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# Fluoxetine and norfluoxetine are potent inhibitors of P450IID6 – the source of the sparteine/debrisoquine oxidation polymorphism

Fluoxetine ((±) - *N* - methyl -3- phenyl -3- ((α,α,α-trifluoro-*p*-tolyl)-oxy))-propylamine hydrochloride) is a potent, selective inhibitor of 5-hydroxytryptamine (5-HT) reuptake and was marketed as an antidepressant in 1987. Two years later it had nearly 20% of the market share for antidepressants in the United States, and it is estimated that physicians in the USA write or renew about 650,000 fluoxetine prescriptions each month (Cowley *et al.*, 1990). However, it has yet to be shown that fluoxetine is effective in the treatment of severely depressed in-patients (The Medical Letter, 1990).

Anecdotal reports of seven patients treated with tricyclic antidepressants (nortriptyline ($n$ = 3), desipramine ($n$ = 3), imipramine ($n$ = 1)) have shown that the plasma concentrations of these drugs increase up to five fold when fluoxetine is added to the treatment (Aranow *et al.*, 1989; Bell & Cole, 1988; Vaughan, 1988). This observation indicates that fluoxetine and/or its active metabolite norfluoxetine are potent inhibitors of the metabolism of tricyclic antidepressants.

A particular cytochrome P450 isoenzyme, now referred to as P450IID6, is the source of the sparteine/debrisoquine oxidation polymorphism (Eichelbaum *et al.*, 1979, Evans *et al.*, 1980). Several tricyclic antidepressants are metabolized predominantly by P450IID6 (Brøsen & Gram, 1989) and several commonly used drugs are potent inhibitors of this enzyme, including quinidine and other antiarrhythmics and some neuroleptics (Brøsen & Gram, 1989). We have recently shown that paroxetine, another selective 5-HT reuptake inhibitor also is a potent inhibitor of P450IID6 having an apparent $K_i$ for 1-hydroxylation of bufuralol in human liver microsomes of about 1 μM (Brøsen *et al.*, 1991a).

The aromatic 2-hydroxylation of imipramine is catalyzed specifically by P450IID6 (Brøsen *et al.*, 1986). We now report the effects of fluoxetine and its active, demethylated metabolite norfluoxetine on the metabolism of imipramine by human liver microsomes. Microsomes from one human liver (HL3) (100 μg protein) were incubated with racemic fluoxetine or racemic norfluoxetine in final concenrations of 0, 0.25, 0.5, 1.0, 1.5 and 2.0 μM and with imipramine in final concentrations of 8, 16 and 32 μM. The incubation conditions and the h.p.l.c. method for the measurement of *N*-desmethylimipramine (desipramine), 2- and 10-OH-imipramine in microsomes are described by Zeugin *et al.* (1990). Untransformed data on the velocities of 2-OH-imipramine formation were analyzed using a non-linear, least-square regression program, which allows an observer-independent weighing of data (Holford, 1990). It was assumed that fluoxetine and norfluoxetine were inhibitors of a single major enzyme catalyzing 2-OH-imipramine formation. This model is described by the following equation:

$$V = \frac{V_{max} \cdot S}{S + K_m(1 + I/K_i)}$$

where $V$ is the velocity of the reaction, $V_{max}$ is the maximal velocity, S is the imipramine concentration, $K_m$ is the apparent Michaelis constant, $K_i$ is the apparent inhibitor constant and I is the concentration of fluoxetine or norfluoxetine.

Analysis of the separate fluoxetine and norfluoxetine data indicated apparent $K_m$ values of 8.7 and 10.3 μM, respectively, and apparent $V_{max}$ values of 7.4 and 6.7 nmol $mg^{-1}$ microsomal protein $h^{-1}$, respectively. The estimated apparent $K_i$ of fluoxetine was 514 nM and that of norfluoxetine was 596 nM (Figure 1). Cornish-Bowden plots were consistent with competitive inhibition for both compounds (data not shown). No effects were observed with regard to formation of desipramine and 10-OH-imipramine.

We have recently shown that levomepromazine inhibits 2-OH-imipramine formation in human liver microsomes with an apparent $K_i$ of about 1 μM (Brøsen *et al.*, 1991b).

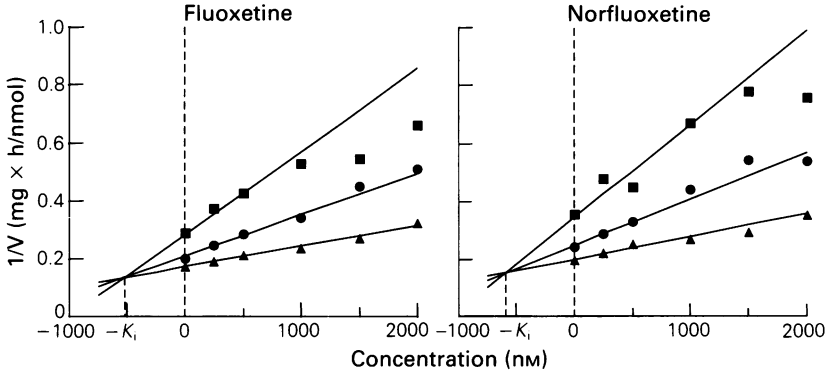


**Figure 1** Dixon plots illustrating the inhibition of the 2-hydroxylation of imipramine (■ 8 μM, ● 16 μM, ▲ 32 μM) by fluoxetine and norfluoxetine in microsomes from a human liver (HL3). Each point represents the mean of a duplicate determination. The straight lines are the best fits obtained when the observed data were analysed by non-linear least squares regression allowing observer-independent weighing of data. $K_i$ Apparent inhibitor constant.

Levomepromazine is a potent inhibitor of P450IID6 *in vivo*. Thus about 50% of levomepromazine treated patients were found to be poor metabolisers of debrisoquine (Syvälathi *et al.*, 1986). The preliminary findings of the present study indicate, that fluoxetine and norfluoxetine are also potent inhibitors of P450IID6, which would explain the large increase in plasma concentrations of desipramine and nortriptyline during coadministration of fluoxetine.

The widespread use of fluoxetine, the long half-lives of both the parent compound (3 days) and of the primary metabolite (7 days) and the potent inhibition of P450IID6 by both compounds indicate that fluoxetine is likely to interact with other drugs that are P450IID6 substrates. Besides tricyclic antidepressants, several neuroleptics (e.g. perphenazine and thioridazine) and some antiarrhythmics (e.g. propafenone and flecainide) have been shown to be metabolized largely or partly by P450IID6 (Brøsen & Gram, 1989).

Significant inter-subject variability in the plasma clearances and half-lives of fluoxetine and norfluoxetine have been observed (Aronoff *et al.*, 1984) which may be related to the sparteine/debrisoquine oxidation polymorphism.

The study was supported by a grant from the Danish Medical Research Council (no. 12-9206). Fluoxetine and norfluoxetine were provided by Eli Lilly Denmark A/S. The study represents a contribution to the aims of COST B1.

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Received 13 November 1990,
accepted 1 March 1991

ADONIS 030652519100156Z

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