Effect of ketoconazole on the pharmacokinetics of imipramine and desipramine in healthy subjects

E. Spina, A. Avenoso, G. M. Campo, M. G. Scordo, A. P. Caputi & E. Perucca[1]

Institute of Pharmacology, University of Messina, Messina, and [1] Clinical Pharmacology Unit, University of Pavia, Pavia, Italy

Aims The aim of the study was to characterize further the role of CYP3A4 in the metabolism of tricyclic antidepressants.

Methods The effect of oral ketoconazole (200 mg day^{-1} for 14 days) on the kinetics of a single oral dose of imipramine (100 mg) and desipramine (100 mg) was evaluated in two groups of six healthy male subjects.

Results Ketoconazole administration was associated with a decrease in imipramine apparent oral clearance (from 1.16 ± 0.21 to 0.96 ± 0.20 l h^{-1} kg^{-1}, mean ± s.d.; $P < 0.02$), a prolongation in imipramine half-life (from 16.7 ± 3.3 to 19.2 ± 5.4 h, $P < 0.05$) and a decrease in area under the curve of metabolically derived desipramine (from 3507 ± 1707 to 3180 ± 1505 nmol l^{-1} h, $P < 0.05$), whereas concentrations of 2-hydroxy-imipramine were unaffected. In the subjects given desipramine, no significant changes in desipramine and 2-hydroxy-desipramine kinetics were observed during ketoconazole treatment.

Conclusions These findings indicate that ketoconazole, a relatively specific inhibitor of CYP3A4, inhibits the *N*-demethylation of imipramine without affecting the 2-hydroxylation of imipramine and desipramine. This interaction, confirms that CYP3A4 plays a role in the demethylation of tricyclic antidepressants.

Keywords: ketoconazole, imipramine, desipramine, CYP3A4, CYP2D6, drug interaction

Introduction

The tricyclic antidepressant imipramine is *N*-demethylated in the liver to the active metabolite desipramine, and both imipramine and desipramine are hydroxylated at position 2 [1]. As shown in Figure 1, different cytochrome P450 (CYP) isoenzymes are involved in the oxidative metabolism of these compounds. While the genetically polymorphic CYP2D6 enzyme is primarily involved in the 2-hydroxylation pathway [2, 3], imipramine demethylation appears to be mediated by at least three different isoforms, namely CYP1A2, CYP2C19 and CYP3A4 [4–7].

The metabolism of tricyclic antidepressants is affected by other drugs which inhibit or induce the hepatic microsomal enzymes [8]. The effect of metabolic modifiers can provide useful information on the enzyme isoforms involved in different biotransformation pathways and their quantitative role. In particular, interaction studies with quinidine and fluvoxamine, selective inhibitors of CYP2D6 and CYP1A2 respectively, have been used to assess the role of these isoenzymes in the 2-hydroxylation of imipramine and desipramine and in the *N*-demethylation of imipramine [9–11]. On the other hand, the stimulation of tricyclic antidepressants metabolism by enzyme inducing agents such as carbamazepine and phenobarbitone may be explained by increased activity of CYP3A4 [12], an enzyme which is partly responsible for imipramine *N*-demethylation [4, 5]. However, recent studies by our group suggest that phenobarbitone and carbamazepine may also induce the 2-hydroxylation of desipramine, a reaction which is largerly catalyzed by CYP2D6 [13, 14]. Since CYP2D6 is considered not to be inducible [15], the effect of anticonvulsants on this pathway may indicate that other P450 isoenzymes such as CYP3A4 contribute to the hydroxylation of desipramine and other tricyclic antidepressants.

To characterize further the role of CYP3A4 in the metabolism of tricyclic antidepressants, we have now evaluated the effect of ketoconazole, a potent and relatively specific inhibitor of CYP3A4 [16, 17], on the single-dose kinetics and disposition of imipramine and desipramine in healthy volunteers.

Correspondence: Dr Edoardo Spina, Institute of Pharmacology, University of Messina, Piazza XX Settembre 4, 98122 Messina, Italy

Methods

Subjects

Twelve non-smoker male subjects aged 24–39 years (body weight 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 and conventional haematology, blood chemistry and urinalysis tests. They were all extensive metabolizers with respect to CYP2D6-related phenotype, as assessed by the dextromethorphan test [18].

Study protocol

Subjects were randomized into two groups of six. Subjects in Group 1 received, at 09.00 h after an overnight fast, a single 100 mg oral dose of imipramine hydrochloride (4×25 mg Tofranil tablets, Geigy, Basel, Switzerland) on

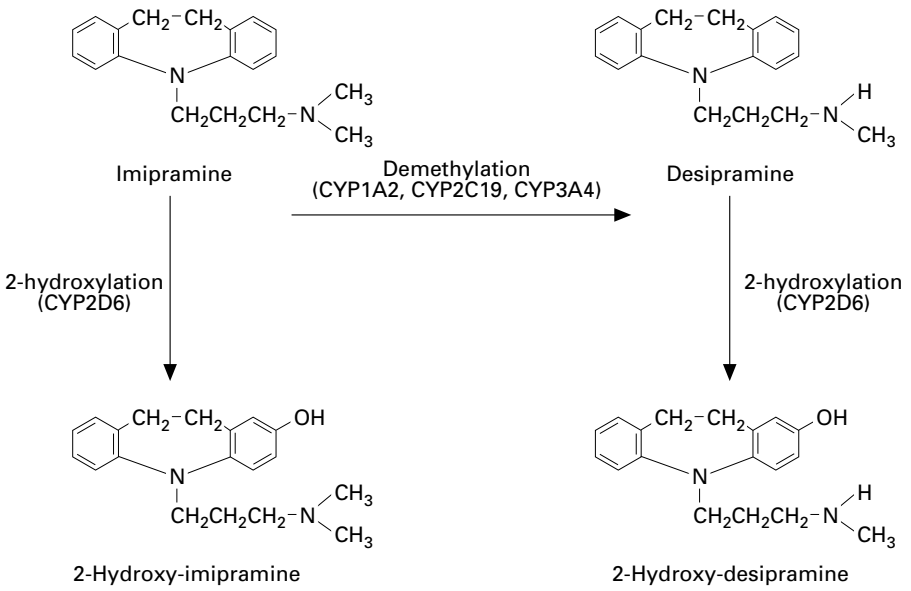

Figure 1 Pathways of imipramine metabolism and possible cytochrome P450 (CYP) enzymes involved (in parenthesis).

two occasions, in a random order, separated by a drug-free interval of at least 4 weeks: (a) in a control session and (b) on the 10th day of a 14-day treatment with ketoconazole (Nizoral, Janssen, Beerse, Belgium), given as a 200 mg tablet once daily at 08.00 h. No food or drinks were allowed for 4 h after dosing. Subjects in Group 2 underwent the same procedures, except that a single 100 mg oral dose of desipramine hydrochloride (4×25 mg Nortimil tablets, Chiesi, Parma, Italy) was given instead of imipramine. Blood samples were drawn in heparinized tubes at time 0 (before the imipramine or desipramine dose) and at 2, 4, 8, 12, 24, 36, 48, 72, and 96 h thereafter. The plasma was frozen at -20° C until assay. As a measure of compliance, plasma ketoconazole concentrations were determined in each subject in the sample drawn at time 0 during the ketoconazole session.

Assay of drugs and metabolites

The concentrations of imipramine, desipramine, 2-hydroxy-imipramine and 2-hydroxy-desipramine in plasma were determined by h.p.l.c. according to Sutfin & Jusko [19]. The sensitivity limits of the assay were 10 nmol l^{-1} for imipramine, 15 nmol l^{-1} for desipramine and 20 nmol l^{-1} for 2-hydroxy-imipramine and 2-hydroxy-desipramine. Plasma ketoconazole concentrations were determined by h.p.l.c. according to Riley & James [20]. Between-day precision was better than 10% for all analytes.

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_z) was calculated by least-squares regression from the log-linear concentration time data pairs and used to calculate the half-life ($t_{1/2,z}$) according to the relationship $t_{1/2,z} = 0.693/\lambda_z$. Areas under the plasma concentration-time curve (AUC) were calculated by the trapezoidal rule and extrapolated to infinity (for metabolites, AUC could be calculated only for up to 24 h). Apparent oral clearance (CL/F) was calculated as Dose/AUC.

Results are reported as means $\pm$ s.d., together with 95% confidence intervals for the mean differences (CI). Statistical comparisons were made by Student's t-test for paired data.

Results

Ketoconazole administration resulted in significantly increased imipramine AUC (from 3795 ± 918 to 4567 ± 1076 nmol l^{-1} h; $P < 0.05$; 95% CI for the difference between mean values: -1458 to -88 nmol l^{-1} h) (Figure 2), decreased CL/F values (from 1.16 ± 0.21 to 0.96 ± 0.20 l h^{-1} kg^{-1}; $P < 0.02$; 95% CI: 0.05 to 0.33 l h^{-1} kg^{-1}) and prolonged half-life (from 16.7 ± 3.3 to 19.2 ± 5.4 h; $P < 0.05$; 95% CI: -5.0 to -0.1 h). The peak concentration of imipramine was also increased during ketoconazole treatment (from 172 ± 21 to 206 ± 23 nmol l^{-1}), but the difference did not reach statistical significance. During intake of ketoconazole, there was a significant decrease in the AUC of metabolically derived desipramine (from 3507 ± 1707 to 3180 ± 1505 nmol l^{-1} h; $P < 0.05$; 95% CI: 69 to 584 nmol l^{-1} h) (Figure 2). Plasma concentrations of 2-hydroxy-imipramine were detectable in all subjects for up to 24 h in both sessions. The 2-hydroxy-imipramine AUC(0, 24h) and the ratios of 2-hydroxy-imipramine AUC(0, 24h) to imipramine AUC(0, 24h) values were not modified by ketoconazole.

The pharmacokinetics of desipramine and 2-hydroxy-desipramine were not significantly modified during ketoconazole administration in subjects from Group 2 (given desipramine). Mean values $\pm$ s.d. (control *vs* ketoconazole session) were 4813 ± 1141 *vs* 4924 ± 1305 nmol l^{-1} h for desipramine AUC, 1.04 ± 0.36 *vs* 1.01 ± 0.41 l h^{-1} kg^{-1} for CL/F, 24.5 ± 4.6 *vs* 23.8 ± 4.1 h for desipramine half-life and 1059 ± 21 *vs* 1012 ± 225 nmol l^{-1} h for the AUC(0, 24h) of metabolically derived 2-hydroxy-desipramine.

Plasma concentrations of ketoconazole were detectable in

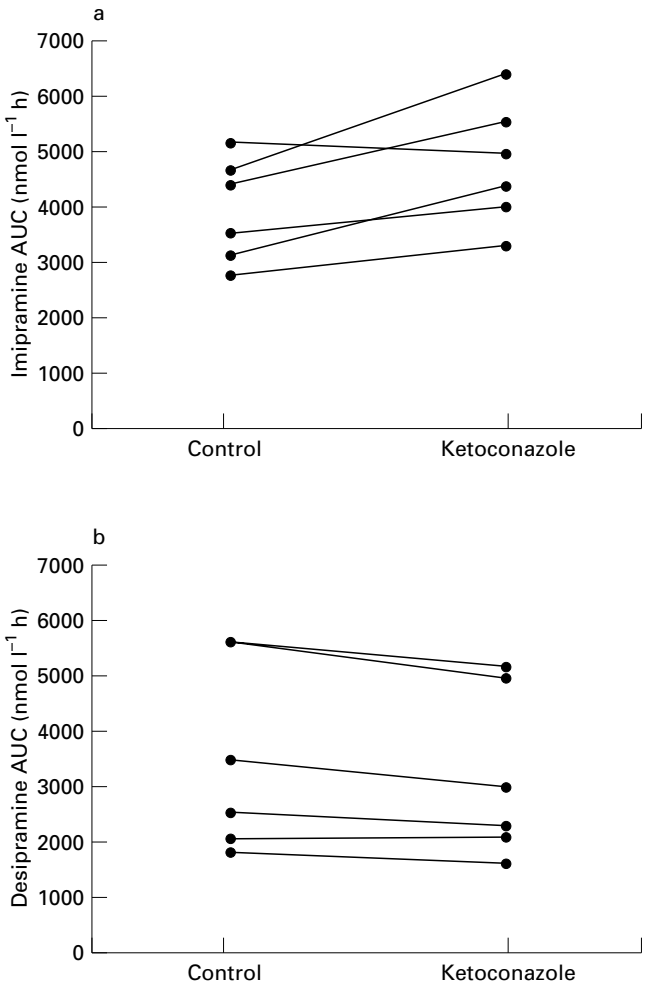

Figure 2 Area under the plasma concentration-time curve (AUC) of imipramine (a) and metabolically derived desipramine (b) in six healthy volunteers after a single oral 100 mg dose of imipramine in a control phase and during treatment with ketoconazole.

all subjects and ranged from 0.96 to 3.38 μmol l^{-1} at the time of imipramine or desipramine administration.

Discussion

The findings of this study indicate that the kinetics of imipramine, but not desipramine, are affected by therapeutic doses of ketoconazole which decreased the apparent oral clearance of imipramine and prolonged its elimination half-life. This suggests inhibition of hepatic metabolism. However, though tricyclic antidepressants are predominantly metabolized in the liver [1], we cannot exclude the possibility of a metabolic inhibition occurring also at gastrointestinal level, as recently reported for ketoconazole and cyclosporine [21]. Interestingly, CYP3A4, the isoform specifically inhibited by ketoconazole, is the most abundant single cytochrome P450 present in human small bowel mucosa [22]. Simultaneous measurement of the two major metabolites in plasma demonstrated that ketoconazole inhibited significantly the demethylation of imipramine without affecting the 2-hydroxylation pathway. In fact, the AUC of the demethylated metabolite desipramine was decreased by the antimycotic agent, whereas the plasma levels of 2-hydroxy-imipramine and the 2-hydroxy-imipramine to imipramine AUC ratios were unaffected.

These findings are consistent with the observations made in the desipramine-treated group. In these subjects, ketoconazole had no effect on desipramine pharmacokinetics and on plasma levels of metabolically derived 2-hydroxy-desipramine, indicating lack of interference with the 2-hydroxylation pathway. Since the 2-hydroxylation of imipramine and desipramine is catalyzed by the same cytochrome P450 (CYP2D6) [3], the data provide additional evidence that the impairment of imipramine elimination by ketoconazole is related mainly to inhibition of *N*-demethylation.

Ketoconazole is a relatively specific inhibitor of CYP3A4, as demonstrated by *in vitro* studies [16, 17] and by its ability to cause clinically important interactions with CYP3A4 substrates such as cyclosporine, terfenadine, midazolam and triazolam [21, 23–25]. The observation that ketoconazole inhibited imipramine demethylation is consistent with *in vitro* data indicating that CYP3A4 is involved in this pathway, although it cannot be completely excluded that at the dose used ketoconazole also affected CYP1A2-mediated *N*-demethylation. In any case, the impairment of imipramine elimination by ketoconazole was less pronounced than that produced in a separate study by the CYP1A2 inhibitor fluvoxamine, suggesting that CYP3A4 plays only a secondary role in imipramine demethylation [10, 11]. There is evidence that CYP2C19 may also contribute to demethylation of tricyclic antidepressants [6, 7]. The inability of ketoconazole to affect the 2-hydroxylation of imipramine and desipramine suggests that CYP3A4 plays no role in this reaction. In agreement with this interpretation, Von Moltke *et al*. [26] found that ketoconazole is a weak inhibitor of desipramine 2-hydroxylation in human liver microsomes.

Our results provide indirect information on the mechanism underlying the inducing effect of phenobarbitone and carbamazepine on the metabolism of tricyclic antidepressants. These anticonvulsants are potent inducers of CYP3A4 [27] and the evidence that this cytochrome is involved in the demethylation of imipramine suggests that the induction of tricyclic antidepressant metabolism by phenobarbital and carbamazepine is mediated at least in part by this isoenzyme. On the other hand, induction of CYP3A4 may not explain the stimulatory effect of anticonvulsants on the 2-hydroxylation of desipramine [13, 14], which, according to the the results of this study, is not mediated by CYP3A4.

In a previous report, the structurally related antimycotic fluconazole, which is also a CYP3A4 inhibitor [16], has been found to increase significantly the plasma concentrations of nortriptyline in a single patient with depression [28]. This observation suggests inhibition of nortriptyline metabolism by fluconazole, although this is difficult to explain on the basis of our findings because nortriptyline, like desipramine, is primarily oxidized by CYP2D6 [1]. Owing to the relatively wide theraputic range of imipramine steady-state plasma concentrations (150–250 μg l^{-1})[1] and to the limited degree of inhibition of imipramine metabolism by ketoconazole observed in our subjects (pharmacokinetic parameters change by about 20%), we believe that this interaction is unlikely to be clinically significant.

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