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Comparative effects of the antimycotic drugs ketoconazole, fluconazole, itraconazole and terbinafine on the metabolism of cyclosporin by human liver microsomes

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Four antimycotic drugs, the azoles ketoconazole, itraconazole and fluconazole, and the allylamine terbinafine have been studied for their effect on the metabolism of cyclosporin by human liver microsomes ($n = 3$) *in vitro*. Ketoconazole caused marked inhibition of cyclosporin hydroxylase (to metabolites M17 and M1) with IC_{50} and K_i values of 0.24 ± 0.01 and 0.022 ± 0.004 μM, respectively. Based on IC_{50} values, itraconazole was ten times less potent (IC_{50} value of 2.2 ± 0.2 μM) and both fluconazole and terbinafine had values above 100 μM. K_i values for itraconazole and fluconazole were 0.7 ± 0.2 and 40 ± 5.6 μM, respectively. No kinetic parameters were calculated for terbinafine because of the lack of inhibitory effects. Based on these data, ketoconazole is confirmed as being a potent inhibitor of cyclosporin metabolism and this has clinical relevance. Although inhibition by fluconazole was much less than that by itraconazole at equimolar concentrations, it should be noted that in patients plasma concentrations of fluconazole are much greater than those of itraconazole. Clinical interactions of cyclosporin with both fluconazole and itraconazole have been reported. In contrast to the azoles, terbinafine does not have the same potential for interaction.

Keywords cyclosporin antimycotic drugs human liver inhibition

Introduction

Although both the azole (e.g. ketoconazole, itraconazole, fluconazole) and allyamine (e.g. terbinafine) antifungals are potent inhibitors of fungal ergosterol synthesis their mode of action is different. Azole antifungals inhibit an enzyme belonging to the superfamily of cytochrome P450, lanosterol 14-demethylase (Van den Bossche *et al.*, 1978, 1980), whereas terbinafine exhibits strong inhibition of a non-cytochrome P-450 enzyme, squalene epoxidase (Schuster, 1985). Enzyme inhibition raises the problem of selectivity due to similar specificities of enzymes catalysing identical steps in mammals and in fungi. This is especially true of some of the azoles. In liver in particular, cytochrome P450 enzymes may be inhibited by azoles and, therefore, competition will exist with all compounds (of endogenous origin or administered) which are also metabolized by these enzymes.

Cyclosporin is extensively metabolised in the liver and Kronbach *et al.* (1988) have shown that a cytochrome P450 isoenzyme P450 IIIA4 catalyses formation of the primary metabolites M17, M21 and M1. Ketoconazole is a potent inhibitor of cyclosporin metabolism by human liver microsomes *in vitro* (Back *et al.*, 1989; Burke *et al.*, 1990) and it is this inhibition that gives a rational basis to the clinically important interactions reported in transplant patients (Cockburn, 1986; Dieperink & Moller, 1982; Ferguson *et al.*, 1982). Fluconazole administration has also been shown to alter cyclosporin pharmacokinetics (Graves *et al.*, 1990; Lazar & Wilner, 1990; Sugar *et al.*, 1989). In contrast, early clinical data suggest that terbinafine will not cause clinically significant drug interactions (Seyffer *et al.*, 1989). The aim of the present study was to compare the effects of four antifungals, ketoconazole, itraconazole, fluconazole and terbinafine on the metabolism of cyclosporin by human liver microsomes *in vitro*.

Methods

Histologically normal livers were obtained from kidney transplant donors (two males, one female; aged 17–27 years). Ethical approval for the study was granted and consent to removal of the liver was obtained from donors' relatives. There was no recorded previous drug usage by the donors. Washed microsomes (105,000 *g*

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pellets) were prepared by sedimentation as described previously (Purba *et al.*, 1987). Cytochrome P-450 was assayed by the method of Omura & Sato (1964) and microsomal protein by the method of Lowry *et al.* (1951).

Cyclosporin hydroxylase activity was determined as described previously (Back *et al.*, 1989), with the following modifications: Incubations contained cyclosporin (CsA; 5 μM, [^{3}H]-CsA; 0.1 μCi, a gift from Sandoz Pharmaceuticals, Basle), and microsomal protein (1.5 mg) and were performed in 10 ml wide neck glass tubes at 37° C with agitation for 20 min. Other reaction constituents and the extraction procedure remained unchanged. [^{3}H]-CSA and metabolites were measured by on-line radiometric detection (FLO-ONE/Beta SERIES A200, Radiomatic, Packard, USA). A good separation of metabolites M17 and M1 was difficult to achieve with this system and hence the enzyme activity determined relates to formation of both the primary hydroxylated metabolites.

CsA and the antifungals were dissolved in methanol which was evaporated to dryness before addition of other reaction constituents. The range of concentration of inhibitors used for calculation of IC_{50} values (concentration of inhibitor producing 50% decrease in enzyme activity) varied according to the drug being used. For fluconazole and terbinafine, the concentration range was 10–100 μM; for itraconazole it was 1–10 μM and for ketoconazole it was 0.1–1 μM. These concentrations were based on preliminary experiments. The V_{max} and K_m values of CsA hydroxylase (CsA concentrations 2.5–20 μM) were determined in the absence and presence of the drugs. The apparent K_i was determined from plots of $^1/_V$ against inhibitor concentrations (I).

Results

At 5 μM CsA, 23.3 ± 1.8% (mean ± s.d.; n = 3) of substrate was metabolised to M17/M1. In incubations without NADPH metabolite formation was less than 1%. Figure 1 shows the inhibition of enzyme activity over a range of antifungal concentrations and the inset table gives the mean IC_{50} values obtained in three livers. Ketoconazole was the most potent inhibitor with an IC_{50} of 0.24 ± 0.01 μM. Itraconazole was ten times less potent with an IC_{50} of 2.2 ± 0.2 μM and IC_{50} values of both fluconazole and terbinafine were well above 100 μM.

Lineweaver-Burk plots for ketoconazole and itraconazole were constructed (not shown) and demonstrated that both compounds competitively inhibit the hydroxylation of CsA, this being reflected by an increase in K_m (1.7 ± 0.2 μM) and virtually no change in V_{max} (82.5 ± 7.3 pmol min^{-1} mg^{-1}). In contrast fluconazole exhibited mixed inhibition.

For an accurate determination of the affinity constant, K_i, Dixon plots were constructed. K_i values for ketoconazole, fluconazole and itraconazole were 0.022 ± 0.004, 40 ± 5.6 and 0.7 ± 0.2 μM, respectively. The K_i values confirmed that ketoconazole was the most potent inhibitor of the four compounds studied. No kinetic parameters were calculated for terbinafine because of the lack of inhibitory effects on CsA metabolism, as shown in Figure 1.

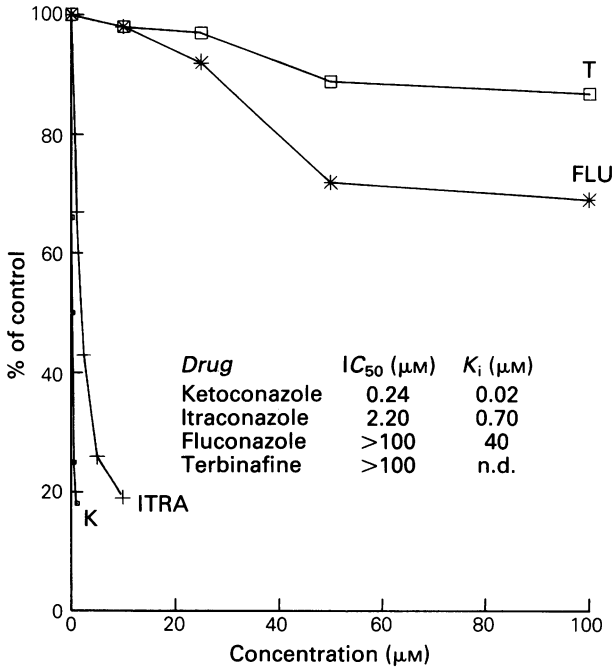

Figure 1 The effect of ketoconazole (K), itraconazole (Itra), fluconazole (Flu) and terbinafine (T) on the metabolism of cyclosporin (to hydroxylated metabolites M17 and M1) by human liver microsomes. Each value is the mean obtained from three different microsomal preparations. Inset are IC_{50} and K_i values. A K_i value was not determined (n.d.) for terbinafine because it did not cause sufficient inhibition. The 100% control value represents metabolism of 23.3 ± 1.8% (mean ± s.d.) of the added cyclosporin.

Discussion

In most patients the major pathway of elimination of cyclosporin is hydroxylation to the metabolite M17 and the blood concentration of M17 can exceed that of the parent drug several fold (Kahan & Grevel, 1988). Inhibition of the hepatic enzyme, cytochrome P450 IIIA4 (Kronbach *et al.*, 1988; Lucey *et al.*, 1990), responsible for catalysing this reaction, can lead to altered cyclosporin pharmacokinetics with important clinical implications. Sustained changes in cyclosporin concentration can result in graft rejection or renal toxicity (Yee & McGuire, 1990). The present *in vitro* study confirms that ketoconazole is a potent inhibitor of CsA metabolism and, thereby, will cause plasma CsA concentrations to increase when coadministered (Dieperink & Moller, 1982; Ferguson *et al.*, 1982; Cockburn, 1986). It has been suggested that the co-administration of ketoconazole could reduce CsA dosage requirements with consequent cost implications (First *et al.*, 1989, 1991), although this has been challenged (Frey, 1990).

The present study also indicates that the two triazole antifungals, itraconazole and fluconazole have a different propensity to inhibit hepatic cytochrome P450, with itraconazole being more potent. Clinical interaction data are somewhat equivocal although the balance of evidence suggests that both itraconazole (Kwan *et al.*, 1987; Trenk *et al.*, 1987) and fluconazole, particularly at higher doses of 200 mg day^{-1}, (Graves *et al.*, 1990; Lazar & Wilner, 1990; Sugar *et al.*, 1989) will cause an elevation of blood CsA concentrations.

Although we have shown that fluconazole is a much less potent inhibitor of cytochrome P450 catalysed CsA metabolism *in vitro* than either itraconazole or ketoconazole (K_i values 40, 0.7, 0.02 μM, respectively) the magnitude of any effect seen clinically depends on the concentration of the antifungal drug at the enzyme site in the liver. In this respect it is important to note that peak plasma fluconazole concentrations (10 μg ml^{-1}; 32 μM) are much greater than those of itraconazole (1.1 μg ml^{-1}; 1.5 μM) (Grant & Cissold, 1989, 1990). Thus, fluconazole and itraconazole may have comparable *in vivo* inhibitory potential.

In vitro terbinafine did not inhibit cyclosporin metabolism, confirming the results of a previous study (Back *et al.*, 1989). The novel structure of terbinafine precludes binding to known cytochrome P450 isoenzymes. It is likely, therefore, not to have the same interaction potential as the azole antifungals (Seyffer *et al.*, 1989).

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