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Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test

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In a previous study we showed that the disposition of clozapine after a single oral dose is unrelated to either debrisoquine or S-mephenytoin hydroxylation polymorphism. The same 14 healthy subjects studied in that investigation were given 150 mg of caffeine. The reciprocal of plasma clozapine AUC (0,24), was correlated with an index of the *N*3-demethylation of caffeine (r_s = 0.84; P = 0.0024), used as a measure of cytochrome P4501A2 (CYP1A2) activity. *N*1- and *N*7-demethylation indices of caffeine also reflect CYP1A2 activity and were also correlated with clozapine clearance (r_s = 0.89 and 0.85; P = 0.0013 and 0.0023; respectively). No significant relationships with xanthine oxidase and *N*-acetyl transferase activity, also assessed by a caffeine test, were found. This study suggests that clozapine is metabolised by CYP1A2 to a major extent.

Keywords clozapine caffeine CYP1A2

Introduction

Clozapine is an atypical neuroleptic with potent antipsychotic efficacy and few parkinsonian side-effects, but is associated with a high incidence of agranulocytosis [1]. Classical neuroleptics such as perphenazine [2], zuclopenthixol [3], thioridazine [4] and haloperidol [5] are metabolised by the polymorphic debrisoquine hydroxylase, CYP2D6 [6]. However, clozapine, which is also eliminated by oxidative metabolism, shows similar disposition in extensive (EM) and poor (PM) metabolisers of debrisoquine [7].

Recently, Jerling *et al.* [8] found that the plasma concentration of clozapine increased markedly in patients who also received fluvoxamine. A similar interaction between tricyclic antidepressants and fluvoxamine has been demonstrated [9, 10]. Spina *et al.* [11] showed that fluvoxamine markedly inhibited the *N*-demethylation of imipramine without affecting the CYP2D6 mediated hydroxylation of desipramine [12]. Brøsen *et al.* [13] have shown *in vitro* that fluvoxamine is a potent inhibitor of phenacetin *O*-deethylation, a reaction catalysed by CYP1A2.

The major metabolic pathway of caffeine is *N*-3-demethylation, which is mainly mediated by CYP1A2 [14, 15]. The *N*-1 and *N*-7-demethylations are catalysed to a small extent by CYP2E1 in addition to CYP1A2 [14, 16]. The polymorphic *N*-acetyl transferase and xanthine oxidase are involved in the further metabolism of caffeine [14, 15]. Caffeine may thus be used as a probe drug for several drug metabolising enzymes. In the present study we performed a caffeine test in subjects previously investigated with respect to clozapine disposition [7].

Methods

In a previous study [7] a single oral 10 mg dose of clozapine was given to five PM of debrisoquine, five PM of S-mephenytoin and five individuals, who were EM of both probe drugs. The pharmacokinetic parameters were similar in the three groups. From that study we have taken reciprocal values of plasma clozapine AUC (0, 24) as an index of oral clearance.

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Fourteen of the 15 healthy Swedish subjects studied [7] took part in the present study, which was conducted 1 year later. Only one subject was a smoker (10–20 cigarettes per day). All were drug free for at least 2 weeks before the study. Informed consent was obtained from all subjects and the study was approved by the Ethics Committee of the Huddinge Hospital.

The subjects abstained from methylxanthine containing food and beverages for 48 h before and during the study. Each subject emptied their bladder and collected a urine sample before administration of caffeine to detect compounds in the urine that might interfere with the analysis.

At 08.00 h 150 mg caffeine was taken in tablet form (Koffein, ACO) as a single oral dose with water. All urine voided during the next 24 h was collected in plastic vessels. The pH was adjusted to 3.5 by 5M hydrochloric acid and 10 ml aliquots were frozen at −20°C until analysis.

Concentrations of caffeine and metabolites in urine were measured by h.p.l.c. according to Carrillo & Benitez [15], and the analyst was blind to the clozapine data. The following molar ratios were calculated to indicate various enzyme activities [15]: Caffeine *N*1-demethylation: 7X + 37U + 37X/137X; Caffeine *N*3-demethylation: AAMU + 1U + 1X + 17U + 17X/137X; Caffeine *N*7-demethylation: 3X + 13U + 13X/137X; Xanthine oxidase: 1U/1X + 1U; *N*-acetylation: AAMU/1X.

AAMU: 5-acetylamino-6-amino-3-methyluracil; 7X: 7-methylxanthine; 1U: 1-methyl uric acid; 3X: 3-methylxanthine; 37U: 3,7-dimethyl uric acid; 1X: 1-methylxanthine; 13U: 1,3-dimethyl uric acid; 37X: 3,7-dimethylxanthine (theobromine); 17U: 1,7-dimethyl uric acid; 17X: 1,7-dimethylxanthine (paraxanthine); 13X: 1,3-dimethylxanthine (theophylline); 137X: 1,3,7-trimethylxanthine (caffeine).

Results

All subjects tolerated the 48 h abstinence from caffeine containing food and beverage, although some subjects had headache. The reciprocal of plasma AUC (0,24) of clozapine [7] correlated with measures of caffeine *N*1-, 3- and 7-demethylations (r_s = 0.89, 0.84 and 0.85; all $P < 0.01$) (Table 1). The relationship with *N*3-demethylation is shown in Figure 1. The three *N*-demethylations were closely intercorrelated (r_s = 0.88–0.92) (Table 1). There was no correlation between clozapine AUC (0,24) and caffeine indices of xanthine oxidase (r_s = −0.32) or *N*-acetyl transferase (r_s = −0.33) activity.

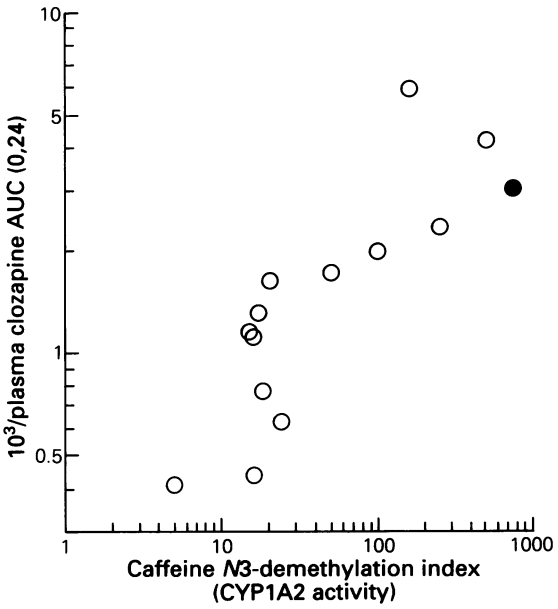

Figure 1 Relationship between reciprocal plasma clozapine AUC (0,24) and a caffeine *N*3-demethylation index of CYP 1A2 activity (r_s = 0.84; n = 14; P = 0.0024). Data for the only smoker are indicated as a filled circle.

The only smoker participating in this study had the highest *N*3-demethylation index and the third highest reciprocal clozapine AUC (0,24) value.

Discussion

The pharmacokinetic interaction observed in patients between fluvoxamine and clozapine [8] suggests that clozapine might be metabolised by CYP1A2, since fluvoxamine was shown to be a potent inhibitor of this isoform of cytochrome P450 *in vitro* [13]. In the present study about 70% of the variance in the oral clearance of clozapine was accounted for by an index of caffeine *N*3-demethylation, used as an *in vivo* marker of CYP1A2 activity. This suggests that clozapine is to a major extent metabolized by CYP1A2, and is consistent with the knowledge that both clozapine disposition [17] and CYP1A2 activity [14, 15] are inducible by smoking. The only smoker

Table 1 Spearman rank correlation coefficients for relationships between the reciprocal of plasma clozapine AUC (0,24) and various enzyme activities measured by a caffeine test

	N1	*N3*	*N7*	*XO*	*NAT*
Reciprocal clozapine AUC (0,24)	0.89**	0.84**	0.85**	−0.32	−0.33
Caffeine *N*1-demethylation (*N*1)		0.88**	0.92***	−0.08	−0.43
Caffeine *N*3-demethylation (*N*3)			0.88**	−0.02	−0.31
Caffeine *N*7-demethylation (*N*7)				−0.28	−0.36
Xanthine oxidase (XO)					0.05

**$P < 0.01$; ***$P < 0.001$; NAT = *N*-acetyl transferase.

in this study had the highest index of CYP1A2 activity and a high oral clearance of clozapine. Jerling *et al.* [8] showed that carbamazepine decreases plasma concentrations of clozapine in patients given both drugs. Carbamazepine is an inducer of CYP3A4 and this enzyme may metabolise clozapine in addition to CYP1A2. Carbamazepine is not an inducer of caffeine metabolism [18] and, therefore, not an inducer of CYP1A2.

Several neuroleptics are metabolised by CYP2D6 and pharmacokinetic interactions have been reported with drugs metabolised by this enzyme including neuroleptics and tricyclic antidepressants [6]. However, measurement of plasma concentrations of clozapine in psychiatric patients indicated no interaction between clozapine and other neuroleptics or tricyclic antidepressants metabolised by CYP2D6 [8]. In theory, the metabolism of clozapine may be inhibited by several drugs metabolised by CYP1A2, such as imipramine, phenacetin, theophylline and possibly caffeine.

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