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 (SSRI) 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 (chlorozoxazone 6-hydroxylase), while norfluoxetine was the only potent inhibitor of CYP3A having IC50 values of 11 μM and 19 μM for testosterone 6β-hydroxylase and cortisol 6β-hydroxylase, respectively.

3 Norfluoxetine, sertraline and fluvoxamine inhibited CYP1A2 (7-ethoxyresorufin O-deethylase) in microsomes from human placenta (IC50 values 29 μM, 35 μM and 80 μM, respectively). Fluvoxamine was a potent inhibitor of CYP1A2-mediated 7-ethoxyresorufin O-deethylase activity (IC50 = 0.3 μM) in human liver.

4 In microsomes from three human livers fluvoxamine potentially inhibited all pathways of theophylline biotransformation, the apparent inhibitor constant, Ki, was 0.07-0.13 μM, 0.05-0.10 μM and 0.16-0.29 μM for inhibition of 1-methylnxanthine, 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 Ki 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, Ki of less than 1 μM [1–3]. Fluvoxamine is a less potent inhibitor of CYP2D6 with Ki of 1.8 to 8.2 μM [1–3]. However, fluvoxamine is a...
potent inhibitor of the \(\alpha\)-deethylation of phenacetin [4] (\(K_i = 0.12 \text{ to } 0.24 \mu\text{M}\)) and of the \(\alpha\)-deethylation of imipramine [1] (\(K_i = 0.14 \mu\text{M}\)), two well-established market reactions for cytochrome P4501A2 (CYP1A2) function in human liver microsomes [5,7]. Fluvoxamine inhibition of P450 isoenzymes other than CYP1A2 and CYP2D6 has not previously been investigated.

These reports showed that steady-state plasma concentrations of fluvoxamine 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 \(\alpha\)-deethylation to 1-methylxanthine (1MX) and 3-methylxanthine (3MX). 1MX is further oxidized to 1-methyluric acid (1MU) by the cyto- plasmatic 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 \(\alpha\)-demethyl- alation pathways and CYP1A2 is believed to catalyze about 80-90% of the \(\alpha\)-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 \(\phi\)armacokinetic interaction between fluvoxamine and theophylline is most likely mediated through inhibition of CYP1A2. The main purpose of the study was to test this hypothesis. To prove that fluvoxamine is a potent and selective inhibitor of CYP1A2, fluvoxamine and the seven other SSSRs 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 CYP1A in theophylline metabolism.

Methods

Chemicals and reagents

Theophylline, theobromine, 1MX, quinidine, cortisol, coumarin, 7-hydroxyxycoumarin 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 6p-hydroxysterosterone was obtained from the Steroid Reference Repository (Professor D. N. Kirk) and \(^{14}\)C-testosterone was obtained from Amersham (Amerham, UK). Other drugs were kindly donated by the following companies: Fluvox- amine: Dukhar B.V. (Weesp, Holland), Paroxetine: Novo-Nordisk (Denmark). Fluoxetine and norfluoxe-
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 mm and 315 mm 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 mmol mg-1 h-1 and the coefficient of variation was < 2%.

Coumarin 7-hydroxylase assay Coumarin is a final concentration of 100 mm was incubated at 37° C with 2/0 mg microsomal protein and a NADPH-generating system in an incubation volume of 500 ml. The reaction was started by the addition of coumarin and the incubation time was 10 min. The formation of 7-hydroxycoumarin was determined by flurometric methods according to Aitio [33]. The quantitation limit was 0.6 nmol mg-1 h-1 and the coefficient of variation was < 6%.

Chloroazoxone 6-hydroxylase assay Chloroazoxone in a final concentration of 40 mm was incubated with 500 mm of microsomal protein in an incubation volume of 500 ml. The reaction was started by the addition of 50 mm NADPH-generating system (concentrations in microsomal suspension: isocitrate dehydrogenase, 1 mm, NADP+, 1 mm, isocitrate, 5 mm, MgCl2, 5 mm) and stopped after 20 min incubation at 37° C by the addition of 350 ml ice-cold zinc sulphate (2% v/v) and cooling on ice. After centrifugation (1000 g, 15 min) the supernatant was stored at -20° C until analysis. 6-hydroxychloroazoxone was assayed by h.p.l.c. The incubation mixture was precipitated with acetonitrile (45:55, v/v). After centrifugation 25 mm supernatant was injected onto a Nucleosil C8 column (particle size 5 mm, 150 mm x 4.6 mm i.d.) eluted at a rate of 1.5 ml min-1 with 25% (v/v) acetonitrile in 7.5 mm phosphate buffer pH 6.5. The effluent was monitored with an ESA Coulometer detector with a 5010 cell set at 280-320 mV at electrode 1 for detection of 6-hydroxychloroazoxone and 750-850 mV at electrode 2 for detection of chloroazoxone. The concentration in microsomal proteins of 6-hydroxychloroazoxone and chloroazoxone were determined by analysis of the voltamograms. The 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 mm) was incubated in a final incubation volume of 750 ml in disodium phosphate buffer (0.1 mm, pH 7.4) with cortisol in a final concentration of 10 mm. The reaction was started by the addition of 75 mm NADPH-generating system and stopped after 30 min incubation at 37° C by the addition of 525 mm ice-cold zinc sulphate (2% v/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 < 5% and the limit of quantitation was 0.01 nmol mg-1 h-1.

Testosterone 6β-hydroxylase assay Microsomal protein (630 mm) was incubated mg 20 min with [14C]-labelled testosterone in a final concentration of 100 mm and an incubation volume of 500 ml. 6β-hydroxysterosterone was measured by the thin layer chromatographic method of Warman 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 SSR1 in final concentrations of 0.1-100 mm.

Theophylline metabolism The metabolites 1MX, 3MX and 13DMU were assayed by an h.p.l.c. method described in detail elsewhere [36]. Briefly, 500 mm 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 mm. 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 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 these theophylline metabolites. Hence, an equation which describes a two-enzyme model was fitted to the data:

$$V = V_{\text{max}} \frac{K_m + [S]}{[S] + L[S]}$$

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 $K_m$). $V_{\text{max}}$ and $K_m$ are the apparent maximal velocity and the apparent Michaelis constant, respectively, of a high affinity enzyme and $L$ is $V_{\text{max}}/K_m$ for the low affinity enzyme(s) and is hence a constant which relates theo-
Figure 1 Endo-1,2,4-tris(3-methylthiophenyl-2-yl)-1,2,4-triazole-3-amine (V) of the formation rate (V) of 3-methylthiophenyl-2-yl)-1,2,4-triazole-3-amine (S) and 1,3-dimethylthiadicarbonyl (S) after incubating microsomes and 3-methylthiophenyl-2-yl)-1,2,4-triazole-3-amine (S) and 1,3-dimethylthiadicarbonyl (S) after incubating microsomes from HIL2 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 values for N-demethylations (fluvoxamine, phenacetin, clozapine and ethanol). For all four inhibitors, a graphic analysis revealed a curved relationship between reciprocal velocity and the inhibitor concentration ([I] (2). An equation which describes a two-enzyme model was therefore fitted to the data:

\[
V = V_{\text{max}} \frac{[S]}{[S] + K_{\text{I}} ([I] + 1)}
\]

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. KI 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, chlorozoxazine, 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 IC50 value was 0.3 μM, which was >30 times less than that of the second strongest inhibitor, paroxetine. Sertraline, litoxetine and norfluoxetine also inhibited EROD activity in liver microsomes, whereas fluvoxamine, desmethylcitalopram and citalopram had weak effects. Norfluoxetine blocked placentar EROD activity completely at a concentration of 100 μM; sertraline was a relatively strong inhibitor, fluvoxamine was a weak inhibitor, whereas citalopram, desmethylcitalopram, fluoxetine, litoxetine and paroxetine were either weak inhibitors or weak stimulators of placental EROD activity. Norfluoxetine blocked cortisol 6β-hydroxylase (CYP1B1) and testosterone 6β-hydroxylase (CYP1B1) activity almost completely at a concentration of 100 μM; fluvoxamine had an IC50 of 40–60 μM. The other SSRIs had either weak or no effects on the CYP3A marker reactions. The residual activity at an inhibitor concentra- tion 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 = 0.857, P < 0.02 (Spearman’s).
Table 1. The effects of eight SSRIs on the formation of 3-methylxanthine (3MX), 1-methyloxanthine (1MX) and 1,3-dimethyluric acid (13DU). From theophylline, 7-ethoxycoumarin O-deethylase (EORD$_{\text{L}}$), cortisol 6β-hydroxylase (C6βOH), intestinone 6β-hydroxylase (THβOH), coumarin 7-hydroxylase (COH), and chlorzoxazone 6-hydroxylase (C60H) activities in human liver microsomes, as well as 7-ethoxycoumarin O-deethylation activity in human placental microsomes (EORD$_{\text{P}}$). The results are the means of duplicate determinations.

<table>
<thead>
<tr>
<th>SSRI</th>
<th>3MX</th>
<th>1MX</th>
<th>13DU</th>
<th>EORD$_{\text{L}}$</th>
<th>EORD$_{\text{P}}$</th>
<th>C6βOH</th>
<th>THβOH</th>
<th>COH</th>
<th>C60H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control activity (nmol mg$^{-1}$ k$^{-1}$)</td>
<td>1.9</td>
<td>3.3</td>
<td>12.8</td>
<td>36.7</td>
<td>7.6</td>
<td>0.28</td>
<td>98.4</td>
<td>39.5</td>
<td>31.9</td>
</tr>
<tr>
<td>% of control activity (100 μM inhibitor concentration)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Fluvoxamine</td>
<td>10</td>
<td>15</td>
<td>56</td>
<td>6</td>
<td>42</td>
<td>34</td>
<td>35</td>
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<td>Paroxetine</td>
<td>29</td>
<td>35</td>
<td>72</td>
<td>9</td>
<td>54</td>
<td>38</td>
<td>60</td>
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<td>46</td>
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<td>9</td>
<td>13</td>
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<td>94</td>
<td>82</td>
<td>66</td>
<td>40</td>
<td>45</td>
<td>71</td>
<td>108</td>
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<tr>
<td>Norfluoxetine</td>
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<td>74</td>
<td>82</td>
<td>37</td>
<td>0</td>
<td>15</td>
<td>5</td>
<td>68</td>
<td>95</td>
</tr>
<tr>
<td>Citalopram</td>
<td>92</td>
<td>95</td>
<td>95</td>
<td>91</td>
<td>82</td>
<td>71</td>
<td>98</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Desmethyl-citalopram</td>
<td>93</td>
<td>97</td>
<td>94</td>
<td>86</td>
<td>172</td>
<td>67</td>
<td>67</td>
<td>81</td>
<td>131</td>
</tr>
</tbody>
</table>

\[K_{\text{IC50}}(\mu M)\]

- Fluvoxamine: 0.2
- Paroxetine: 0.2
- Centriline: 0.2
- Lithium: 0.3
- Fluoxetine: 10
- Norfluoxetine: 10
- Citalopram: 10
- Desmethyl-citalopram: 10

Human liver microsomes from HL1.
Human liver microsomes from HL4.
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 propylene glycol were found to inhibit C60H activity, with $K_{\text{IC50}}$ values of 1.3 μM and 20 μM, respectively. None of the SSRIs inhibited chlorzoxazone 6-hydroxylase (C60H) activity or coumarin 7-hydroxylase (COH) activity ($K_{\text{IC50}} > 100$ μM).

**Theophylline metabolism**

Edadie-Hofstee plots [37] showed that the formation of 1MX, 3MX and 13DU 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 log likelihoods [37] of the estimations using the biphasic model (Equation 1) ranged from 16.2 to 20.2, 3.8 to 9.8 and -10.1 to -5.9 for 3MX, 1MX and 13DU, 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 log likelihood values of -19.6 to -12.0 for fits to the data for formation of 13DU. Fluvoxamine had marked inhibitory effect on the formation of all three theophylline metabolites. Thus, the formation of 1MX, 3MX and 13DU 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 13DU, respectively. The $K_{i}$ values and the apparent $V_{\text{max}}$ 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 several 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-ethoxycoumarin as a probe. For eight SSRIs the $r^2$ was 0.952 ($P < 0.01$) for the correlation with 3MX formation, $r^2 = 0.827$ ($P < 0.05$) for 1MX and $r^2 = 0.917$ ($P < 0.01$) for the correlation with 13DU 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 chromatographic peaks it was not possible to determine a $K_{i}$ value for phenacetin inhibition of 13DU formation.

Clorazepate, 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 fluoroxamine of the formation of 3-methylcholine (3MX), 1-methylcholine (1MX) or 1,3-dimethylcholine (1,3DMU) from theophylline in human liver microsomes. Mean values and ranges determined with microsomes from three human livers (HL1, HL2 and HL3) are shown.

<table>
<thead>
<tr>
<th></th>
<th>V_{max} \textsuperscript{a} (nmol mg\textsuperscript{-1} h\textsuperscript{-1})</th>
<th>K_{m} \textsuperscript{a} (mM)</th>
<th>K_{i} ^{\dagger} (\mu M)</th>
<th>L \textsuperscript{\dagger} (\mu M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3MX</td>
<td>1.9 (1.4-2.6)</td>
<td>0.6 (0.4-0.8)</td>
<td>0.07 (0.05-0.10)</td>
<td>0.03 (0.02-0.05)</td>
</tr>
<tr>
<td>1MX</td>
<td>3.1 (2.6-4.3)</td>
<td>0.6 (0.4-0.8)</td>
<td>0.10 (0.07-0.13)</td>
<td>0.3 (0.2-0.4)</td>
</tr>
<tr>
<td>1,3DMU</td>
<td>28 (17-33)</td>
<td>8.5 (6.2-11.3)</td>
<td>0.24 (0.16-0.29)</td>
<td>3.8 (3.3-4.5)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}V_{max}, K_{m} and K_{i} refer to the high affinity site.

\textsuperscript{\dagger}The constant which relates the theophylline concentration to the velocity via the low affinity enzyme.

![Figure 3](image-url) Inhibition of the 8-hydroxylation of theophylline in microsomes from HL1 as a function of a) ethanol concentration and ethanol concentration plus 50 \mu M fluoroxamine, and b) fluoroxamine concentration and fluoroxamine concentration plus 34 \mu M ethanol. Each point represents the mean of duplicate determinations.

Discussion

We have shown that fluoroxamine is a potent inhibitor of the metabolism of 7-ethoxresorufin in human liver microsomes (CYP1A2), but that it causes weak or no inhibition of testosterone and cortisol (CYP3A), chloroxazone (CYP2E1), coumarin (CYP2A6) and placental 7-ethoxresorufin (CYP1A1) metabolism. Fluoroxamine is a weak inhibitor of meophenyl hydroxylase (CYP2C9) in vivo (Broş, personal communication), but inhibition of CYP2C9 has not been investigated. The results strongly suggest that CYP1A2 is the only isofrom which is inhibited potently by fluoroxamine.

The study also showed that fluoroxamine, as expected, is a potent inhibitor of the formation of 1MX, 3MX and 1,3DMU 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 \mu M reported for the O-demethylation of phenacetin [4], 0.14 \mu M for the N-demethylation of imipramine [1], as well as an IC_{50} value of 0.3 \mu M for the O-demethylation of ethoxyresorufin reported here. Thus, our data provide strong support for the hypothesis that the pharmacokinetic interaction between fluoroxamine and theophylline is caused by inhibition of CYP1A2, and that this isoform is important for all

60 \mu M (45-80 \mu M) for inhibition of the formation of 1MX, 3MX and 1,3DMU, 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 \mu M. Ethanol does not exhibit any non-specific inhibition of P450 enzymes in concentrations up to 86 \mu M (0.5%) [1, data not shown]. At a concentration of 86 \mu M ethanol decreased the formation of 1,3DMU to 58% (Figure 3a), whereas the demethylation pathways were not affected. When fluoroxamine was added in a final concentration of 50 \mu M to all incubations containing ethanol in concentrations from 0.17 to 86 \mu M, 8-hydroxylation of theophylline was decreased to 15% of control activity (Figure 3a). Fluoroxamine was re-tested in the concentration range 0.1-100 \mu M with ethanol in a final concentration of 34 \mu M. In combination, the two drugs decreased 8-hydroxylation to 10% of control activity as compared with a decrease of 1,3DMU formation to 56% when fluoroxamine was incubated alone (Figure 3b). For K_{i} determinations ethanol was incubated in concentrations from 0.17 \mu M to 3.4 \mu M. 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 \mu M for incubation with ethanol alone and 200 \mu M when fluoroxamine was added.

For technical reasons it was only possible to test chloroxazone in concentrations up to 10 \mu M. 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 \mu M) (data not shown). The IC_{50} values were 60 \mu M for inhibition of 1MX and 3MX formation and >100 \mu M for the inhibition of 1,3DMU formation.

Quinidine did not inhibit the metabolism of theophylline (data not shown).
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 chloroxazone, 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 chloroxazone (510 µM) [28], and for the inhibition of the N-demethylation of N-nitosodimethylamine (310 µM) [45]. For technical reasons it was not possible to test chloroxazone 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 in 13DMU formation [13]. Ketoconazole is an inhibitor of several P450s, but it 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-hydroxylation of these two compounds are indeed catalyzed by the same P450 [29–31]. Norfluroxetine 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 SSRIs 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 [43], 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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