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Selective serotonin reuptake inhibitors and theophylline metabolism in human liver microsomes: potent inhibition by fluvoxamine

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1 Fluvoxamine and seven other selective serotonin reuptake inhibitors (SRRI) were tested for their ability to inhibit a number of human cytochrome P450 isoforms (CYPs).

2 None of the drugs showed potent inhibition of CYP2A6 (coumarin 7-hydroxylase) or CYP2E1 (chlorzoxazone 6-hydroxylase), while norfluoxetine was the only potent inhibitor of CYP3A having IC_{50} values of 11 μM and 19 μM for testosterone 6β-hydroxylase and cortisol 6β-hydroxylase, respectively.

3 Norfluoxetine, sertraline and fluvoxamine inhibited CYP1A1 (7-ethoxyresorufin *O*-deethylase) in microsomes from human placenta (IC_{50} values 29 μM, 35 μM and 80 μM, respectively). Fluvoxamine was a potent inhibitor of CYP1A2-mediated 7-ethoxyresorufin *O*-deethylase activity (IC_{50} = 0.3 μM) in human liver.

4 In microsomes from three human livers fluvoxamine potently inhibited all pathways of theophylline biotransformation, the apparent inhibitor constant, K_i, was 0.07–0.13 μM, 0.05–0.10 μM and 0.16–0.29 μM for inhibition of 1-methylxanthine, 3-methylxanthine and 1,3-dimethyluric acid formation, respectively. Seven other SSRIs showed either weak or no inhibition of theophylline metabolism.

5 Ethanol inhibited the formation of 1,3-dimethyluric acid with a K_i value of 300 μM, a value which is consistent with inhibition of CYP2E1. Ethanol and fluvoxamine both inhibited 8-hydroxylation by about 45% and, in combination, the compounds decreased the formation of 1,3-dimethyluric acid by 90%, indicating that CYP1A2 and CYP2E1 are equally important isoforms for the 8-hydroxylation of theophylline.

6 It is concluded that pharmacokinetic interaction between fluvoxamine and theophylline is due to potent inhibition of CYP1A2.

Keywords fluvoxamine selective serotonin reuptake inhibitors microsomes theophylline

Introduction

The selective serotonin reuptake inhibitors (SSRI) are a new group of antidepressants and include the compounds citalopram, fluoxetine, fluvoxamine, paroxetine and sertraline. All SSRIs are eliminated by oxidation and may, therefore, inhibit the oxidative metabolism of other drugs. Indeed fluoxetine, its active metabolite norfluoxetine and paroxetine are potent inhibitors of cytochrome P4502D6 (CYP2D6) in human liver microsomes, having apparent inhibition constants, K_i of less than 1 μM [1–3]. Fluvoxamine is a less potent inhibitor of CYP2D6 with K_is of 1.8 to 8.2 μM [1–3]. However, fluvoxamine is a

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potent inhibitor of the *O*-deethylation of phenacetin [4] (K_i ~ 0.12 to 0.24 μM) and of the *N*-demethylation of imipramine [1] (K_i ~ 0.14 μM), two well-established marker reactions for cytochrome P4501A2 (CYP1A2) function in human liver microsomes [5–7]. Fluvoxamine inhibition of P450 isoforms other than CYP1A2 and CYP2D6 has not previously been investigated.

Three case reports showed that steady-state plasma concentrations of theophylline in asthmatic patients increased by a factor of two to three during concomitant fluvoxamine intake, leading to severe toxic reactions [8–10]. This suggests that fluvoxamine inhibits theophylline metabolism *in vivo*. Theophylline (1,3-dimethylxanthine) is eliminated almost exclusively by cytochrome P450 mediated hepatic oxidation. 8-hydroxylation to 1,3-dimethyluric acid (13DMU) accounts for about half of the total theophylline clearance [11], and the remainder is due to *N*-demethylation to 1-methylxanthine (1MX) and 3-methylxanthine (3MX). 1MX is further oxidized to 1-methyluric acid (1MU) by the cytoplasmatic enzyme xanthine oxidase [12]. There is strong direct and indirect evidence that CYP1A2 is involved in all the major pathways of theophylline metabolism [6, 13–16]. The inducing effect of cigarette smoking is greatest on the two *N*-demethylation pathways and CYP1A2 is believed to catalyze about 80–90% of the *N*-demethylations and about 50% of the 8-hydroxylation *in vitro* [17]. There is some evidence that the remainder of the 8-hydroxylation is catalyzed by CYP2E1 and CYP3A [13]. Thus, the pharmacokinetic interaction between fluvoxamine and theophylline is most likely mediated through inhibition of CYP1A2. The main purpose of this study was to test this hypothesis. To prove that fluvoxamine is a potent and selective inhibitor of CYP1A2, fluvoxamine and the seven other SSRIs were screened for their possible inhibition of CYP1A1, CYP1A2, CYP2A6, CYP2E1 and CYP3A. A second purpose of the study was to substantiate the role of CYP2E1 and CYP3A in theophylline metabolism.

Methods

Chemicals and reagents

Theophylline, theobromine, 1MX, quinidine, cortisol, coumarin, 7-hydroxycoumarin and 7-ethoxyresorufin were purchased from Sigma (Missouri, USA). 3MX and 13DMU were purchased from Fluka AG (Buchs, Switzerland), and testosterone and ethanol (96%) were purchased from Merck (Darmstadt, Germany). Unlabelled 6β-hydroxytestosterone was obtained from the Steroid Reference Repository (Professor D. N. Kirk) and [4-^{14}C]-testosterone was obtained from Amersham (Amersham, UK). Other drugs were kindly donated by the following companies: Fluvoxamine: Duphar B.V. (Weesp, Holland). Paroxetine: Novo-Nordisk (Denmark). Fluoxetine and norfluoxetine: Eli Lilly A/S (Denmark). Clozapine: Sandoz Ltd (Basel, Switzerland). Chlorzoxazone: Nycomed DAK A/S (Denmark). 6-hydroxychlorzoxazone: Courtesy F. Guengerich & McNiel Consumer Products (USA). Citalopram and desmethylcitalopram: Lundbeck A/S (Denmark). Sertraline: Pfizer Inc. (USA). Litoxetin: Synthélabo (France). Ketoconazole: Janssen Pharma (Beerse, Belgium). Phenacetin: Hopkins and Williams Ltd (Chadwell Heath, UK). Resorufin: Eastman Kodak Co. (Rochester, NY, USA). 6β-hydroxycortisol: Steraloids (Wilton, NH, USA). All drugs were dissolved in distilled water except for norfluoxetine, sertraline and clozapine which were dissolved in 10 mM HCl. In the 7-ethoxyresorufin, coumarin and testosterone metabolite assays, sertraline and norfluoxetine were dissolved in 5% v/v ethanol. Chorzoxazone was dissolved in 6 mM KOH. Cortisol and testosterone were dissolved in 0.5% and 5% v/v ethanol, respectively. 7-ethoxyresorufin was dissolved in 0.2% v/v methanol/DMSO (1:1) and coumarin was dissolved in 1% v/v acetone. Other chemicals were of high analytical grade and were obtained from either Merck or Sigma.

Human microsomes

Whole human livers were obtained from kidney donor patients shortly after circulatory arrest. The livers were immediately cut into small pieces, frozen in dry ice and stored at −80° C. Microsomes from three human livers (HL1, HL2, and HL3) used in the theophylline, chorozoxazone and cortisol metabolite assays were prepared by a standard technique [18], and the microsomal protein concentration was measured by the method of Lowry *et al.* [19]. Microsomes from human liver (HL4) for ethoxyresorufin, coumarin and testosterone metabolite assays were isolated as described by Mäenpää *et al.* [20], and protein concentration was determined according to Bradford [21].

A human placenta (HP) was obtained after normal vaginal delivery 1 month before term from a mother who had smoked 10 cigarettes daily during pregnancy. The placental microsomes were prepared according to Hakkola *et al.* [22]. CYP1A1 activity was shown to be increased in the placenta [22].

Enzyme and drug assays

7-ethoxyresorufin, coumarin, chlorzoxazone, cortisol and testosterone metabolism CYP1A2 activity was determined by 7-*O*-deethylation of ethoxyresorufin to resorufin in human liver microsomes (HL1) [16, 23, 24], and CYP1A1 activity was determined by 7-*O*-deethylation of ethoxyresorufin to resorufin in human placental microsomes [16, 25]. CYP2A6 activity was determined by the 7-hydroxylation of coumarin in human liver microsomes (HL4) [26, 27]. CYP2E1 activity was determined by the 6-hydroxylation of chlorzoxazone in human liver microsomes (HL1) [28]. Assessment of CYP3A activity was carried out by measuring the 6β-hydroxylation of cortisol and testosterone

in human liver microsomes (HL1 and HL4, respectively) [29–31]. The concentrations of substrates in the assays were chosen to be around the K_m values for the respective metabolic reactions.

7-ethoxyresorufin O-deethylase assay Resorufin formation was determined by a fluorometric method [32]. Incubation mixtures containing NADPH-generating system, 7-ethoxyresorufin in a final concentration of 1 μM and 315 μg of microsomal protein from either human liver or human placenta were incubated for 5 min at 37° C. The reaction was started by the addition of microsomes. The quantitation limit was 1.2 nmol mg^{-1} h^{-1} and the coefficient of variation was < 2%.

Coumarin 7-hydroxylase assay Coumarin in a final concentration of 100 μM was incubated at 37° C with 210 μg microsomal protein and a NADPH-generating system in an incubation volume of 500 μl. The reaction was started by the addition of microsomes and the incubation time was 10 min. The formation of 7-hydroxycoumarin was determined by fluorometric methods according to Aitio [33]. The quantitation limit was 0.6 nmol mg^{-1} h^{-1} and the coefficient of variation was < 6%.

Chlorzoxazone 6-hydroxylase assay Chlorzoxazone in a final concentration of 40 μM was incubated with 500 μg of microsomal protein in an incubation volume of 500 μl. The reaction was started by the addition of 50 μl NADPH-generating system (concentrations in microsomal suspension: isocitrate dehydrogenase, 1 u ml^{-1}, $NADPNa_2$, 1 mM, isocitrate, 5 mM, $MgCl_2$, 5 mM) and stopped after 20 min incubation at 37° C by the addition of 350 μl ice-cold zinc sulphate (2% w/v) and cooling on ice. After centrifugation (1000 *g*, 15 min) the supernatant was stored at −20° C until analysis. 6-hydroxychlorzoxazone was assayed by h.p.l.c. The incubation mixture was precipitated with acetonitrile (45:55; v:v). After centrifugation 25 μl supernatant was injected onto a Nucleosil C_{18} column (particle size 5 μm, 150 mm × 4.6 mm i.d.) eluted at a rate of 1.5 ml min^{-1} with 25% (v:v) acetonitrile in 12.5 mM phosphate buffer pH 6.5. The effluent was monitored with an ESA Coulochem detector with a 5010 cell set at 280–320 mV at electrode 1 for detection of 6-hydroxychlorzoxazone and 750–850 mV at electrode 2 for detection of chlorzoxazone. Dynamic voltamograms of 6-hydroxychlorzoxazone and chorzoxazone were determined prior to each series of analysis because optimal electrode settings could vary 50 mV between runs. For quantification external standardization was used with a six point calibration curve for each 20 samples. The variation between the reference factor was less than 2%. The coefficient of variation was <2% and the limit of quantitation was 0.1 nmol mg^{-1} h^{-1}.

Cortisol 6β-hydroxylase assay Microsomal protein (750 μg) was incubated in a final incubation volume of 750 μl in disodium phosphate buffer (0.1 M, pH 7.4) with cortisol in a final concentration of 10 μM. The reaction was started by the addition of 75 μl NADPH-generating system and stopped after 30 min incubation at 37° C by the addition of 525 μl ice-cold zinc sulphate (2% w/v) and cooling on ice. After centrifugation (1000 *g*, 15 min) the supernatant was kept at −20° C until analysis. 6β-hydroxycortisol was analyzed by h.p.l.c. [34]. The coefficient of variation was 3% and the limit of quantitation was 0.01 nmol mg^{-1} h^{-1}.

Testosterone 6β-hydroxylase assay Microsomal protein (630 μg) was incubated for 20 min with [^{14}C]-labelled testosterone in a final concentration of 100 μM and an incubation volume of 500 μl. 6β-hydroxytestosterone was measured by the thin layer chromatographic method of Waxman *et al.* [35] with slight modifications as described in detail previously [20]. The quantitation limit was 0.3 nmol mg^{-1} h^{-1} and the coefficient of variation was <10%.

All marker drugs were incubated in duplicate with SSRI in final concentrations of 0.1–100 μM.

Theophylline metabolism

The metabolites 1MX, 3MX and 13DMU were assayed by an h.p.l.c. method described in detail elsewhere [36]. Briefly, 500 μg of microsomal protein were preincubated for 5 min at room temperature with theophylline in final concentrations ranging from 0.2 to 18 mM. In the inhibition assays, 1.6 mM theophylline was incubated in duplicate with putative inhibitors in final concentrations ranging from 0.1–100 μM. The quantitation limits were 0.15 nmol mg^{-1} h^{-1}, 0.06 nmol mg^{-1} h^{-1} and 0.09 nmol mg^{-1} h^{-1} for the formation of 1MX, 3MX and 13DMU, respectively, and coefficients of variation for the assays were <12% for all three metabolites.

Analysis of kinetic data

The velocities of formation of 1MX, 3MX and 13DMU were investigated with microsomes from three livers using theophylline in final concentrations from 0.2 to 18 mM. For all three metabolites the relation between velocity and the ratio of velocity to substrate concentration was curvilinear [37] (Figure 1), indicating that at least two distinct enzymes are responsible for the formation of each of three theophylline metabolites. Hence, an equation which describes a two-enzyme model was fitted to the data:

$$V = V_{max} \frac{[S]}{K_m + [S]} + L \cdot [S] \quad (1)$$

This assumes that each of the three metabolites is formed in parallel by a high affinity enzyme (low K_m) and a low affinity enzyme (high K_m). V_{max} and K_m are the apparent maximal velocity and the apparent Michaelis constant, respectively, of a high affinity enzyme and L is V_{max}/K_m for the low affinity enzyme(s) and is hence a constant which relates theo-

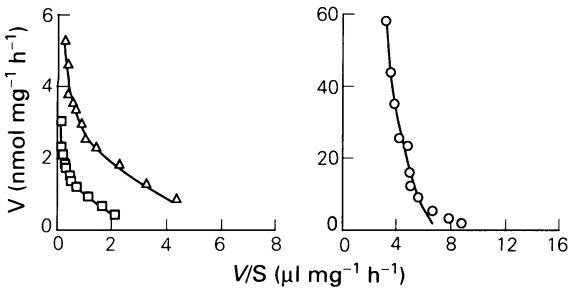

Figure 1 Eadie-Hofstee plots of the formation rate (V) of 3-methylxanthine (□), 1-methylxanthine (△) and 1,3-dimethyluric acid (○) after incubating microsomes from HL2 with theophylline in concentrations (S) from 0.2–18 mM. Each point represents the mean of duplicate determinations and the lines represent the best fits according to Equation 1.

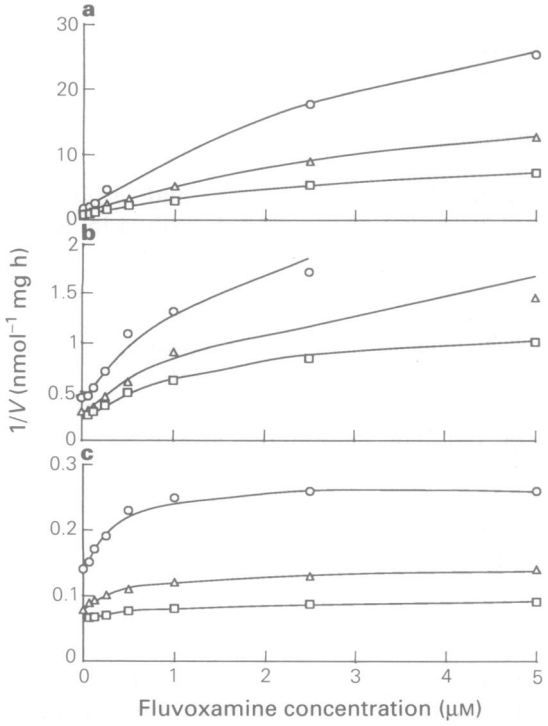

Figure 2 The effect of fluvoxamine on the formation of a) 3-methylxanthine, b) 1-methylxanthine and c) 1,3-dimethyluric acid in microsomes from HL1 (Dixon plots). Theophylline concentrations: (○) 0.8 mM, (△) 1.6 mM and (□) 2.4 mM. Each point represents the mean of duplicate determinations and the lines represent the best fits according to Equation 2.

phylline concentration (S) to velocity via the low affinity enzyme(s). Equation 1 was fitted to the data using an iterative curve fitting programme based on non-linear regression analysis [38]. Furthermore, a one-enzyme model was fitted to all data, and a model including two saturable enzymes and a linear low affinity enzyme was fitted to the data for 8-hydroxylation. Goodness-of-fit was evaluated by comparison of loglikelihood values [38].

At final concentrations of up to 100 µM a series of drugs were screened for their inhibitory effect on theophylline metabolism. Four of the drugs (fluvoxamine, phenacetin, clozapine and ethanol) displaying inhibition were re-tested in a broader concentration range with theophylline concentrations about the K_m values for N-demethylations (fluvoxamine, phenacetin and clozapine) and 8-hydroxylation (ethanol). For all four inhibitors, a graphic analysis revealed a curvilinear relationship between reciprocal velocity and the inhibitor concentration [39] (Figure 2). An equation which describes a two-enzyme model was therefore fitted to the data:

$$V = V_{max} \frac{[S]}{[S] + K_m \left(\frac{[I]}{K_i} + 1\right)} + L \cdot [S] \qquad (2)$$

According to this model, the formation of the three theophylline metabolites proceeds in parallel via a high affinity enzyme showing competitive inhibition and a low affinity enzyme showing linear kinetics in the concentration range tested. K_i is the apparent inhibitor constant for inhibition of the high affinity site and I is the inhibitor concentration. The equation was fitted to the data using an iterative method [38].

Results

SSRI inhibition of 7-ethoxyresorufin, coumarin, chlorzoxazone, cortisol and testosterone metabolism

The SSRIs were tested for their ability to inhibit a number of cytochrome P450 isoforms assessed by the use of well-established marker reactions. The effects of eight SSRIs on the metabolism of the model drugs are shown in Table 1. As expected, fluvoxamine was found to be a potent inhibitor of 7-ethoxyresorufin O-deethylase (EROD) activity in liver microsomes (CYP1A2). The IC_{50} value was 0.3 µM, which was >30 times less than that of the second strongest inhibitor, paroxetine. Sertraline, litoxetin and norfluoxetine also inhibited EROD activity in liver microsomes, whereas fluoxetine, desmethylcitalopram and citalopram had weak effects. Norfluoxetine blocked placental EROD activity completely at a concentration of 100 µM; sertraline was a relatively strong inhibitor, fluvoxamine was a weak inhibitor, whereas citalopram, desmethylcitalopram, fluoxetine, litoxetin and paroxetine were either weak inhibitors or weak stimulators of placental EROD activity. Norfluoxetine blocked cortisol 6β-hydroxylase (C6βOH) and testosterone 6β-hydroxylase (T6βOH) activities almost completely at a concentration of 100 µM; fluvoxamine had an IC_{50} of 40–60 µM. The other SSRIs had either weak or no effects on the CYP3A marker reactions. The residual activity at an inhibitor concentration of 100 µM (% of velocity without inhibitor) of the formation of 6β-hydroxycortisol and 6β-hydroxytestosterone showed a highly significant correlation for eight SSRIs: $r_s = 0.857$, $P < 0.02$ (Spearman's).

Table 1 The effects of eight SSRIs on the formation of 3-methylxanthine (3MX), 1-methylxanthine (1MX) and 1,3-dimethyluric acid (13DMU) from theophylline, 7-ethoxyresorufin *O*-deethylase ($EROD_{HL}$), cortisol 6β-hydroxylase (C6βOH), testosterone 6β-hydroxylase (T6βOH), coumarin 7-hydroxylase (COH), and chlorzoxazone 6-hydroxylase (C6OH) activities in human liver microsomes, as well as 7-ethoxyresorufin *O*-deethylase activity in human placental microsomes ($EROD_{HP}$). The results are the means of duplicate determinations

SSRI	*3MX*[a]	*1MX*[a]	*13DMU*[a]	$EROD_{HL}$[b]	$EROD_{HP}$[c]	*C6βOH*[a]	*T6βOH*[b]	*COH*[b]	*C6OH*[a]
Control activity (nmol mg^{-1} h^{-1})	1.9	3.3	12.8	36.7	7.6	0.28	98.4	39.5	31.9
% of control activity (100 μM inhibitor concentration)									
Fluvoxamine	10	15	56	6	42	34	35	94	95
Paroxetine	29	35	72	9	54	38	60	77	99
Sertraline	76	46	77	9	13	47	54	64	83
Litoxetin	38	45	69	23	156	58	59	96	67
Fluoxetine	100	90	94	82	66	40	43	71	108
Norfluoxetine	71	74	82	37	0	15	5	68	95
Citalopram	92	95	95	91	82	71	98	92	92
Desmethylcitalopram	93	97	94	86	172	67	67	81	131
IC_{50} (μM)									
Fluvoxamine	0.2	0.2		0.3	80	40	60		
Paroxetine	50	50		10	>100	70	>100		
Sertraline	>100	90		20	35	90	>100		
Litoxetin	50	50		15	>100	>100	>100		
Fluoxetine	>100	>100		>100	>100	60	75		
Norfluoxetine	>100	>100		50	29	19	11		
Citalopram	>100	>100		>100	>100	>100	>100		
Desmethylcitalopram	>100	>100		>100	>100	>100	>100		

[a]Human liver microsomes from HL1.
[b]Human liver microsomes from HL4.
[c]Human placental microsomes (predominantly CYP1A1 activity).

Two known inhibitors of CYP2E1 were used as positive controls to assure the reliability of the assay. Both ethanol and propylenglycol were found to inhibit C6OH activity, with IC_{50} values of 1.5 mM and 20 mM, respectively. None of the SSRIs inhibited chlorzoxazone 6-hydroxylase (C6OH) activity or coumarin 7-hydroxylase (COH) activity (IC_{50} > 100 μM).

Theophylline metabolism

Eadie-Hofstee plots [37] showed that the formation of 1MX, 3MX and 13DMU exhibited biphasic kinetics with microsomes from HL1, HL2 and HL3 (Figure 1, results for HL2). For the formation of all three theophylline metabolites the biphasic model (Equation 1) fitted the data best. The loglikelihoods [37] of the estimations using the biphasic model (Equation 1) ranged from 16.2 to 20.2, 8.8 to 9.8 and −10.1 to −5.9 for 3MX, 1MX and 13DMU, respectively, as compared with 1.0 to 5.9, −4.2 to −0.5 and −18.7 to −14.0 using the equation for the one-enzyme model. The equation for two saturable enzymes and a linear low affinity site gave loglikelihood values of −19.6 to −12.0 for fits to the data for formation of 13DMU.

Fluvoxamine had marked inhibitory effect on the formation of all three theophylline metabolites. Thus, the formation of 1MX, 3MX and 13DMU was decreased to 10%, 15% and 55% of control, respectively. Dixon plots of fluvoxamine inhibition kinetics confirmed the biphasic model (Figure 2) and both Dixon and Cornish-Bowden plots were consistent with competitive inhibition. The apparent K_i values for fluvoxamine inhibition of the high affinity enzyme were 0.07–0.13 μM, 0.05–0.10 μM and 0.16–0.29 μM for formation of 1MX, 3MX and 13DMU, respectively. The K_i values and the apparent V_{max} and K_m values for the high affinity site and the value of L, representing the linear low affinity site, are shown in Table 2. The IC_{50} values of seven other SSRIs ranged from 50 to >100 μM (Table 1).

The residual activity at an inhibitor concentration of 100 μM (% of velocity without inhibition) of the formation of the three theophylline metabolites correlated well with similar results obtained using 7-ethoxyresorufin as a probe. For eight SSRIs the r_s was 0.952 ($P < 0.01$) for the correlation with 1MX formation, $r_s = 0.827$ ($P < 0.05$) for the correlation with 3MX formation and $r_s = 0.917$ ($P < 0.01$) for the correlation with 13DMU formation.

Phenacetin was a weak inhibitor of all three theophylline oxidation pathways. Equation 2 was fitted to the data and the apparent K_i values were 80 μM (65–115 μM) and 95 μM (60–140 μM) for inhibition of 1MX and 3MX formation, respectively, given as mean values and ranges determined at three theophylline concentrations. Because of interfering chomatographic peaks it was not possible to determine a K_i value for phenacetin inhibition of 13DMU formation.

Clozapine, another CYP1A2 substrate [40–43], was also a weak inhibitor of the formation of all three theophylline metabolites. The apparent K_i values were 55 μM (30–95 μM), 55 μM (50–60 μM) and

Table 2 Kinetic parameters describing the inhibition a) by fluvoxamine of the formation of 3-methylxanthine (3MX), 1-methylxanthine (1MX) or 1,3-dimethyluric acid (13DMU) from theophylline in human liver microsomes. Mean values and ranges determined with microsomes from three human livers (HL1, HL2 and HL3) are shown

	V_{max}[a] (nmol mg^{-1} h^{-1})	K_m[a] (mM)	K_i[a] (μM)	L[b] (μl mg^{-1} h^{-1})
3MX	1.9 (1.4–2.6)	0.6 (0.4–0.8)	0.07 (0.05–0.10)	0.03 (0.02–0.05)
1MX	3.1 (2.6–4.3)	0.6 (0.4–0.8)	0.10 (0.07–0.13)	0.3 (0.2–0.4)
13DMU	28 (17–33)	8.5 (6.2–11.3)	0.24 (0.16–0.29)	3.8 (3.3–4.4)

[a] V_{max}, K_m and K_i refer to the high affinity site.
[b] The constant which relates the theophylline concentration to the velocity via the low affinity enzyme.

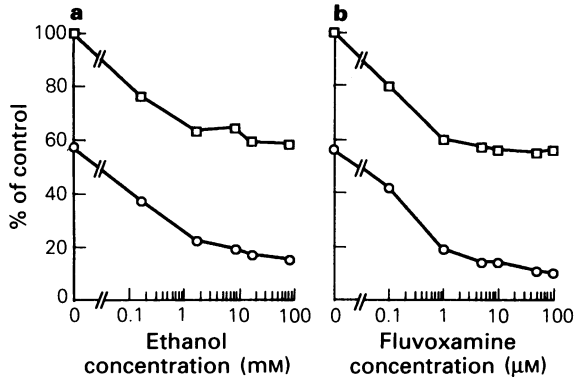

Figure 3 Inhibition of the 8-hydroxylation of theophylline in microsomes from HL1 as a function of a) ethanol concentration (□) and ethanol concentration plus 50 μM fluvoxamine (○), and b) fluvoxamine concentration (□) and fluvoxamine concentration plus 34 mM ethanol (○). Each point represents the mean of duplicate determinations.

60 μM (45–80 μM) for inhibition of the formation of 1MX, 3MX and 13DMU, respectively (given as mean values and ranges determined at three theophylline concentrations).

CYP2E1 inhibitors Ethanol was screened as an inhibitor of theophylline metabolism in concentrations from 0.17 to 86 mM. Ethanol does not exhibit any non-specific inhibition of P450 enzymes in concentrations up to 86 mM (0.5%) [1, data not shown]. At a concentration of 86 mM ethanol decreased the formation of 13DMU to 58% (Figure 3a), whereas the demethylation pathways were not affected. When fluvoxamine was added in a final concentration of 50 μM to all incubations containing ethanol in concentrations from 0.17 to 86 mM, 8-hydroxylation of theophylline was decreased to 15% of control activity (Figure 3a). Fluvoxamine was re-tested in the concentration range 0.1–100 μM with ethanol in a final concentration of 34 mM. In combination, the two drugs decreased 8-hydroxylation to 10% of control activity as compared with a decrease of 13DMU formation to 56% when fluvoxamine was incubated alone (Figure 3b). For K_i determinations ethanol was incubated in concentrations from 0.17 mM to 3.4 mM. Dixon plots of the data showed biphasic kinetics (data not shown), and the apparent K_i value for the inhibition of 8-hydroxylation by ethanol was 300 μM for incubation with ethanol alone and 200 μM when fluvoxamine was added.

For technical reasons it was only possible to test chlorzoxazone in concentrations up to 10 μM. At this concentration 8-hydroxylation of theophylline was decreased to 80% of control, whereas the demethylation pathways were not affected.

CYP3A inhibitors Ketoconazole inhibited the formation of all three theophylline metabolites only at the highest concentration used (100 μM) (data not shown). The IC_{50} values were 60 μM for inhibition of 1MX and 3MX formation and >100 μM for the inhibition of 13DMU formation.

Quinidine did not inhibit the metabolism of theophylline (data not shown).

Discussion

We have shown that fluvoxamine is a potent inhibitor of the metabolism of 7-ethoxyresorufin in human liver microsomes (CYP1A2), but that it causes weak or no inhibition of testosterone and cortisol (CYP3A), chlorzoxazone (CYP2E1), coumarin (CYP2A6) and placental 7-ethoxyresorufin (CYP1A1) metabolism. Fluvoxamine is a weak inhibitor of mephenytoin hydroxylase (CYP2C19) *in vivo* (Brøsen, personal communication), but inhibition of CYP2C9 has not been investigated. The results strongly suggest that CYP1A2 is the only isoform which is inhibited potently by fluvoxamine.

The study also showed that fluvoxamine, as expected, is a potent inhibitor of the formation of 1MX, 3MX and 13DMU from theophylline. Most likely this high affinity site is CYP1A2 [13, 17]. The K_i values (Table 2) were similar to published values of 0.12–0.24 μM reported for the *O*-deethylation of phenacetin [4], 0.14 μM for the *N*-demethylation of imipramine [1], as well as an IC_{50} value of 0.3 μM for the *O*-deethylation of ethoxyresorufin reported here. Thus, our data provide strong support for the hypothesis that the pharmacokinetic interaction between fluvoxamine and theophylline is caused by inhibition of CYP1A2, and that this isoform is important for all

the major pathways of theophylline metabolism. In agreement with this, we showed that phenacetin was a weak inhibitor of the formation of all three theophylline metabolites. Furthermore the neuroleptic drug, clozapine, a putative substrate for CYP1A2 [40–43], was also a weak inhibitor of the three major pathways.

Even at high concentrations fluvoxamine decreased the formation of 13DMU to 50–60% of the control value (Figure 3), and the seven other SSRIs decreased 13DMU formation to an even lesser extent (Table 1). The residual activity is mediated by P450s other than CYP1A2, and the results of the present study suggest these include CYP2E1. Thus, the formation of 13DMU *in vitro* is highly correlated with the microsomal content of immunoreactive CYP2E1 protein [13]. None of the SSRIs was found to inhibit the 6-hydroxylation of chlorzoxazone, indicating that they do not interact with CYP2E1. Ethanol is a substrate for CYP2E1 and, hence, also an inhibitor of this P450 [44]. The K_i value reported here for ethanol inhibition of 13DMU formation is in agreement with the previously published K_i values for ethanol inhibition of the 6-hydroxylation of chlorzoxazone (510 μM) [28], and for the inhibition of the *N*-demethylation of *N*-nitrosodimethylamine (310 μM) [45]. For technical reasons it .was not possible to test chlorzoxazone as an inhibitor of 8-hydroxylation in sufficiently high concentrations to determine a K_i or IC_{50} value.

Previous studies gave contradictory results with regard to the role of CYP3A isoforms in 13DMU formation [13]. Ketoconazole is an inhibitor of several P450s, but is a particular potent inhibitor of CYP3A isoforms with an apparent K_i value of 0.1 μM in human liver microsomes [46]. We found that ketoconazole was a weak inhibitor of all three oxidative pathways of theophylline metabolism, suggesting that CYP3A is not important for the formation of 13DMU. Norfluoxetine, the pharmacologically active metabolite of fluoxetine, was found to be a relatively potent inhibitor of CYP3A. A strong correlation was found between results obtained with testosterone and cortisol as model substrates for CYP3A function, confirming that the 6β-hydroxylations of these two compounds are indeed catalyzed by the same P450 [29–31]. Norfluoxetine was only a weak inhibitor of the three metabolic pathways of theophylline, and this further suggests that CYP3A is not involved in the 8-hydroxylation of theophylline.

Lack of inhibition by the potent CYP2D6 inhibitor, quinidine, confirmed the result of an earlier study showing that CYP2D6 is not involved in the biotransformation of theophylline [47].

In conclusion, we have confirmed that fluvoxamine is a potent and selective inhibitor of CYP1A2, and that among the SSRI it is the only drug with this property. This mechanism is the source of the pharmacokinetic interaction between fluvoxamine and theophylline as well as other drugs, such as clozapine [40, 42], propranolol [48] and imipramine [49, 50]. Our data also illustrate that fluvoxamine could be an important probe for the assessment of the role of CYP1A2 in the metabolism of drugs and other xenobiotics in humans, in addition to furafylline [51, 52]. An advantage of fluvoxamine over furafylline is that it is widely available both for *in vitro* and *in vivo* studies.

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