Inhibition of CYP2C9 by selective serotonin reuptake inhibitors *in vitro*: studies of phenytoin *p*-hydroxylation

Jürgen Schmider, David J. Greenblatt, Lisa L. von Moltke, Dmitry Karsov & Richard I. Shader

Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, Boston MA

Aims Inhibition of cytochrome P450 (CYP) activity by selective serotonin reuptake inhibitors (SSRIs) has frequently been reported with regard to pathways mediated by CYP2D6, CYP3A4/5, and CYP1A2. Little data exist on the capability of SSRIs to inhibit CYP2C9.

Methods We investigated the effect of SSRIs on *p*-hydroxylation of phenytoin (PPH), an established index reaction reflecting CYP2C9 activity, in an *in vitro* assay using liver tissue from six different human donors.

Results In control incubations (without inhibitor), 5-(*p*-hydroxy-phenyl)-5-phenylhydantoin (HPPH) formation rates were: V_{max} 0.023 nmol min^{-1} mg^{-1}; K_m 14.3 μM. Average inhibition constants (K_i) differed significantly among the SSRIs, with fluvoxamine having the lowest K_i (6 μM) followed by R-fluoxetine (13 μM), norfluoxetine (17 μM), RS-fluoxetine (19 μM), sertraline (33 μM), paroxetine (35 μM), S-fluoxetine (62 μM), and desmethylsertraline (66 μM). Thus, assuming comparable molar concentrations at the site of inhibition, fluvoxamine can be expected to have the highest probability of interfering with the metabolism of CYP2C9 substrates. S-fluoxetine is on average a 5 fold weaker CYP2C9 inhibitor than either R-fluoxetine or the racemic mixture.

Conclusions These findings are consistent with published case reports describing SSRI-related increments in plasma phenytoin levels. Because phenytoin has a narrow therapeutic index, plasma levels should be closely monitored when SSRIs are coadministered.

Keywords: phenytoin, cytochrome P-450, CYP2C9, metabolism, *in vitro*, human liver microsomes, fluoxetine, norfluoxetine, sertraline, desmethylsertraline, fluvoxamine, paroxetine, selective serotonin reuptake inhibitor

Introduction

Selective serotonin reuptake inhibitors (SSRIs) have frequently been reported to cause pharmacokinetic interactions with numerous other drugs. Most studies have implicated pathways mediated by CYP2D6 [1–3], CYP3A4/5 [4–6], and CYP1A2 [7, 8]. No systematic data exist on the capability of SSRIs to inhibit CYP2C9, the cytochrome that mediates the metabolism of frequently coadministered drugs such as phenytoin, tolbutamide, S-warfarin, ibuprofen, diclofenac and naproxen. Some case reports indicate reduced clearance of phenytoin when coadministered with fluoxetine [9–12]. Since clearance of phenytoin predominantly depends on CYP2C9-mediated para-hydroxylation, this suggests that SSRIs have the capacity to inhibit this CYP as well. In addition, there is evidence that the stereoisomers of fluoxetine exhibit a differential inhibitory pattern on CYP mediated metabolism. We investigated the effect of R-fluoxetine (R-FLU), S-fluoxetine (S-FLU), the racemic mixture RS-fluoxetine (RS-FLU), norfluoxetine (NOR), sertraline (SERT), desmethylsertraline (DES), fluvoxamine (FX), and paroxetine (PX) on *p*-hydroxylation of phenytoin (PPH), an established index reaction reflecting CYP2C9 activity [13–15], in an *in vitro* assay using liver tissue from six different human donors.

Correspondence: Dr Jürgen Schmider, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA.
Phone: +617 636 6997, Fax: +617 636 6738.

Methods

Chemical reagents and reaction cofactors were purchased from standard commercial sources as described previously [3–6, 15]. Chlorzoxazone, PPH, and its metabolite were purchased from Sigma (St Louis MO, USA). RS-FLU and NOR were kindly provided by Lilly, Indianapolis IN, USA; R-FLU and S-FLU by Sepracor, Marlboro MA, USA; SERT and DES by Pfizer, Groton CT, USA; FX by Solvay, Marietta GA, USA; and PX by Smithkline Beecham, Philadelphia PA, USA.

Liver samples from six different human donors with no known liver disease were obtained from the International Institute for the Advancement of Medicine (Exton, PA, USA), or the Liver Tissue Procurement and Distribution System, University of Minnesota, Minneapolis, MN. The tissue was partitioned and kept at −80° C until the time of microsome preparation. Microsomes were prepared by

differential ultracentrifugation; microsomal pellets were suspended in 0.1 M potassium phosphate buffer containing 20% glycerol and again stored at −80° C until use. The protein concentration of microsome samples was determined using the Bicinchoninic Acid Protein Assay (BCA-Pierce). Bovine serum albumin was used as standard.

Varying quantities of phenytoin, with or without the SSRIs, in methanol solution were added to a series of incubation tubes and evaporated to dryness (40° C, mild vacuum). Concentrations of PPH ranged from 0 to 250 μM (9 data points). PPH at concentrations of 10, 25, and 50 μM was incubated with the SSRIs at two different concentrations each: R-FLU, RS-FLU, and NOR at 50 and 100 μM; S-FLU and PX at 100 and 200 μM; SERT and DES at 75 and 150 μM), FX at 20 and 40 μM. Incubation mixtures containing 50 mM potassium phosphate buffer (pH adjusted to 7.5 at 25° C), 5 mM Mg^{++}, 0.5 mM $NADP^{+}$, and an isocitrate/isocitric dehydrogenase regenerating system were preincubated at 37° C for 5 min, and reactions were initiated by the addition of microsomes. Final volumes of the incubation mixture were 250 μl, with a microsomal protein concentration of 0.8 mg ml^{-1} (linear up to 1 mg ml^{-1}). Incubations were performed for 60 min at 37° C (linear range: 10–70 min), then stopped with the addition of 100 μl acetonitrile and cooling on ice. Chlorzoxazone was then added as an internal standard, and the mixture was spun at 16000 *g* for 5 min in a Micro-MB centrifuge. Supernatants were injected into the HPLC. All incubations were done in duplicate. Calibration curves were prepared by adding incubation buffer and internal standard to known amounts of 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (HPPH), yielding a final volume of 250 μl.

The h.p.l.c. system consisted of a Lambda-Max Model 480 LC ultraviolet spectrophotometer detector (Waters Associates, Milford, MA), set at a wavelength of 210 nm. A 30 cm × 3.9 mm stainless steel C_{18} μBondapack column was used for separation. The mobile phase consisted of 30% acetonitrile and 70% 50 mM potassium phosphate buffer; the flow rate was 1.3 ml min^{-1}. Samples were injected directly for assay. The SSRIs did not interfere with the assay. Addition of protein to the calibration samples did not alter metabolite recovery. Height ratios were determined utilizing the internal standard method, and height ratios of duplicate samples were averaged. Average coefficient of variation for duplicate control curve data points was 5.6% (s.d. 5.3) and for inhibitor data points 9.3% (s.d. 8.9).

Data points consisting of reaction velocities (V) at varying concentrations of the substrate PPH (S) without inhibitors were fitted by derivative-free iterative nonlinear least-squares regression to the Michaelis Menten equation, from which K_m, the Michaelis-constant, and V_{max}, the maximal HPPH formation rate, were determined. Utilizing the predetermined kinetic parameters, the Michaelis Menten equation consistent with competitive inhibition was fitted to data points for HPPH formation in the presence of the metabolic inhibitor (total of six data points for each SSRI) by the same computerized process. The iterated variable was K_i, the inhibition constant.

Statistical differences in K_i among SSRIs were examined by one-way analysis of variance with repeated measurements (ANOVA) and the Student-Newman-Keuls test.

Results

A consistent mechanism of inhibition for the various SSRIs could not be clearly established. However, in the majority of cases inhibition was explained by a competitive mechanism, or competitive inhibition could not be excluded. To obtain inhibition constants that allow comparison of all SSRIs for their potency to inhibit CYP2C9-mediated PPH hydroxylation, the Michaelis Menten equation consistent with competitive inhibition was fitted to all inhibition data (Figure 1). The derived kinetic parameters and inhibition constants are displayed in Table 1.

All eight SSRI components inhibited HPPH formation. They differed significantly in mean values of K_i, based on ANOVA ($F=12.231$, $P<0.0001$). FX was the most potent inhibitor, having the lowest average K_i value differing significantly from SERT, DES, PX, and S-FLU (Student-Newman-Keuls test). R-FLU demonstrated significantly stronger inhibition (Student-Newman-Keuls test) on the hydroxylation of PPH compared with S-FLU which had a five fold higher average K_i (Table 1).

Discussion

HPPH formation rate patterns were consistent with Michaelis Menten kinetics as was reported previously [13]. PPH kinetics *in vivo* are well-established as nonlinear within the usual therapeutic range. The relatively low mean K_m value for PPH hydroxylation *in vitro* may in part explain this phenomenon.

Inhibition patterns for the SSRIs were not consistent among the liver tissue samples investigated. To compare the inhibitory potency of the SSRIs, a Michaelis Menten

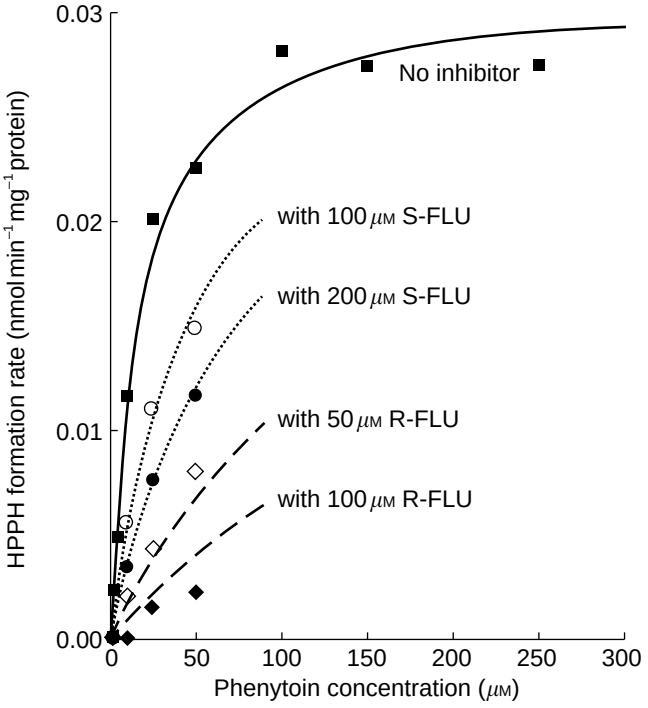

Figure 1 Effect of S-fluoxetine and R-fluoxetine on HPPH formation rate with microsomal preparations from a representative liver sample (HL 5). Lines represent functions of best fit as determined by nonlinear regression.

Table 1 Inhibition of phenytoin hydroxylation by SSRIs. Kinetic parameters and inhibition constants

	HL 1	*HL 2*	*HL 3*	*HL 4*	*HL 5*	*HL 6*	*Mean*	*s.d.*
V_{max}	0.017	0.025	0.028	0.016	0.031	0.022	0.023	0.006
K_m	9.4	17.3	12.9	16.3	17.5	12.5	14.3	3.2
K_i for:								
RS-FLU	11	24	17	24	19	21	19**	5
S-FLU	40	108	36	72	57	56	62	26
R-FLU	9	25	6	18	5	14	13**	7
NOR	12	28	13	22	5	24	17**	9
SERT	16	34	33	33	38	43	33**	9
DES	38	45	70	56	73	113	66	27
FX	2	5	23	4	2	3	6*	8
PX	18	57	22	40	39	33	35	14

V_{max} in [nmol min^{-1} mg^{-1} protein]; K_m and K_i in [μM]; s.d. = standard deviation. Statistical differences in K_i among SSRIs were examined by analysis of variance with repeated measures: $F = 12.231$, $P < 0.0001$; *post hoc* test: Student-Neuman-Keuls. *different from SERT, PX, S-FLU, and DES; **different from S-FLU and DES.

equation consistent with competitive inhibition [16] was fitted to the control and the inhibition data. The resulting derived kinetic parameters and inhibition constants do not reflect the exact underlying biological mechanisms but do explain the actual formation rates with reasonable accuracy (Figure 1) thereby allowing comparison of the inhibition constants for the SSRIs. On the average, FX is the strongest inhibitor of HPPH formation followed by R-FLU, NOR, RS-FLU, SERT, PX, S-FLU, and DES with increasing values for K_i (Table 1).

These *in vitro* findings are consistent with *in vivo* observations. Although controlled kinetic studies of PPH coadministered with FLU are not published, case reports strongly suggest that FLU may potentially impair PPH kinetics *in vivo* [9–12]. Steady state plasma concentrations of FLU and NOR during chronic treatment with FLU in humans [17] may produce intrahepatic concentrations of FLU and NOR that approach *in vitro* K_i values for PPH hydroxylation, based on data indicating substantial hepatic uptake of SSRIs and related compounds [3, 5, 18–24]. Since PPH clearance *in vivo* depends mainly on HPPH formation [25], impaired HPPH formation by FLU and NOR may produce an increase in steady-state PPH levels. Chronic treatment with SERT, on the other hand, produces plasma levels of SERT and DES that are considerably lower than FLU and NOR [17]; this, together with the higher K_i values of SERT and DES, reduce the likelihood of an *in vivo* interaction of SERT with PHH. A controlled clinical pharmacokinetic study demonstrated no significant interaction of SERT, 200 mg daily, with PPH [26].

Little data exist on stereoselective inhibition of drug metabolism by fluoxetine enantiomers. It has previously been reported that the enantiomers of FLU and NOR display differential inhibition of CYP2D6 mediated bufuralol 1′-hydroxylation [27] and desipramine hydroxylation [28], with S-FLU being an order of magnitude stronger than R-FLU. However, R-FLU appears to be a stronger inhibitor of hexobarbitone metabolism in the mouse [29], a reaction probably mediated by CYP2C isoforms. Our data indicate that R-FLU, in contrast to its weaker properties as a CYP2D6 inhibitor, is a 5 to 6 fold stronger inhibitor of PPH-hydroxylation *in vitro* compared with S-FLU.

It is likely that the relative inhibitory capacity of the NOR enantiomers parallels that of the FLU enantiomers, as is the case with bufuralol 1′-hydroxylation [27]. Thus R-NOR is probably also a stronger inhibitor of PPH para hydroxylation than S-NOR. The racemic mixture of NOR has a similar average value for K_i as RS-FLU (Table 2). S-FLU is therefore less likely to cause pharmacokinetic interactions if coadministered with phenytoin. We assume that the pharmacokinetic interactions of FLU and PPH probably are attributable mainly to the R-component of the racemic mixture.

In conclusion, careful monitoring of PPH plasma levels is mandatory when phenytoin and SSRIs are coadministered, particularly because phenytoin has a narrow therapeutic index. Caution is also advised for the coadministration of SSRIs with other drugs where clearance is dependent on CYP2C9-mediated metabolism (e.g. tolbutamide, S-warfarin, ibuprofen, diclofenac, naproxen). In the case of warfarin, for example, anecdotal reports suggest the possibility of warfarin potentiation by coadministration of FLU [30, 31]. Prescribing information for fluvoxamine [32] indicates that coadministration of fluvoxamine approximately doubles plasma warfarin concentrations and prolongs prothrombin times.

This work was supported in part by a grant from Sepracor, Inc., and by Grant MH-34223 from the Department of Health and Human Services. Dr Schmider was the recipient of a Merck Sharp & Dohme International Fellowship in Clinical Pharmacology. Dr von Moltke is the recipient of a Scientist Development Award (K21-MH-01237) from the Department of Health and Human Services.

References

1 Skjelbo E, Brøsen K. Inhibitors of imipramine metabolism by human liver microsomes. *Br J Clin Pharmacol* 1992; **34**: 256–261.

2 Spina E, Pollicino AM, Avenoso A, Campo GM, Perucca E, Caputi AP. Effect of fluvoxamine on the pharmacokinetics of imipramine and desipramine in healthy subjects. *Ther Drug Monit* 1993; **15**: 243–246.
3 von Moltke LL, Greenblatt DJ, Cotreau-Bibbo MM, Duan SX, Harmatz JS, Shader RI. Inhibition of desipramine hydroxylation *in vitro* by serotonin-reuptake-inhibitor antidepressants, and by quinidine and ketoconazole: a model system to predict drug interactions *in vivo*. *J Pharmacol Exp Ther* 1994; **268**: 1278–1283.
4 von Moltke LL, Greenblatt DJ, Harmatz JS, *et al.* Triazolam biotransformation by human liver microsomes *in vitro*: effects of metabolic inhibitors, and clinical confirmation of a predicted interaction with ketoconazole. *J Pharmacol Exp Ther* 1996; **276**: 370–379.
5 von Moltke LL, Greenblatt DJ, Cotreau-Bibbo MM, Harmatz JS, Shader RI. Inhibitors of alprazolam metabolism *in vitro*: effect of serotonin-reuptake-inhibitor antidepressants, ketoconazole and quinidine. *Br J Clin Pharmacol* 1994; **38**: 23–31.
6 Schmider J, Greenblatt DJ, von Moltke LL, Harmatz JS, Shader RI. N-demethylation of amitriptyline *in vitro*: role of cytochrome P-450 3A (CYP3A) isoforms, and effect of metabolic inhibitors. *J Pharmacol Exp Ther* 1995; **275**: 592–597.
7 Brøsen K, Skjelbo E, Rasmussen BB, Poulsen HE, Loft S. Fluvoxamine is a potent inhibitor of cytochrome P4501A2. *Biochem Pharmacol* 1993; **45**: 1211–1214.
8 Rasmussen BB, Mäenpää J, Pelkonen O, *et al.* Selective serotonin reuptake inhibitors and theophylline metabolism in human liver microsomes: potent inhibition by fluvoxamine. *Br J Clin Pharmacol* 1995; **39**: 151–159.
9 Jalil P. Toxic reaction following the combined administration of fluoxetine and phenytoin: two case reports [letter]. *J Neurol Neurosurg Psychiat* 1992; **55**: 412–413.
10 Shader RI, Greenblatt DJ, von Moltke LL. Fluoxetine inhibition of phenytoin metabolism. *J Clin Psychopharmacol* 1994; **14**: 375–376.
11 Woods DJ, Coulter DM, Pillans P. Interaction of phenytoin and fluoxetine. *N Z Med J* 1994; **107**: 19.
12 Darley J. Interaction between phenytoin and fluoxetine. *Seizure* 1994; **3**: 151–152.
13 Doecke CJ, Veronese ME, Pond SM, *et al.* Relationship between phenytoin and tolbutamide hydroxylations in human liver microsomes. *Br J Clin Pharmacol* 1991; **31**: 125–130.
14 Tassaneeyakul W, Veronese ME, Birkett DJ, *et al.* Co-regulation of phenytoin and tolbutamide metabolism in humans. *Br J Clin Pharmacol* 1992; **34**: 494–498.
15 Schmider J, Greenblatt DJ, von Moltke LL, *et al.* Characterization of six *in vitro* reactions mediated by human cytochrome P450: Application to the testing of cytochrome P450-directed antibodies. *Pharmacology* 1996; **52**: 125–134.
16 Segel IH. *Enzyme kinetics: behavior and analysis of rapid equilibrium and steady state enzyme systems*. eds.: New York: Wiley & Sons, Inc., 1975: 100–160.
17 von Moltke LL, Greenblatt DJ, Duan SX, Harmatz JS, Wright CE, Shader RI. Inhibition of terfenadine metabolism *in vitro* by azole antifungal agents and by selective serotonin reuptake inhibitor antidepressants: relation to phamacokinetic interactions *in vivo*. *J Clin Psychopharmacol* 1996; **16**: 104–112.
18 Shader RI, von Moltke LL, Schmider J, Harmatz JS, Greenblatt DJ. The clinician and drug interactions—an update. *J Clin Psychopharmacol* 1996; **16**: 197–201.
19 Schmider J, Greenblatt DJ, von Moltke LL, Shader RI. Relationship of *in vitro* data on drug metabolism to *in vivo* pharmacokinetics and drug interactions; implications for diazepam disposition in humans. *J Clin Psychopharmacol* 1996; **16**: 267–272.
20 von Moltke LL, Greenblatt DJ, Court MH, Duan SX, Harmatz JS, Shader RI. Inhibition of alprazolam and desipramine hydroxylation *in vitro* by paroxetine and fluvoxamine: comparison with other serotonin-reuptake-inhibitor antidepressants. *J Clin Psychopharmacol* 1995; **15**: 125–131.
21 Greenblatt DJ, von Moltke LL, Schmider J, Harmatz JS, Shader RI. Inhibition of human cytochrome P450–3A isoforms by fluoxetine and norfluoxetine: *in vitro* and *in vivo* studies. *J Clin Pharmacol* 1996; **36**: 792–798.
22 Bailey DN, Shaw RF. Interpretation of blood and tissue concentrations in fatal self-ingested overdose involving amitriptyline: an update (1978–1979). *J Anal Toxicol* 1980; **4**: 232–236.
23 Fraser AD, Isner AF, Perry RA. Distribution of trimipramine and its major metabolites in a fatal overdose case. *J Anal Toxicol* 1987; **11**: 168–170.
24 Levine B, Jenkins AJ, Smialek JE. Distribution of sertraline in postmortem cases. *J Anal Toxicol* 1994; **18**: 272–274.
25 Nation RL, Evans AM, Milne RW. Pharmacokinetic drug interactions with phenytoin (Part I). *Clin Pharmacokin* 1990; **18**: 37–60.
26 Rapeport WG, Muirhead DC, Williams SA, Cross M, Wesnes K. Absence of effect of sertraline on the pharmacokinetics and pharmacodynamics of phenytoin. *J Clin Psychiat* 1996; **57[suppl 1]**: 24–28.
27 Stevens JC, Wrighton SA. Interaction of the enantiomers of fluoxetine and norfluoxetine with human liver cytochromes P450. *J Pharmacol Exp Ther* 1993; **266**: 964–971.
28 von Moltke LL, Greenblatt DJ, Duan SX, Harmatz JS, Aberg G, Shader RI. Differential inhibition of desipramine hydroxylation *in vitro* by the enantiomers of fluoxetine. *Clin Pharmacol Ther* 1995; **37**: 205.
29 Fuller RW, Perry KW. Comparison of fluoxetine and norfluoxetine enantiomers as inhibitors of hexobarbitone metabolism in mice. *J Pharm Pharmacol* 1992; **44**: 1041–1042.
30 Hanger HC, Thomas F. Fluoxetine and warfarin interactions. *N Z Med J* 1995; **108**: 157.
31 Woolfrey S, Gammack NS, Dewar MS, Brown PJ. Fluoxetine-warfarin interaction. *Br Med J* 1993; **307**: 241.
32 *Physician's Desk Reference, 1996*. eds.: Oradell NJ: Medical Economies Co., 1996: 2545.

(*Received 7 February 1997, accepted 27 June 1997*)

