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FLUVOXAMINE IS A POTENT INHIBITOR OF CYTOCHROME P4501A2

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Abstract—Fluvoxamine is a new antidepressant and selectively inhibits serotonin reuptake (SSRI). The present study demonstrates that fluvoxamine is a very potent inhibitor of the high-affinity O-deethylation of phenacetin, which is catalysed by cytochrome P4501A2 (CYP1A2), in microsomes from three human livers. Thus, the apparent inhibitor constant of fluvoxamine, K_i, ranged from 0.12 to 0.24 μM. Seven other SSRIs, citalopram, *N*-desmethylcitalopram, fluoxetine, norfluoxetine, paroxetine, sertraline and litoxetin either did not inhibit or were weak inhibitors of the O-deethylation of phenacetin. Our findings explain the mechanism of the pharmacokinetic interactions between fluvoxamine and drugs that are metabolized by CYP1A2, e.g. theophylline and imipramine.

Fluvoxamine is a new antidepressant, a selective serotonin reuptake inhibitor (SSRI§), which is eliminated primarily via oxidation in the liver [1]. Fluvoxamine is a very potent inhibitor of one of the cytochrome P450s (CYP1A2, CYP2C, CYP2D6 and CYP3A4 nomenclature as used by Nebert *et al.* [2]) which catalyse the N-demethylation of imipramine in human liver microsomes with an apparent inhibitor constant, K_i, of 0.14 μM [3]. The apparent K_i of fluvoxamine for inhibition of the 2-hydroxylation of imipramine, a process which is catalysed by the sparteine/debrisoquine oxygenase CYP2D6 [2], was 4 μM [3]. The N-demethylation of imipramine is partially determined by the mephenytoin oxidation polymorphism *in vivo*, suggesting a role of a P450 in the 2C subfamily for this reaction [4]. However, the role of 2C isozymes was not confirmed *in vitro* [3, 5]. In microsomes from nine human livers (HLs) there was a statistically significant, positive correlation between the content of two other isozymes of cytochrome P450, the CYP1A2 and CYP3A4 [2] and the maximal velocity of *N*-desmethylimipramine formation (H. Kroemer, personal communication). This finding has been confirmed in an independent study [5]. Cigarette smoking induces the levels of CYP1A2 in HL [6] and clinical studies have suggested that the N-demethylation of imipramine proceeds more rapidly in smokers than in non-smokers [7]. Thus, both the *in vitro* and the *in vivo* data suggest that CYP1A2 is an important enzyme for the N-demethylation of imipramine, and when we combined the results of the various studies we suspected that fluvoxamine might be a potent inhibitor of the human CYP1A2.

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§ Abbreviations: HL, human liver; SSRI, selective serotonin reuptake inhibitors.

Theophylline is also metabolized by CYP1A2 [8], and three case reports suggest inhibition of theophylline metabolism by fluvoxamine [9–11], lending further support to this notion. On this basis a study was performed on the effects of fluvoxamine on the high-affinity O-deethylation of phenacetin, a well established marker reaction for the CYP1A2 function in human liver microsomes [6, 12].

MATERIALS AND METHODS

Chemicals. Phenacetin and paracetamol were obtained from Hopkins and Williams Ltd (Chadwell Heath, U.K.). Fluvoxamine: Duphar, B.V. (Weesp, Holland). Citalopram and *N*-desmethylcitalopram: H. Lundbeck A/S (Denmark). Fluoxetine and norfluoxetine: Eli Lilly A/S (Denmark). Sertraline: Pfizer Inc. (U.S.A.). Litoxetin: Synthélabo (France). Paroxetine: Novo-Nordisk (Denmark).

Other chemicals were of high analytical grade and supplied by Merck (Darmstadt, Germany).

Liver microsomes. Whole HLs were obtained from three kidney donor patients shortly after circulary arrest. The livers were immediately cut into slices, frozen in dry ice and stored at −80°. Microsomes were prepared by a standard technique [13], and the protein concentration was measured by the method of Lowry *et al.* [14].

Incubation conditions. Microsomes were incubated in a final incubation volume of 500 μL in a disodium phosphate buffer (100 mM; pH 7.4) using 100 μg of microsomal protein. Stock solutions of 50 μL phenacetin and 50 μL of fluvoxamine were pre-incubated for 5 min at room temperature. Microsomes from the three livers were incubated with phenacetin in final concentrations of 1, 2.5, 5, 10 and 100 μM and fluvoxamine in final concentrations of 0, 0.1, 0.25, 0.5, 1.0, 5.0, 10, 20, 40 and 100 μM. The reaction was started by adding 50 μL of an NADPH-generating system (concentrations in microsomal suspension: isocitrate dehydrogenase, 1 U/mL, $NADPNa_2$, 1 mM, isocitrate, 5 mM,

Table 1. Inhibition* of the formation of paracetamol by fluvoxamine from three HLs

	V_{max} (nmol/mg/hr)	K_m (μM)	K_i (μM)	L† (μL/mg/hr)
HL1	202 (196–209)	57 (55–60)	0.18 (0.11–0.27)	13.2
HL2	96 (89–101)	47 (43–53)	0.24 (0.17–0.27)	6.1
HL3	27 (18–30)	14 (10–26)	0.12 (0.05–0.18)	3.1

* V_{max}, K_m, K_i refer to the high affinity side (Eqn 1).

A complete set of data were determined at each of the phenacetin concentrations of 1, 2.5, 5, 10 and 100 μM, and the means (range) of the five separate determinations are given for each constant in each of the three livers.

† Determined according to Eqn 1 at a phenacetin concentration of 100 μM.

Table 2. Effects on the formation of paracetamol by eight SSRIs in HL1

SSRI	IC50 (μM)
Fluvoxamine	0.2
Citalopram	>100
N-Desmethylcitalopram	>100
Fluoxetine	>100
Norfluoxetine	>100
Paroxetine	45
Sertraline	70
Litoxetin	60

IC_{50} is the concentration of the inhibitor which reduces paracetamol formation by 50%.

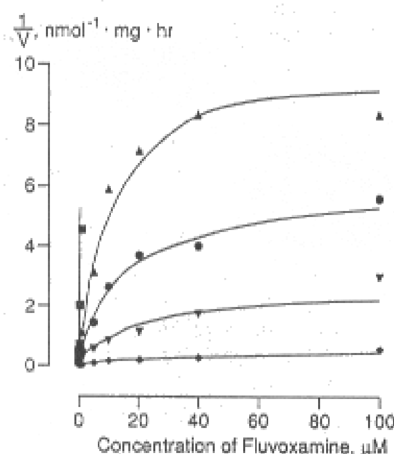

Fig. 1. The effect of fluvoxamine on the O-deethylation of phenacetin in HL1 (Dixon plots). Phenacetin concentrations: (■) 1 μM, (▲) 2.5 μM, (●) 5 μM, (▼) 10 μM and (◆) 100 μM. The lines represent the best fits according to Eqn 1.

$MgCl_2$, 5 mM). Incubations were carried out at 37° in a shaking water bath in air, and the reactions were stopped after 10 min by adding 100 μL of ice-cold $ZnSO_4$ (0.1 M). After centrifugation at 900 *g* for 10 min the supernatant was kept at −20° until analysis. Additional experiments were performed with microsomes from HL1 and phenacetin in a final concentration of 10 μM and seven other SSRIs: citalopram, *N*-desmethylcitalopram, fluoxetine, norfluoxetine, paroxetine, sertraline and litoxetin in final concentrations of 0, 0.01, 0.1, 0.5, 1.0, 10 and 100 μM. All incubations were carried out in duplicate. Less than 10% of the substrate was consumed during the incubations. Paracetamol formed by O-deethylation of phenacetin was analysed by HPLC with electrochemical detection [15]. Thus, an aliquot (100 μL) of the incubation mixture was mixed with an equal volume of 2 N perchloric acid. Twenty microlitres of the supernatant were injected into the HPLC column after centrifugation. For measurement of paracetamol a Spherisorb ODS, 5 μm 15 cm column was eluted with phosphate buffer pH 4.4 (methanol 92/8; v/v) and the conductivity of the effluent was monitored with an ESA Coluchem II electrochemical detector equipped with a 5010 analytical cell set of 150 mV (electrode 1) and 350 mV (electrode 2) and a 0.5 μA full range deflection. The assay limit of paracetamol was 40 femtomol and the interday coefficient of variation 5%.

RESULTS

A graphical analysis revealed a curvilinear relationship between the reciprocal velocity of the paracetamol formation and the fluvoxamine concentration (Fig. 1). Hence, an equation which describes a two-enzyme model was fitted to the data:

$$V = \frac{V_{max} \times S}{K_m\left(1 + \frac{C_I}{K_i}\right) + S} + LS. \qquad (1)$$

According to this model, the O-deethylation of phenacetin proceeds in parallel via a high-affinity enzyme, alias the CYP1A2, showing inhibition and a low-affinity enzyme showing no inhibition. K_m is the Michaelis constant, V_{max} is the maximal velocity and K_i is the apparent inhibitor constant for inhibition of the high-affinity site, C_I is the fluvoxamine concentration, S is the phenacetin concentration and L is a constant which relates S to the velocity via the low-affinity enzyme [3]. The equation was fitted to the data using an iterative method [16]. The mean

apparent K_m ranged from 14 to 57 μM and the mean apparent V_{max} for paracetamol formation in the three human livers, HL1, HL2 and HL3 ranged from 27 to 202 nmol/mg/hr (Table 1). The apparent K_i for fluvoxamine inhibition of the high-affinity O-deethylation of phenacetin ranged from 0.12 to 0.24 μM (Fig. 1 and Table 1). The IC_{50} values of seven other SSRIs ranged from 45 to >100 μM (Table 2).

DISCUSSION

It has previously been shown that the kinetics of phenacetin O-deethylation is biphasic [17–19] and indeed this was confirmed in the present study (Fig. 1). We report apparent K_m values for the high-affinity site (Table 1) which are in agreement with previously published values [17–19].

The present study shows that fluvoxamine is a very potent inhibitor of the formation of paracetamol from phenacetin via the high-affinity site, alias the CYP1A2 function [6, 12]. Our findings ought to be confirmed by studying the effects of fluvoxamine on purified or expressed CYP1A2. The K_i values for O-deethylation (Table 1) are almost identical to the value of 0.14 μM reported for N-demethylation of imipramine [3]. This is consistent with the assumption that the two oxidations are catalysed by the same P450. Also, in agreement with the present study citalopram, *N*-desmethylcitalopram, fluoxetine and norfluoxetine did not inhibit the N-demethylation of imipramine, and in addition paroxetine is a weak inhibitor of this oxidation [3]. For technical reasons (interfering peaks on the chromatogram) it was not possible to investigate the effects of sertraline and litoxetin on the metabolism of imipramine *in vitro*.

CYP1A2 is a constitutively expressed enzyme which is induced by polycyclic aromatic hydrocarbons [6]. CYP1A2 is a major enzyme activating a number of heterocyclic amines into their proximate carcinogenic and/or mutagenic forms [12]. Recent studies have shown that CYP1A2 also catalyses the oxidation of uroporphyrinogen to uroporphyrin and, hence, that the enzyme may play a role in the development of uroporphyria [20]. More important in the present context, CYP1A2 is a major enzyme catalysing the biotransformation of a number of drugs such as phenacetin [6, 12], caffeine and theophylline [8] and imipramine [5]. It is suggested that fluvoxamine has the potential for causing important drug–drug interactions when given in combination with either of these drugs. Indeed, this has already been demonstrated for theophylline [9–11] and for imipramine [21]. On the basis of the much weaker *in vitro* inhibition of CYP1A2 reported here (Table 2) it is unlikely that other SSRIs cause similar problems.

Furaphylline which is an antiasthmatic drug of the methylxanthine group is also a potent inhibitor of phenacetin O-deethylation *in vitro* [22], and it has been reported that caffeine accumulates to a toxic level due to potent inhibition of its metabolism in coffee drinking furaphylline-treated volunteers [23]. Similar interaction studies with fluvoxamine and caffeine are warranted.

During concomitant fluvoxamine intake, the steady-state plasma levels of propranolol, clomipramine and amitriptyline may increase by up to seven times [1, 24]. This suggests that CYP1A2 is a major enzyme catalysing the biotransformation of these drugs. Thus, if the very potent inhibition is specific for CYP1A2 then it is possible that fluvoxamine will become an important tool for the assessment of the role of the isozyme for the oxidation of drugs and other xenobiotics in humans.

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