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The effects of selective serotonin reuptake inhibitors and their metabolites on S-mephenytoin 4′-hydroxylase activity in human liver microsomes

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The inhibitory effects of four selective serotonin reuptake inhibitors (SSRIs), fluoxetine, sertraline, paroxetine and citalopram, and three metabolites (norfluoxetine, demethylcitalopram and didemethylcitalopram), on S-mephenytoin 4′-hydroxylation activities in human liver microsomes were studied. The 4′-hydroxylation of S-mephenytoin, a representative substrate toward CYP2C19, was competitively inhibited by all the SSRIs and their metabolites studied. The mean K_i values of fluoxetine, norfluoxetine, sertraline, paroxetine, citalopram, demethylcitalopram and didemethylcitalopram were 5.2, 1.1, 2.0, 7.5, 87.3, 55.8 and 7.7 μM, respectively. The findings suggest that some SSRIs and their metabolites with a low K_i value (e.g., fluoxetine, norfluoxetine) may reduce the clearance of drugs metabolized by this isoform of P450, thereby resulting in a possible drug-drug interaction, when administered simultaneously. In addition, SSRIs and their metabolites examined herein may be substrates toward CYP2C19.

Keywords S-mephenytoin 4′-hydroxylase (CYP2C19) citalopram fluoxetine paroxetine sertraline metabolites (norfluoxetine, demethylcitalopram and didemethylcitalopram)

Introduction

Citalopram, fluoxetine, paroxetine and sertraline are selective serotonin reuptake inhibitors (SSRIs), which preferentially bind to the presynaptic serotonin reuptake carrier in the central nervous system [1, 2]. These drugs have been marketed in Europe and North America as a new type of antidepressant and reported to possess significantly fewer side-effects than tricyclic antidepressants [1, 3–5]. SSRIs such as fluoxetine [3], paroxetine [4] and sertraline [5] are extensively metabolized by the liver, whereas citalopram is partially eliminated unchanged by the kidneys [1, 6]. Although the enzyme(s) responsible for the metabolism of SSRIs has not yet been fully characterized, *in vitro* human liver microsomal studies have suggested that an isoform of cytochrome P450, CYP2D6, is involved in the metabolism of SSRIs [7–9], being in agreement with an *in vivo* human panel study [10].

CYP2D6 shows genetically determined polymorphism in humans [11, 12]. The large interindividual variability in plasma concentrations of several tricyclic antidepressants seen in patients treated with similar doses has been attributed mainly to this polymorphism [13–16]. However, *in vivo* and *in vitro* studies have shown that imipramine and amitriptyline are also metabolized by another form of P450 (CYP2C19) which also shows the genetically determined polymorphism in humans [15, 17–20]. Thus, both the polymorphisms of CYP2C19 and CYP2D6 are considered to contribute to the variable plasma levels of imipramine or amitriptyline in patients treated with either of those tricyclics [15]. There is also the possibility that CYP2C19 may also be involved in the metabolism of SSRIs in addition to CYP2D6. In accordance with this possibility, Shindrup *et al.* [21] reported that plasma levels of citalopram were significantly higher in poor metabolizers than in extensive metabolizers of S-mephenytoin, suggesting that the metabolism of citalopram is mediated, at least partly, via CYP2C19.

In the present study we have assessed the inhibitory effects of four SSRIs, citalopram, fluoxetine, paroxetine and sertraline and three metabolites (norfluoxetine, demethylcitalopram and didemethylcitalopram), on the 4′-hydroxylase activity of S-mephenytoin in human liver microsomes. This approach was adopted for mephenytoin hydroxylase by Inaba *et al.* [22].

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Methods

Drugs

Citalopram, demethylcitalopram and didemethylcitalopram were kindly supplied by Lundbeck Ltd. (Copenhagen-Valby, Denmark), fluoxetine and norfluoxetine by Eli Lilly and Co. Ltd (Indianapolis, USA), paroxetine by Smith-Kline Beecham Pharmaceuticals (London, UK) and sertraline by Invicta Pharmaceuticals (Sandwich, UK). *Rac*-mephenytoin and 4′-hydroxymephenytoin were kindly donated by Dr Küpfer (University of Berne, Berne, Switzerland). S-mephenytoin was separated from the racemic mixture of mephenytoin by a Chiralcel OJ column (10 μm, 4.6 × 250 mm, Daicel Chemical Co. Ltd, Tokyo, Japan) as reported by Yasumori *et al.* [23].

Human liver microsomes

The four human liver samples were obtained from patients who underwent a partial hepatectomy at the Division of General Surgery, International Medical Center of Japan (Tokyo, Japan). Surgery was performed for the removal of metastatic tumour(s) from the liver. The use of the human liver for the study had been approved by the Institutional Ethics Committee. Liver microsomes were prepared as described previously [24, 25]. The mean (±s.d.) activity of S-mephenytoin 4′-hydroxylation in the four human liver microsomes was 0.054 ± 0.037 nmol mg^{-1} protein min^{-1}, when 100 μM was used as a substrate concentration.

Incubation conditions

Microsomes (0.025 mg protein) were incubated at 37 °C for 60 min with S-mephenytoin (50, 100 and 200 μM) and an NADPH generating system in the presence or absence of SSRIs in a final volume of 250 μl. The concentrations of SSRIs and their metabolites used were 0, 30, 100, 200 and 300 μM for citalopram and demethylcitalopram, 0, 10, 25, 30 and 50 μM for didemethylcitalopram, sertraline, paroxetine and fluoxetine, and 0, 1, 5, 10 and 30 μM for norfluoxetine. The reactions were stopped by the addition of 100 μl acetonitrile. After the termination of the reactions, 50 μl of cyclobarbitone solution (1.25 μg ml^{-1} in methanol) was added as an internal standard. The mixture was centrifuged at 10 000 ***g*** for 5 min, and then the supernatant was injected into the high-performance liquid chromatography (h.p.l.c.) system.

Determination of 4′-hydroxymephenytoin

Determination of 4′-hydroxymephenytoin was carried out by an h.p.l.c. method as reported previously [24]. The mobile phase consisted of acetonitrile and a sodium phosphate buffer (0.05 M, pH 4.0) in the proportion of 25/75 (by v/v), and was delivered to a CAPCELL PAK C_{18} AG120 column (Shiseido Co. Ltd, Tokyo, Japan; 4.6 mm × 25 cm) at a flow rate of 0.8 ml min^{-1}. The eluate was monitored at a wavelength of 204 nm. The calibration curve was generated from 25 to 100 ng ml^{-1} by processing the authentic standard substance through the entire procedures.

The formation rate of 4′-hydroxymephenytoin was linear at 37 °C for up to 90 min, when 200 μM of S-mephenytoin and 0.1 mg ml^{-1} of microsomal protein were present. A linear relationship was also observed between the rate of metabolite production in 60 min and protein concentration for up to 1.0 mg ml^{-1}. Less than 0.5% of the substrate was metabolized during the incubation for 60 min. The limits of detection and quantitation were 2 and 5 pmol mg^{-1} protein min^{-1}, respectively. The intra- and inter-assay CVs were less than 6.4 and 8.0%, respectively.

Data analysis

Inhibition patterns were determined by a visual inspection of double reciprocal plots for S-mephenytoin concentration *vs* the velocity of the reaction. Apparent K_m in each concentration of the putative inhibitors was estimated by the linear regression analysis by using unweighted raw data following a simple Michaelis–Menten kinetic approach. The K_i (inhibition constant) values were determined from unweighted linear regression analysis using an equation consistent with the competitive inhibition [26];

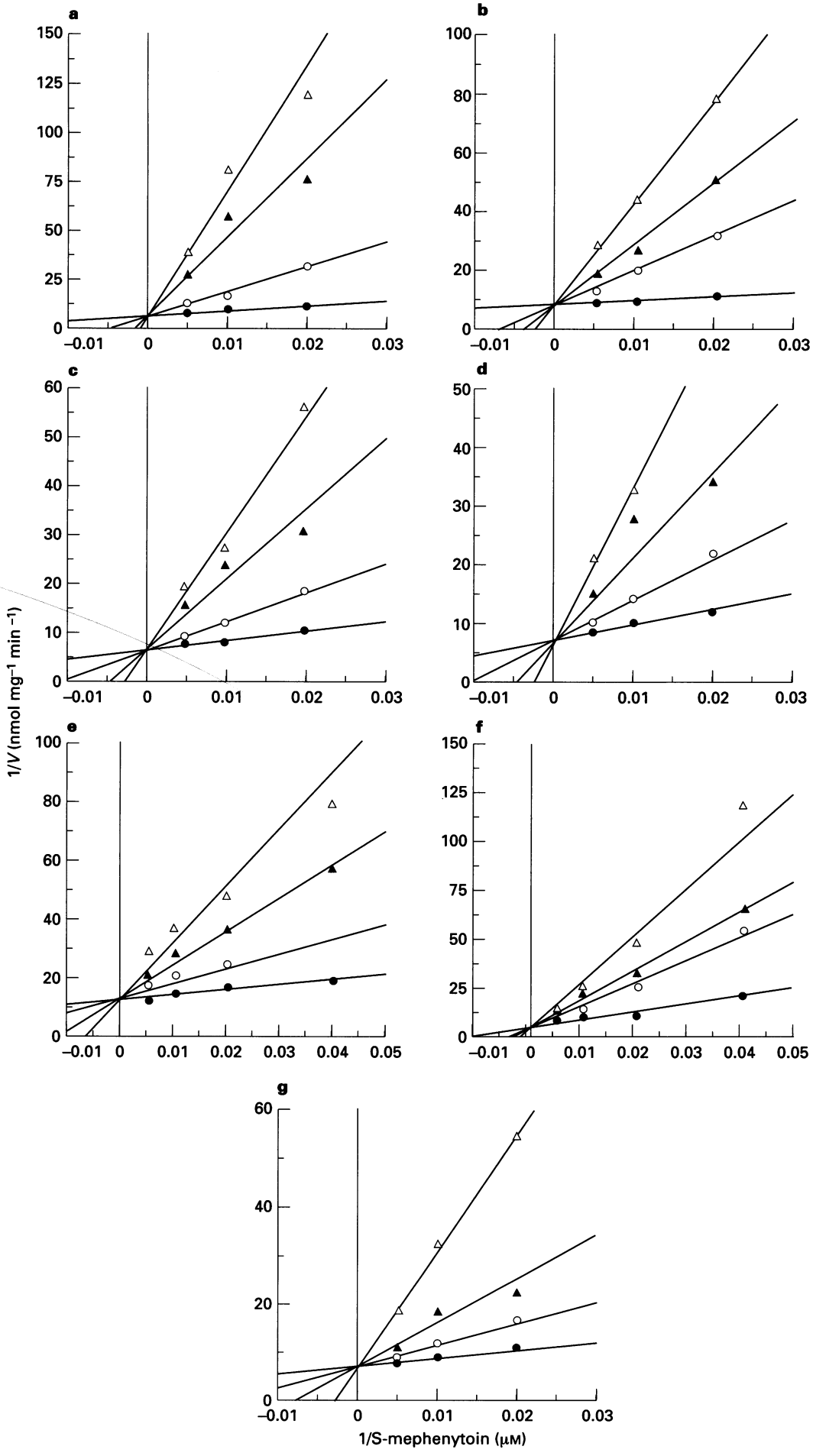
$$\text{Apparent } K_m = K_m + K_m \cdot I/K_i$$

where K_m = substrate concentration at which the reaction velocity is equal half of the maximum velocity in the absence of an inhibitor; apparent $K_m = K_m$ in the presence of an inhibitor; I = inhibitor concentration; K_i = inhibition constant.

Results

All SSRIs and their metabolites examined in the present study inhibited S-mephenytoin 4′-hydroxylase activities in human liver microsomes. However, the magnitude of the inhibition differed among them: the inhibition

Figure 1 Representative Lineweaver-Burk plots for the inhibition of S-mephenytoin 4′-hydroxylase activities: by a) sertraline ● 0 μM, ○ 10 μM, ▲ 25 μM, △ 50 μM; b) fluoxetine ● 0 μM, ○ 10 μM, ▲ 25 μM, △ 50 μM; c) norfluoxetine ● 0 μM, ○ 1 μM, ▲ 5 μM, △ 10 μM; d) paroxetine ● 0 μM, ○ 10 μM, ▲ 25 μM, △ 50 μM; e) citalopram ● 0 μM, ○ 100 μM, ▲ 200 μM, △ 300 μM; f) demethylcitalopram ● 0 μM, ○ 100 μM, ▲ 200 μM, △ 300 μM; and g) didemethylcitalopram ● 0 μM, ○ 10 μM, ▲ 25 μM, △ 50 μM in human liver microsomes.


a
b
c
d
e
f
g
1/V (nmol mg⁻¹ min⁻¹)
1/S-mephenytoin (μM)

percentage values of S-mephenytoin 4′-hydroxylation, when 100 μM of S-mephenytoin was used as a substrate, were 62.8, 82.6, 73.3, 52.4, 5.7, 13.2 and 61.2% for 30 μM of fluoxetine, norfluoxetine, sertraline, paroxetine, citalopram, demethylcitalopram and didemethylcitalopram, respectively.

The inhibition occurred in a competitive manner for all SSRIs and their metabolites as shown in Figure 1. The mean (±s.d.) K_i values obtained from four experiments are listed in Table 1. Norfluoxetine showed the most potent inhibition with a K_i value of 1.1 μM, followed by sertraline (K_i = 2.0 μM) and fluoxetine (K_i = 5.2 μM). Didemethylcitalopram and paroxetine showed a K_i value of around 7 μM. The mean K_i values of citalopram and demethylcitalopram were more than 55 μM, indicating that both are weaker inhibitors of CYP2C19.

Discussion

All the SSRIs and their metabolites examined inhibit the 4′-hydroxylation of S-mephenytoin competitively in human liver microsomes. The K_i values of three SSRIs and the two metabolites (norfluoxetine and didemethylcitalopram), except for citalopram and demethylcitalopram, were comparable to or less than those of omeprazole (2 μM), diazepam (12 μM) and R-mephobarbitone (34 μM), which are substrates of CYP2C19 [25, 27, 28]. Since omeprazole has been reported to reduce the clearance of diazepam and to slow the elimination rate of desmethyldiazepam [29–31], both of which are metabolized *in vivo* by CYP2C19 [30–32], fluoxetine, norfluoxetine and paroxetine may interfere with the CYP2C19-mediated metabolism of drugs (e.g. diazepam, imipramine) and may cause drug–drug interactions when co-administered. In fact, fluoxetine has been reported to decrease the clearance of diazepam when administered simultaneously [33]. Not only the parent drug, fluoxetine, but also its metabolite, norfluoxetine, may be involved in this drug–drug interaction.

Since the mean K_i values of citalopram and its major metabolite, demethylcitalopram [1, 21], are much greater (>55 μM) compared with other SSRIs and norfluoxetine, citalopram may not interfere with the metabolism of other drugs mediated via CYP2C19. This idea is supported by the recent finding that treatment with citalopram caused no significant change in the S/R ratio of mephenytoin in healthy volunteers [21]. However, fluoxetine and citalopram are chiral compounds that are marketed as racemic mixtures. Since the inhibition of CYP2D6-mediated bufuralol 1′-hydroxylation is greater with the S-form than the R-form of fluoxetine and norfluoxetine [26], it should be kept in mind that the inhibitory potency of individual enantiomers of fluoxetine, citalopram and their metabolites may differ between each other for CYP2C19. This aspect of the enantioselective effects of SSRIs and their metabolites on the CYP2C19-mediated metabolism of drugs definitely requires further studies.

Table 1 K_i values of serotonin reuptake inhibitors (sertraline, fluoxetine, paroxetine and citalopram) and their metabolites (norfluoxetine, demethylcitalopram and didemethylcitalopram) for S-mephenytoin 4′-hydroxylase (CYP2C19) activities in human liver microsomes

Compounds	K_i (μM)
Sertraline	2.0±0.6
Fluoxetine	5.2±3.2
Norfluoxetine	1.1±0.8
Paroxetine	7.5±5.1
Citalopram	87.3±32.0
Demethylcitalopram	55.8±13.7
Didemethylcitalopram	7.7±3.7

The values are expressed as mean±s.d. (n=4)

The inhibition pattern of all SSRIs and their metabolites occurred in a competitive manner. This finding suggests that these compounds bind to the substrate binding site of CYP2C19, although this contention may not necessarily indicate that they are substrates for CYP2C19. However, because the K_i value should be regarded as the K_m of a compound, when the inhibition pattern obtained occurs in a competitive fashion, our findings suggest that the SSRIs examined in the present study may be good substrates of CYP2C19. To our knowledge, there has been no report describing a possible involvement of CYP2C19 in the metabolism of SSRIs, except for that of Sindrup *et al.* [21] showing that the oral clearance of citalopram decreased in poor metabolizers of S-mephenytoin as compared with that in extensive metabolizers. Therefore, further *in vitro* and *in vivo* studies to clarify a possible involvement of CYP2C19 in the metabolism of SSRIs is necessary.

In conclusion, the results of the present study have shown that the four SSRIs, fluoxetine, sertraline, paroxetine and citalopram and their metabolites (norfluoxetine, demethylcitalopram and didemethylcitalopram) inhibit S-mephenytoin 4′-hydroxylation competitively in human liver microsomes. Because the mean K_i values of fluoxetine, norfluoxetine, sertraline and paroxetine are much (i.e. about 8 to 80 times) lower compared with those of citalopram and its major metabolite (Table 1), they may interfere with the metabolism of drugs mediated via CYP2C19, thereby resulting in the possible drug–drug interactions when co-administered. In addition, SSRIs and their metabolites examined herein may be substrates toward CYP2C19. Obviously, further studies are required to confirm these suggestions.

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