in Table 1. The median of the total caffeine clearance (eq. 1) was 107 ml min^{-1} in Period A and 21 ml min^{-1} in Period B. All the N-demethylations clearances were statistically significantly lower in Period B than in Period A, whereas the decrease in the 8-hydroxylation clearance of 137X (eq. 5) from 1.2 to 0.7 ml min^{-1} was not statistically significant. The median renal clearance was unchanged at approximately 1 ml min^{-1} .

The median of the partial N-demethylation, 8-hydroxylation and renal clearances of the three primary demethylated metabolites formed from caffeine are listed in Table 2. Only the N7-demethylation clearance of 17X (1X formation) and the renal clearance of 17X changed statistically significantly during fluvoxamine.

Figure 2 shows the plasma concentration of caffeine and its three primary demethylated metabolites without and with concomitant intake of fluvoxamine. Thus, the median half-life of caffeine was 5 h without fluvoxamine but increased to 31 h with concomitant intake of fluvoxamine, the median difference was 29 h and the 95% confidence interval was [14; 54], which was

Table 1. The total and partial clearances^a of caffeine in eight healthy subjects without (Period A) and with (Period B) concomitant intake of fluvoxamine

Subject no.	Clearance (ml min^{-1})											
	Total clearance		N3-demethylation		N1-demethylation		N7-demethylation		8-hydroxylation		Renal clearance	
	Period A	Period B	Period A	Period B	Period A	Period B	Period A	Period B	Period A	Period B	Period A	Period B
1	141	5.4	67	3.7	18	13	9.4	5.0	0.5	0.2	1.0	0.6
2	100	14	57	8.1	58	11	25	6.4	0.5	0.6	1.4	1.4
3	156	42	67	32	36	34	26	22	2.3	1.4	0.8	1.0
4	68	10	27	3.0	4.4	1.6	6.3	2.9	1.3	0.7	1.3	0.7
5	90	42	30	19	9.2	6.4	10	8.8	1.5	1.5	1.2	2.6
6	113	28	31	9.3	10	4.9	6.4	5.5	0.4	0.4	0.3	0.4
7	148	40	58	10	25	4.1	22	5.3	2.7	0.8	7.2	1.8
8	98	7.4	35	3.1	24	15	17	8.7	1.0	0.3	1.0	1.0
Median	107	21	46	8.7	21	8.5	14	6.0	1.2	0.7	1.1	1.1
Median difference	-90		-35		-6.4		-6.2		-0.4		-0.1	
95% confidence interval	[-114; -66]		[-50; -21]		[-26; -2.8]		[-14; -2.2]		[-1.2; 0.0]		[-1.5; 0.7]	

^a Calculated according to eqs 1-6.

Table 2. The median of the partial N-demethylation, 8-hydroxylation and renal clearances^a of the three primary demethylated metabolites formed from caffeine, paraxanthine (17X), theobromine (37X) and theophylline (13X) in eight healthy subjects without (Period A) and with (Period B) concomitant intake of fluvoxamine

	Clearance (ml min ⁻¹)		Median difference	
	Period A Median (range)	Period B Median (range)	[95% confidence interval]	
N7-demethylation of 17X	41 (32-71)	19 (12-49)	-24	[-37; -14]
8-hydroxylation of 17X	12 (7.2-22)	8.4 (7.7-15)	-1.5	[-7.5; 0.1]
Renal of 17X	6.6 (2.7-14)	5.3 (2.2-7.8)	-2.3	[-6.8; -0.3]
N3-demethylation of 37X	31 (14-61)	8.6 (3.4-48)	-15	[-38; 11]
8-hydroxylation of 37X	0.8 (0.2-35)	0.9 (0.1-4.5)	-0.6	[-16; 1.8]
Renal of 37X	9.4 (4.6-14)	9.7 (3.4-14)	-1.3	[-4.8; 2.3]
N1-demethylation of 13X	83 (18-196)	57 (24-184)	-11	[-64; 41]
8-hydroxylation of 13X	19 (11-22)	9.0 (5.2-34)	-5.0	[-13; 5.0]
Renal of 13X	28 (12-90)	23 (7.8-58)	-8.0	[-37; 0.0]

^a Calculated according to eqs 7-15.

statistically significant. The medians, median differences and [95% confidence interval] for AUC (reported as $\mu\text{M}\cdot\text{h}$) of the three N-demethylated metabolites and caffeine were: 37X: Median 59 in period A (0-24 h) vs 137 in Period B (0-24 h), median difference 77 [-50; 387]; 17X: Median 104 in period A (0-24 h) vs 76 in Period B (0-24 h), median difference -31 [-54; -9]; 13X: Median 15 in period A (0-24 h) vs 17 in Period B

(0-24 h), median difference 3 [0; 5]; 137X: Median 154 in period A (0-24 h) vs 435 in Period B (0-24 h), median difference 276 [147; 385].

The median of the volume of distribution was 49 l without and 43 l with concomitant intake of fluvoxamine, which was not statistically significant (median difference: -1.0 l, 95% confidence interval [-16; 11]).

Table 3 shows the medians of the 24 h urinary

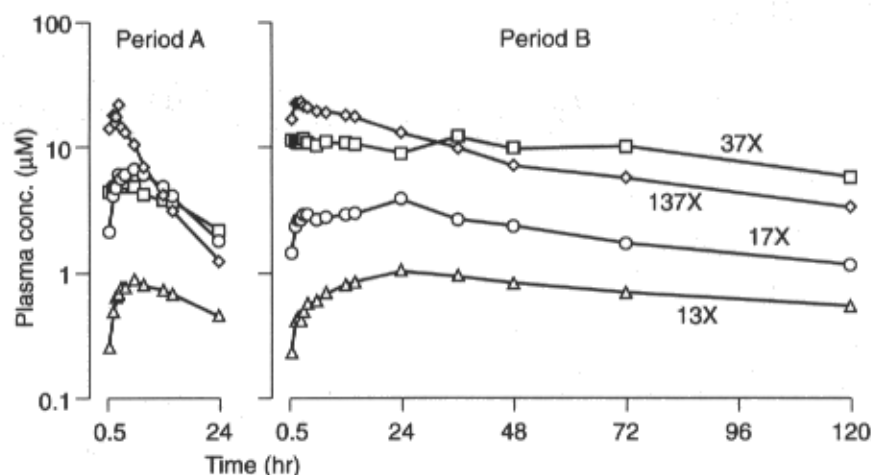


Fig. 2. The plasma concentrations of caffeine and its primary demethylated metabolites without (Period A) and with (Period B) concomitant intake of fluvoxamine.

Table 3. The urinary recoveries (median and range) of caffeine (137X) and 14 of its metabolites without (Period A) and with (Period B) concomitant intake of fluvoxamine. (For abbreviations, see legend to Fig. 1)

Compound	0-24 h recovery (% of dose)		Median difference [95% confidence interval]	
	Period A Median (range)	Period B Median (range)		
Caffeine (137X)	1.0 (0.3-3.4)	3.0 (1.1-4.4)	1.7	[0.5; 2.7]
Paraxanthine (17X)	4.1 (1.8-6.5)	1.9 (0.8-4.1)	-1.6	[-3.0; -0.6]
Theophylline (13X)	2.8 (0.9-6.2)	2.4 (1.0-4.7)	-0.5	[-2.0; 0.8]
Theobromine (37X)	2.6 (0.9-14)	5.3 (0.5-33)	2.5	[-3.5; 17]
137U	1.2 (0.4-1.7)	1.3 (0.6-2.0)	0.2	[-0.1; 0.5]
17U	7.2 (4.5-10)	3.8 (2.8-8.3)	-3.0	[-5.6; -0.3]
13U	1.7 (1.0-2.0)	1.3 (0.6-1.9)	-0.3	[-0.9; 0.3]
37U	0.4 (0.1-6.2)	0.6 (0.1-4.4)	0.1	[-2.5; 1.0]
1X	6.1 (1.1-19)	1.8 (0.5-3.2)	-5.8	[-12; -0.5]
1U	10 (5.6-20)	3.3 (1.4-5.9)	-8.3	[-14; -3.0]
3X	3.0 (1.6-16)	4.9 (0.5-21)	0.5	[-5.9; 6.9]
3U	2.7 (0.0-4.4)	2.5 (0.4-3.5)	0.2	[-0.8; 0.8]
7X	6.1 (3.5-34)	5.6 (2.2-14)	-1.2	[-14; 2.2]
7U	2.3 (0.7-7.2)	1.8 (0.4-4.2)	-0.6	[-2.8; 0.8]
AFMU	4.4 (3.4-19)	1.5 (0.8-11)	-3.3	[-5.8; -2.7]
Total	70 (41-138)	44 (27-85)	-14	[-50; 6.7]

recoveries of caffeine and 14 of its metabolites in Period A and in Period B. Thus, 70% (range 41-138%) of the dose was excreted in Period A and 44% (range 27-85%) was excreted in Period B, but the difference was not statistically significant (median difference: -14%, 95% confidence interval [-50%; 6.7%]). The total recovery in Period B (0-48 h) was 94% (median) (range 47-156%). The recoveries of all the N3-demethylation products (17X, 17U, 1X, 1U and AFMU) were statistically significantly lower in Period B than in Period A (Table 3). For all the metabolites arising from the N1- and N7-demethylation pathways, there were no statistically significant changes in the recoveries. The recovery of caffeine itself was statistically significantly increased during intake of fluvoxamine (Table 3).

The four different urinary metabolic caffeine ratios determined in the 5 h urine samples all decreased during intake of fluvoxamine from 3.3 to 2.1, 2.1 to 0.4, 5.7 to 1.1, and from 24.7 to 2.3 for (AFMU + 1X + 1U)/17U; 17X/137X; (17X + 17U)/137X; (AFMU + 1X + 1U + 17X + 17U)/137X (eqs 18-21), respectively (Fig. 3). The corresponding median differences [95% confidence interval] were: -1.4 [-3.3; -0.2]; -1.6, [-2.1; -1.0]; -4.6 [-8.2; -2.1]; -16 [-51; -6.0], respectively. Also the 6 h plasma 17X/137X ratio decreased during intake of fluvoxamine from 0.7 to 0.2, median difference: -0.5, 95% confidence interval [-0.7; -0.3].

There was no statistically significant correlations between any of the 5 h urinary caffeine metabolic ratios

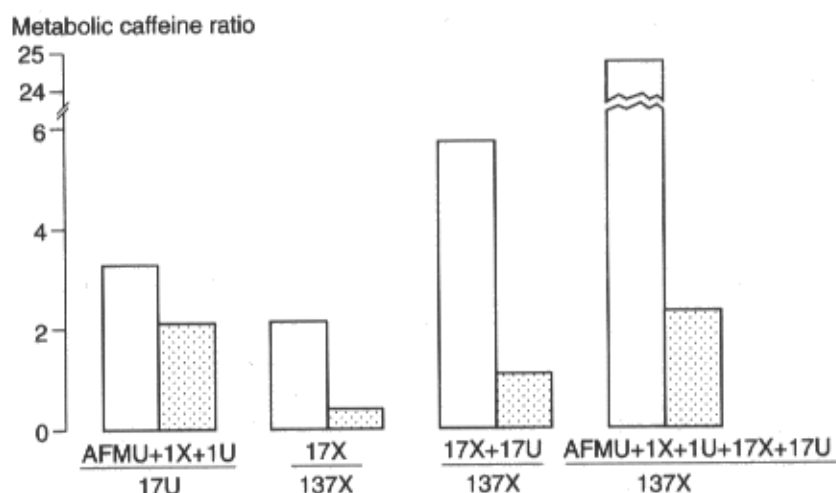


Fig. 3. Different urinary caffeine metabolic ratios performed in a urine sample collected after 5 h without (Period A, □) and with (Period B, ▨) concomitant intake of fluvoxamine (AFMU + 1X + 1U)/17U (Campbell *et al.*, 1987b); 17X/137X (Kadlubar *et al.*, 1990); (17X + 17U)/137X (Butler *et al.*, 1992); (AFMU + 1U + 1X + 17U + 17X)/137X—Modified from (Carrillo & Benitez, 1994). For abbreviations see legend to Fig. 1.

and the total or the partial N3-demethylation clearances of caffeine (Table 4). However, the 6 h plasma 17X/137X ratio correlated statistically significantly with the total and the partial N3-demethylation clearances both without and with concomitant intake of fluvoxamine (Table 4).

Nearly all volunteers reported mild fatigue during intake of fluvoxamine.

Discussion

This study demonstrates that fluvoxamine as expected is a very potent inhibitor of the CYP1A2 mediated metabolism of caffeine. The degree of inhibition is similar or even more pronounced than that previously reported for other CYP1A2 substrates such as propranolol (Benfield & Ward, 1986), imipramine (Spina *et al.*,

1992), theophylline (Donaldson *et al.*, 1994) and clozapine (Hiemke *et al.*, 1994; Jerling *et al.*, 1994). It was shown in an earlier study (Tarrus *et al.*, 1987) that the methylxanthine furafylline results in a 10-fold increase in caffeine levels and elimination half-life. Subsequent *in vitro* studies confirmed that furafylline like fluvoxamine is a potent inhibitor of CYP1A2 (Sesardic *et al.*, 1990). Our data confirm that caffeine is almost exclusively (>99%) eliminated by metabolism and that N3-demethylation is the primary oxidative pathway although not quite as prominent as the 80% reported previously (Lelo *et al.*, 1986). We report that both the total clearance and the N3-demethylation clearance decreased by more than 80% during fluvoxamine (Table 1), and this is consistent with CYP1A2 being a major enzyme responsible for the biotransformation of caffeine (Campbell *et al.*, 1987a; Butler *et al.*,

Table 4. The correlations between total caffeine clearances of N3-demethylation clearances and the four different 5 h urinary metabolic caffeine ratios or a 6 h plasma ratio in eight healthy subjects

	Total clearance		N3-demethylation clearance	
	Period A	Period B	Period A	Period B
	R(s)-values			
Urine				
(AFMU + 1X + 1U)/17U	0.333	0.405	0.476	0.423
17X/137X	0.119	0.524	0.071	0.685
(17X + 17U)/137X	0.476	0.595	0.524	0.589
(AFMU + 1X + 1U + 17X + 17U)/137X	0.429	0.535	0.548	0.625
Plasma:				
17X/137X	0.786*	0.833**	0.905**	0.946**

* Significance level (two-tailed test) $p < 0.05$.

** Significance level (two-tailed test) $p < 0.01$.

1989). The volume of distribution did not change during fluvoxamine, and accordingly, the median of the elimination half-life increased from 5 to 31 h (Fig. 2). The N1- and N7-demethylations of caffeine as well as the N7-demethylation of paraxanthine were also inhibited by fluvoxamine although to a lesser degree than the inhibition of caffeine N3-demethylation (Tables 1 and 2). Thus, our data support the suggestion that CYP1A2 also catalyses the formation of 37X, 13X and 1X (Fig. 1) but that other P450s are equally important (Gu *et al.*, 1992). The results of the present pharmacokinetic investigation neither supports a role of CYP1A2 for the 8-hydroxylation of caffeine nor the demethylations and hydroxylations of 37X and 13X (Table 2). The latter is somewhat surprising since fluvoxamine is a potent inhibitor of both the 8-hydroxylation and the N1-demethylation of theophylline in human liver microsomes *in vitro* (Rasmussen *et al.*, 1995) but the reason for this discrepancy is not clear.

Fluvoxamine is a moderate inhibitor of CYP2D6 *in vitro* (Crewe *et al.*, 1992; Skjelbo & Brosten, 1992) and *in vivo* (Spina *et al.*, 1992). It is also a moderate inhibitor of CYP2C19 *in vivo* (data to be published), but does not inhibit CYP1A1, CYP2A6, CYP2E1 or CYP3A4 *in vitro* (Rasmussen *et al.*, 1995). To our knowledge, inhibition of CYP2C19 has not been examined *in vitro*. Thus, CYP1A2 seems to be the only P450 that is inhibited very potently by fluvoxamine. Hence, our study confirms that it is a major enzyme catalysing the biotransformation of caffeine in particular the N3-demethylation, and that assessment of this pathway may serve as a biomarker for CYP1A2.

Indeed, all four urinary metabolic ratios for caffeine N3-demethylation decreased statistically significantly with concomitant intake of fluvoxamine (Fig. 3), and this confirms that they all are measures of CYP1A2. None of the four ratios correlated with either the total or the N3-demethylation clearance (Table 4). An important reason is that the sample number was low ($n = 8$) and the CYP1A2 variability was limited within the sample, and hence, the possibility of a type-2 error must be considered.

Caffeine is an adenosine receptor antagonist and, in very high concentrations, caffeine also rules out the direct release of intracellular calcium and inhibits the cyclic nucleotide phosphodiesterases (Fredholm, 1995). Hence, the pharmacodynamic effects of caffeine include elevation of blood pressure, increased plasma renin and increased plasma catecholamine levels. Thus, the symptoms of caffeine intoxication are tremor, restlessness, palpitations, nausea, sleeplessness and increased anxiogenic effects. Further, it has been shown (Charney *et al.*, 1985) that patients with panic disorders have increased sensitivity to the CNS-effects of

caffeine. Psychiatric patients often consume high quantities of caffeine-containing beverages (Winstead, 1976), and although not shown directly in the present pharmacokinetic investigation, our data clearly point to the possibility of caffeine intoxication during fluvoxamine treatment. Caffeine intoxication during fluvoxamine treatment could be a disregarded adverse effect leading to discontinuation of the drug. It is, therefore, recommended that patients on fluvoxamine should restrict their intake of caffeine, tea and coca-cola or drink decaffeinated coffee, but further clinical studies are required.

In conclusion, we report that fluvoxamine is, as expected, a very potent inhibitor of caffeine metabolism and our data provide additional evidence that fluvoxamine is a useful tool for the quantitative determination of the role of CYP1A2 in drug oxidation (Brosten *et al.*, 1993; Rasmussen *et al.*, 1995).

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