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Disposition of clozapine in man: lack of association with debrisoquine and S-mephenytoin hydroxylation polymorphisms

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A large interindividual variability has previously been demonstrated in the bioavailability, steady-state plasma concentrations and clearance of clozapine, an atypical neuroleptic drug. To evaluate the importance of genetic factors in the metabolism of clozapine, its disposition after a single oral dose of 10 mg was studied in 15 healthy Caucasian volunteers. Five of the subjects were poor metabolisers (PM) of debrisoquine, five were PM of S-mephenytoin, and the remaining five were extensive metabolisers (EM) of both probe drugs. There was a 10-fold interindividual variation in C_{max} and a 14-fold variation in AUC(0, 24) of clozapine among the 15 subjects studied. The mean (s.d.) C_{max} was 117 (81) nmol l^{-1} and the mean AUC(0,24) value was 890 (711) nmol l^{-1} h. The value of $t_{½,z}$ varied 3-fold with a mean (s.d.) of 13.3 (5.0) h. There were no significant differences in the plasma concentrations or any of the pharmacokinetic parameters of clozapine between PM and EM of debrisoquine, or between the two S-mephenytoin hydroxylation phenotypes. We conclude that neither of the major genetic polymorphisms of oxidative drug metabolism contribute to the large interindividual variability in clozapine pharmacokinetics.

Keywords clozapine debrisoquine S-mephenytoin polymorphism pharmacokinetics

Introduction

Clozapine, a dibenzodiazepine derivative, is an atypical neuroleptic with potent antipsychotic properties. It has been reported to be effective in 30% of otherwise 'treatment-resistant' patients, with effects on positive as well as negative schizophrenic symptoms [1]. It also differs from most other neuroleptics in having a low frequency of extrapyramidal side-effects. However, its use is limited by a high incidence of agranulocytosis [2].

At least 80% of a dose of clozapine is excreted in the urine and faeces as metabolites [2]. These include products formed by *N*-oxidation and *N*-demethylation of the piperazine ring, as well as hydroxy metabolites [3, 4]. The plasma concentrations of clozapine at steady-state vary widely between patients treated with the same oral dose [5, 6, 7]. This variation is only partially related to gender, age and smoking behaviour [8].

The metabolism of the neuroleptics perphenazine [9], zuclopenthixol [10], thioridazine [11], and haloperidol [12] have been shown to cosegregate with the polymorphic hydroxylation of debrisoquine [13]. Five to ten per cent of Caucasians are poor metabolisers (PM) of debrisoquine [14, 15] and are characterised by the absence of a specific hepatic cytochrome P450 isoenzyme, CYP2D6 [16]. The hydroxylation of S-mephenytoin is also polymorphic, 3% of Caucasians being PM of this drug [17]. The aim of the present study was to determine whether the metabolism of clozapine is associated with either the debrisoquine or the S-mephenytoin hydroxylation polymorphism.

Methods

Fifteen healthy Swedish subjects (seven women and eight men), aged 22 to 60 years and weighing 52 to 90 kg took part in the study. They were recruited from a population of over 1000 healthy volunteers previously phenotyped with respect to the debrisoquine and S-mephenytoin hydroxylation polymorphisms [18]. The debrisoquine hydroxylation phenotype was determined according to Mahgoub *et al.* [19] after oral intake of 10

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mg debrisoquine hemisulphate (Declinax, Hoffman-LaRoche & Co. AG, Basel, Switzerland). Urine was collected for 8 h after drug intake and analysed for debrisoquine and 4-hydroxydebrisoquine by gas chromatography [20]. The metabolic ratio (MR) of debrisoquine was defined as the ratio between the urinary recovery of debrisoquine and that of 4-OH-debrisoquine in the 8 h urine sample. Subjects with an MR greater than 12.6 were classified as PM (14, 15). The S-mephenytoin hydroxylation phenotype was determined simultaneously with that of debrisoquine according to Sanz *et al.* [21]. The S/R enantiomeric ratio of mephenytoin was determined by gas chromatography in the 0–8 h as well as 24–32 h urine samples collected after the intake of 100 mg racemic mephenytoin (Mesantoin, Sandoz, Basel, Switzerland). Subjects with an S/R ratio of about 1 in both 0–8 and 24–32 h urines were classified as PM, while extensive metabolisers (EM) all had ratios below 0.65 [21].

The 15 subjects were chosen to include five who were PM of debrisoquine and EM of mephenytoin, five who were PM of mephenytoin and EM of debrisoquine, and five who were EM of both probe drugs. All EM of debrisoquine had a debrisoquine MR below 1.0 and, therefore, represented rapid metabolisers within the EM phenotype. All subjects were healthy as assured by the medical history, physical examination, electrocardiogram, and routine laboratory analysis. The PM and EM groups did not differ with respect to age or weight. Only one of the subjects (a PM of mephenytoin) 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 at Huddinge Hospital.

After an overnight fast, the volunteers ingested a single oral dose of 10 mg clozapine in capsule form. As clozapine is not available in dose units less than 25 mg, the 10 mg capsules were prepared by the Pharmacy Department at Huddinge Hospital after grounding of 25 mg clozapine tablets (Leponex, Sandoz, Basel, Switzerland). The 10 mg dose of clozapine was chosen after a pilot study in two healthy volunteers (EM of both debrisoquine and mephenytoin) who received a single oral 50 mg and 25 mg dose of clozapine, respectively. Both subjects experienced marked sedation and orthostatic hypotension with dizziness. Therefore, a dose of 10 mg clozapine was chosen for the study.

Venous blood samples (20 ml) were drawn before and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 32, 48 and 72 h after drug intake. Plasma was separated after centrifugation and stored frozen at −20° C until analysed for clozapine. The volunteers stayed in the hospital during the first 12 h after drug intake. Blood pressure, heart rate and side effects were recorded regularly during the study.

Plasma concentrations of clozapine were measured by gas chromatography with mass spectrometry [6]. Propyl-*N*-norclozapine was used as an internal standard. The limit of assay was 1.5 nmol l^{-1} and the coefficient of variation at this concentration was 7.2%.

The terminal elimination half-life ($t_{½,z}$) was calculated by least squares linear regression analysis of the log plasma drug concentration-time data beyond 12 h. AUC values up to 24 h (AUC(0,24)) were estimated using the linear trapezoidal rule. Student's *t*-test for unpaired data and the Spearman rank correlation test were used for statistical analysis. A *P* value of 0.05 or less was regarded as statistically significant.

Results

After a single 10 mg oral dose of clozapine all subjects except one had measurable plasma drug concentrations at 24 h after drug intake. In four subjects, the concentrations could be measured up to 32 h, in five subjects up to 48 h and in one subject up to 72 h. The mean plasma concentrations of clozapine in EM and PM of S-mephenytoin and in EM and PM of debrisoquine were similar (Figure 1). Eleven of the 15 12 h samples were reanalysed 6 months later with similar results, confirming that the pronounced interindividual variation was not due to variation in the analytical method.

The pharmacokinetic parameters of clozapine are summarised in Table 1. In one subject in whom the plasma concentration of clozapine at 24 h was below the limit of determination, a value of 1.5 nmol l^{-1} was assigned. There was a 10-fold variability in C_{max} (range 29–312 nmol l^{-1}) and a 14-fold variation in AUC(0,24) values (range 169–2443 nmol l^{-1} h) among the 15 subjects studied. C_{max} was reached 1 to 4 h after drug intake. A second peak in the plasma concentration was observed in 11 subjects during the first 12 h after drug intake (data not shown). The terminal elimination half-life varied from 8 to 22 h. Kinetic parameters in PM and EM of debrisoquine, and PM and EM of S-mephenytoin were similar (Table 1).

No significant differences were found in the pharmacokinetic parameters of clozapine between male and female subjects. There was no correlation between any of the kinetic parameters and age or body-weight.

All subjects complained of tiredness after the 10 mg dose of clozapine. Other frequently reported side-effects were dry mouth, slight dizziness and unclear speech. No

Table 1 Mean (± s.d.) pharmacokinetic parameters of clozapine (10 mg p.o.) in poor (PM) and extensive metabolisers (EM) of debrisoquine and of S-mephenytoin

	All subjects (n = 15)	*Debrisoquine hydroxylation phenotype*			*S-Mephenytoin hydroxylation phenotype*		
		EM (n = 10)	*PM* (n = 5)	P	*EM* (n = 10)	*PM* (n = 5)	P
t_{max} (h)	2.2 ± 1.0	2.2 ± 1.1	2.2 ± 0.8	NS	2.1 ± 1.0	2.4 ± 1.1	NS
C_{max} (nmol l^{-1})	117 ± 81	125 ± 94	100 ± 50	NS	110 ± 85	131 ± 79	NS
$t_{½,z}$ (h)	13.3 ± 5.0	13.4 ± 5.1	12.9 ± 5.4	NS	13.8 ± 5.5	12.3 ± 4.4	NS
AUC(0,24) (nmol l^{-1} h)	890 ± 711	943 ± 849	785 ± 355	NS	799 ± 620	1075 ± 917	NS

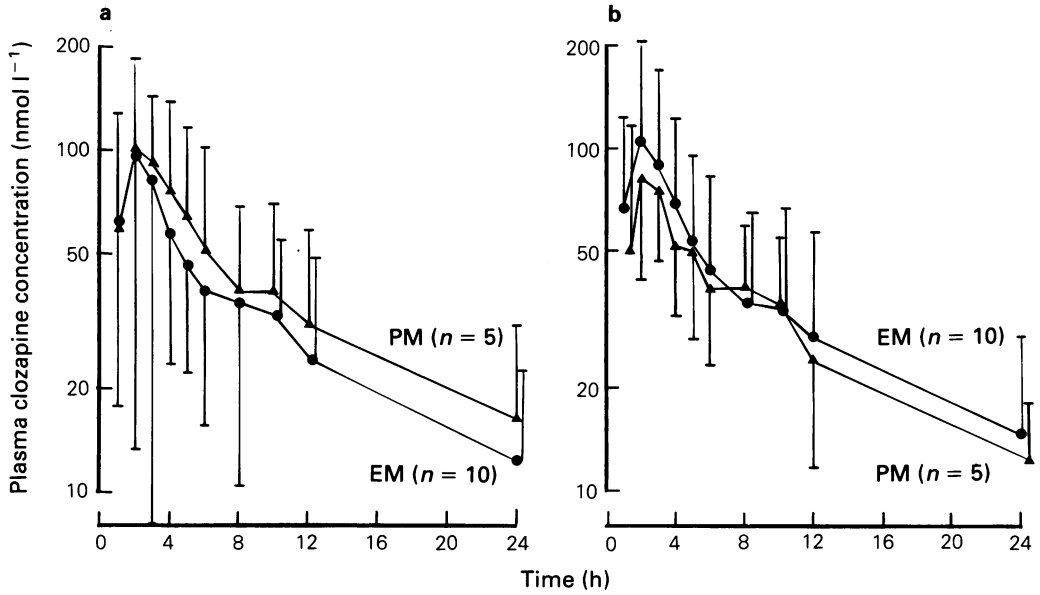

Figure 1 Plasma concentrations of clozapine (mean, s.d.) after a single oral dose of 10 mg clozapine in PM and EM of S-mephenytoin (a) and of debrisoquine (b). The plasma concentration in one subject (EM of both probe drugs) was below the limit of sensitivity (1.5 nmol l^{-1}) at 24 h after drug intake. The value of 1.5 nmol l^{-1} was assigned for this missing value for the calculation of the mean. There were no statistically significant differences in the plasma concentrations between the phenotypes at any time point.

relationship between these side-effects and the plasma concentrations of clozapine was apparent. However, no formal rating of side-effects was performed. One subject had transient bradycardia (< 60 beats min^{-1}) at about 2 h after clozapine intake. This subject was a PM of S-mephenytoin, and had the second highest C_{max} value (252 nmol l^{-1}), occurring at 2 h and coinciding with the bradycardia.

Discussion

Fischer *et al.* [22] have reported *in vitro* data, using human liver microsomes and recombinant RT2D6 cells specifically expressing CYP2D6, showing that clozapine is metabolised by CYP2D6. Thus, it was metabolised in RT2D6 cells to several unidentified metabolites, but not to the *N*-oxide and the *N*-demethyl product, the main metabolites of clozapine found in both human liver microsomes and in human plasma during clozapine therapy. The formation of the unidentified metabolites in RT2D6 cells was inhibited by dextromethorphan and by quinidine, whereas *N*-oxidation and *N*-demethylation was not inhibited by quinidine in human liver microsomes [22]. Although the power of the present study is low, our results suggest that the overall pharmacokinetics of clozapine *in vivo* are not influenced significantly by polymorphic variation in CYP2D6 or S-mephenytoin hydroxylase activities. However, the possible clinical relevance of minor metabolites formed by polymorphic enzymes cannot be excluded, as they might possess pharmacological and/or toxic properties.

The affinity of clozapine for CYP2D6 as reported by Fischer and co-workers [22] might also have consequences for neurotransmittor function and/or drug metabolism locally in the brain since this enzyme is present in the brain [23] and has been associated with a dopamine transporter [24, 25]. Clozapine is an atypical neuroleptic with respect to its effects on dopaminergic receptors, antipsychotic efficacy and low degree of extrapyramidal side-effects [26]. It also differs from other antipsychotic drugs in having a low affinity for sigma opioid receptors [27]. It has been suggested that the sigma binding site is a cytochrome P450 enzyme [28], presumably CYP2D6 [29], rather than a neurotransmitter receptor. That clozapine has a low affinity for the sigma receptor and also is the only neuroleptic drug studied so far whose metabolism is not to a major extent dependent on CYP2D6 is in line with this hypothesis. Further evidence for a possible association between CYP2D6 and dopaminergic neurotransmission comes from Brøsen and co-workers [30], who recently reported that there might be an increased sensitivity to extrapyramidal side-effects (acute dystonia, akathisia) of dopaminergic antagonists in PM of debrisoquine.

In most subjects, a second peak was seen in the plasma concentration-time curve during the first 12 h after clozapine intake. To our knowledge enterohepatic recirculation has not been described for clozapine, but it is known that about 30% of an oral dose is recovered in faeces [31]. The disposition of clozapine was biphasic with a terminal half-life of 8 to 22 h. These values are similar to those reported for schizophrenic patients [7, 32].

In conclusion, we suggest that the large interindividual variability in the pharmacokinetics of clozapine is not associated with the polymorphic hydroxylation of either debrisoquine or S-mephenytoin.

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