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The effect of carbamazepine on the 2-hydroxylation of desipramine

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Abstract The effect of carbamazepine (CBZ, 200 mg twice daily for 28 days) on the kinetics of a single oral dose of desipramine (DMI, 100 mg) was investigated in six healthy volunteers. Compared with a control session, treatment with CBZ caused a marked increase in DMI apparent oral clearance (from 1.05 ± 0.40 to 1.38 ± 0.52 l h per kg, means ± SD, $P < 0.01$) and a significant shortening in DMI half-life (from 22.1 ± 3.5 to 17.8 ± 3.5 h, $P < 0.01$). The amount of 2-hydroxy-desipramine (2-OH-DMI) excreted in urine over a 24-h period was significantly increased during CBZ intake (from 75 ± 15 to 92 ± 16 μmol, $P < 0.01$). These findings suggest that CBZ induces the 2-hydroxylation of DMI, a reaction primarily catalyzed by the polymorphic CYP2D6 isozyme. This interaction may have considerable practical significance.

Key words Desipramine · Carbamazepine · CYP2D6 Hydroxylation · Enzyme induction

Introduction

Tricyclic antidepressants are metabolized in the liver by oxidation (*N*-demethylation and/or ring hydroxylation), followed by conjugation with glucuronic acid (Gram 1974). Different cytochrome P450 isozymes are probably involved in the oxidative metabolism of these compounds and, in particular, the hydroxylation reactions are catalyzed by a specific cytochrome P450 isozyme known as CYP2D6 (Dahl and Bertilsson 1993; Spina and Caputi 1994). This is the source of the debrisoquine/sparteine genetic polymorphism, a monogenic trait which is expressed in the population as two phenotypes, the extensive metabolizer and the poor metabolizer phenotype (Brosen and Gram 1989; Eichelbaum and Gross 1990). Poor metabolizers, who represent 5–10% of a Caucasian population, exhibit a severely impaired capacity to metabolize several commonly used drugs, including certain neuroleptics, beta-blockers and antiarrhythmics, and show virtual absence of the polymorphic isozyme in their livers (Zanger et al. 1988; Gonzalez et al. 1991).

The activity of CYP2D6 may be influenced by other drugs which inhibit or induce hepatic microsomal enzymes. While CYP2D6-mediated reactions are highly susceptible to inhibition by other substrates or inhibitors of the enzyme (Brosen and Gram 1989; Eichelbaum and Gross 1990), the effect of enzyme-inducing agents is controversial. Earlier studies reported that classical enzyme inducers such as phenobarbital and rifampicin exert little or no influence on the activity of this isozyme (Eichelbaum et al. 1986; Leclercq et al. 1989). On the other hand, a recent report by our group indicates that phenobarbital may induce the CYP2D6-related 2-hydroxylation of desipramine (Spina et al., submitted).

Carbamazepine (CBZ) is an anticonvulsant agent which is also used for the treatment of psychiatric disorders such as manic-depressive illness (Post et al. 1983). CBZ is not metabolized via CYP2D6 (Eichelbaum et al. 1985) and, like barbiturates, is an enzyme inducer which stimulates its own metabolism and that of other drugs (Perucca et al. 1984). It has been reported that CBZ may decrease plasma concentrations of tricyclic antidepressants, suggesting stimulation of their metabolism (Brown et al. 1990; Leinonen et al. 1991; Brosen and Kragh-Sorensen 1993; Jerling et al. 1994). Since biotransformation of tricyclic compounds involves multiple pathways, probably mediated by different P450 isozymes, these studies do not

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provide any information on the specific reaction(s) being induced.

The aim of the present investigation was to evaluate the effect of CBZ treatment on the single-dose kinetics of the tricyclic antidepressant desipramine (DMI), whose major metabolic route, 2-hydroxylation, reflects the activity of the CYP2D6 isoenzyme (Spina et al. 1984, 1987; Birgersson et al. 1986).

Materials and methods

Subjects

Six non-smoker male subjects, aged 24–38 years, weighing 67–81 kg, gave their informed consent to take part in the study, which was approved by a local ethics committee. All subjects were healthy as assessed by physical examination, ECG, conventional hematology (complete blood cell count), blood chemistry, and urinalysis tests. They were all extensive metabolizers with respect to the CYP2D6-related phenotype, as assessed by the dextromethorphan test (Schmid et al. 1985).

Study protocol

Each subject received at 8:00 a.m. after an overnight fast a single 100-mg oral dose of desipramine hydrochloride (4 × 25-mg Nortimil tablets, Chiesi, Parma, Italy) on two separate occasions: (a) in a control session and (b) on day 24 of 28-day treatment with CBZ (Tegretol, Geigy, Basel, Switzerland, 1 × 200 mg tablets twice daily). A drug-free interval of at least 2 weeks elapsed between the two sessions. No food or drinks were allowed for 4 h after dosing. Blood samples were drawn in heparinized tubes at times 0 (pre-dose), 2, 4, 8, 12, 24, 36, 48, 72 h. Urine was collected from 0 to 24 h after administration of DMI. The plasma samples and an aliquot of urine were kept frozen at −20° C until assay.

Drug and metabolite assay

The concentrations of DMI and 2-hydroxydesipramine (2-OH-DMI) in plasma and urine were determined by HPLC according to Sutfin and Jusko (1979) with appropriate modifications (Spina et al. 1992). Urine samples were hydrolyzed with glucuronidase arylsulfatase prior to extraction, in order to determine total (free + conjugated) metabolite concentration. The sensitivity limits of the assay in plasma and urine were 15 nmol/l for DMI and 30 nmol/l for 2-OH-DMI.

Plasma CBZ concentrations were determined by the enzyme- multiplied immunoassay technique (Syva, Palo Alto, Calif., USA).

Pharmacokinetic and statistical analysis

Peak concentrations (C_{max}) and times of peak (T_{max}) were derived directly from the experimental values. The rate constant of the terminal elimination phase (λ) was calculated by least-squares regression from the log-linear concentration time data pairs and used to calculate the half-life ($t_{1/2}$) according to the relationship $t_{1/2} = 0.693/\lambda$. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule and extrapolated to infinity. Apparent oral clearance (CL/F) was calculated as Dose/AUC. Clearance values were calculated by assuming complete oral bioavailability (F), and may therefore represent overestimates. DMI renal clearance and metabolic clearance by 2-hydroxylation ($CL_{2\text{-OH-DMI}}$) were calculated, respectively, by dividing the 24-h recovery of unchanged drug or 2-OH-DMI in urine by the AUC of parent drug in plasma (0–24). Calculation of clearance by 2-hydroxylation is based on the assumption that the rate limiting step in urinary 2- OH-DMI excretion is its rate of formation and not its rate of conjugation.

Results are reported as means ± SD. Statistical comparisons were made by the Student's *t* test for paired data.

Results

The plasma concentration profiles of DMI (means ± SD) in the two study sessions are shown in Fig. 1, and pharmacokinetic parameters are summarized in Table 1. As compared with the control session, treatment with CBZ was associated with a marked decrease in plasma DMI concentrations at all sampling times. During CBZ coadministration, DMI AUC values decreased from 4731 ± 1574 to 3602 ± 1235 nmol/l/h ($P < 0.01$), DMI half-life decreased from 22.1 ± 3.5 to 17.8 ± 3.5 h ($P < 0.01$) and DMI apparent oral clearance increased from 1.05 ± 0.40 to 1.38 ± 0.52 l/h per kg ($P < 0.01$). Metabolically derived 2-OH-DMI could not be detected in most plasma samples in both sessions. The amount of 2-OH-DMI excreted in urine (0–24 h) increased significantly during treatment with CBZ from 75 ± 15 to 92 ± 16 μmol ($P < 0.01$), which corresponds, respectively, to 23 ± 5% and 28 ± 5% of the administered dose. The metabolic clearance of DMI

Table 1 Pharmacokinetic parameters of desipramine (means ± SD, $n = 6$) after a single oral 100 mg dose of the drug (corresponding to 330 μmol) in a control session and during coadministration of carbamazepine. For abbreviations, see Materials and methods

Parameters	Control	Carbamazepine	*P* value
C_{max} (nmol/l)	152 ± 46	127 ± 37	< 0.05
t_{max} (h)	5.3 ± 2.1	5.3 ± 2.1	NS
$t_{1/2}$ (h)	22.1 ± 3.5	17.8 ± 3.5	< 0.01
AUC (nmol/l h)	4731 ± 1574	3602 ± 1235	< 0.01
CL/F (l/h per kg)	1.05 ± 0.40	1.38 ± 0.52	< 0.01
Urinary recovery of DMI (μmol)	3.2 ± 0.9	2.7 ± 0.7	NS
Urinary recovery of 2-OH-DMI (μmol)	75 ± 15	92 ± 16	< 0.01
Renal clearance of DMI (l/h per kg)	0.019 ± 0.006	0.019 ± 0.005	NS
$CL_{2\text{-OH-DMI}}$ (l/h per kg)	0.47 ± 0.22	0.65 ± 0.25	< 0.01

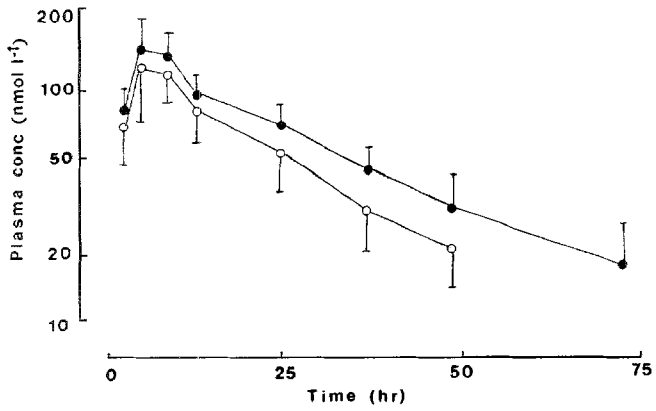

Fig. 1 Plasma concentrations of desipramine (means ± SD, $n = 6$) after a single oral dose of 100 mg of the drug in a control session (●) and during treatment with carbamazepine (○).

by 2-hydroxylation also underwent a significant increase from 0.47 ± 0.22 to 0.65 ± 0.25 l/h per kg ($P < 0.01$). The renal clearance of DMI was very small and similar in the two study sessions.

Plasma concentrations of CBZ in a sample taken on 24 day of CBZ administration before the morning dose ranged from 14.5 to 22.8 μmol/l.

The DMI dose was well tolerated by all subjects. During the first week of CBZ intake, two subjects complained of drowsiness and one reported decreased concentration ability.

Discussion

The present study clearly indicates that the rate of DMI elimination is increased during CBZ treatment. In fact, compared with the control session, CBZ coadministration was associated with decreased peak plasma DMI concentrations, increased DMI oral clearance and shortened DMI half-life, suggesting induction of pre-systemic and systemic metabolism of the antidepressant. As 2-hydroxylation represents the major metabolic route of DMI biotransformation, it is reasonable to assume that the increase in DMI elimination was due, at least in part, to induction of this metabolic pathway. This interpretation is supported by the finding of increased metabolic clearance by 2-hydroxylation and increased urinary recovery of 2-hydroxylated metabolite observed during concurrent CBZ intake. These results confirm previous clinical observations (Brown et al. 1990; Leinonen et al. 1991; Brosen and Kragh-Sorensen 1993; Jerling et al. 1994) of increased metabolism of tricyclic antidepressants by CBZ, and demonstrate that this effect involves, at least in part, an induction of hydroxylation pathways.

In vivo and in vitro studies have shown that the 2- hydroxylation of DMI is catalyzed by the polymorphic CYP2D6 isozyme (Spina et al. 1984, 1987; Birgersson et al. 1986). The activity of this enzyme is predominantly under genetic control, and the suggestion has been made that it is only marginally affected by enzyme induction (Eichelbaum et al. 1986; Leclercq et al. 1989). The present study and another recent investigation by our group (Spina et al., submitted) provide suggestive evidence that enzyme inducing anticonvulsant such as CBZ and phenobarbital stimulate, to some extent, the CYP2D6-mediated 2-hydroxylation of DMI. Theoretically, since other low-affinity and not yet identified P450 isozymes may contribute to the hydroxylation of DMI and other tricyclic antidepressants (Brosen 1990), it cannot be excluded that the inducing effect of anticonvulsants affects predominantly alternative isozymes different from CYP2D6. The study of the effect of CBZ on DMI disposition in poor metabolizers of debrisoquine could be of help in discriminating between these two possibilities. These subjects have a genetically determined inability to express a functional CYP2D6 and would not be expected to exhibit accelerated DMI clearance after CBZ treatment if the drug acts by increasing the level of this enzyme. Conversely, modifications of DMI disposition of the same magnitude as those observed in extensive metabolizers would become evident if other isozymes were involved. Since all subjects treated with CBZ were extensive metabolizers, such a differential responsiveness to induction could not be tested in the present study. However, the absence of significant changes in DMI disposition after phenobarbital treatment, recently observed in a poor metabolizer subject, provides indirect evidence that CYP2D6 may be the actual isozyme which is induced in extensive metabolizers (Spina et al., submitted).

In view of the increasing use of CBZ in the treatment of psychiatric disorders, the interaction of this compound with DMI (and, potentially, other tricyclic antidepressants metabolized by the same pathway) may have important practical implications. It should be noted that in this study the increase in oral DMI clearance was considerable despite the fact that CBZ was used at a relatively small dosage. Since enzyme induction by CBZ is known to be dose dependent (Perucca et al. 1984), the magnitude of interaction could be even greater when larger CBZ dosages are prescribed. Overall, these data suggest that larger daily doses of tricyclic antidepressants may be required to achieve therapeutically effective plasma concentrations in psychiatric patients receiving concurrent treatment with CBZ (Brosen and Kragh-Sorensen 1993). Besides antidepressants, the metabolism of haloperidol, another substrate of CYP2D6 (Llerena et al. 1992), is probably induced by CBZ treatment as suggested by decreased plasma neuroleptic levels when these drugs are used in combination (Jann et al. 1985).

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