Calcium channel antagonists and cyclosporine metabolism: *in vitro* studies with human liver microsomes

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The effects of four Ca^{2+} channel antagonists on the metabolism of cyclosporine (CsA) by human liver microsomes ($n = 4$) *in vitro* have been examined. Nicardipine produced marked inhibition of both M17 and M21 ($\text{IC}_{50} = 7.0 \mu\text{M}$) formation. In contrast nifedipine produced less than 20% inhibition of M17 and M21 even at the highest concentration examined ($50 \mu\text{M}$). Diltiazem data were comparable to those for nifedipine. Verapamil ($50 \mu\text{M}$) produced 30 and 28% inhibition of M17 and M21 formation, respectively. These findings give a basis to the increase in CsA blood concentrations seen in transplant patients who are also given nicardipine.

Keywords cyclosporine Ca^{2+} channel antagonists human liver inhibition of metabolism

Introduction

The immunosuppressant cyclosporine (CsA) is widely used to prevent the rejection of transplanted organs and, increasingly, to treat diseases of autoimmune origin. CsA undergoes extensive hepatic metabolism in man to mono- and dihydroxylated as well as *N*-demethylated products (Ptachcinski *et al.*, 1986). The major primary oxidative metabolites are M1, M17 and M21, with further oxidation of M1 or M17 generating dihydroxylated metabolites. One area of concern with CsA use has been the relatively large number of clinically important interactions with other drugs which have been reported (Castelao *et al.*, 1988; Cockburn, 1986; Yee *et al.*, 1988). Interactions can be anticipated if drugs that enhance or inhibit CsA metabolism are coadministered. Thus the enzyme inducing agents phenytoin, phenobarbitone and rifampicin all decrease CsA blood levels while ketoconazole, erythromycin, diltiazem, nicardipine and verapamil have been reported to increase CsA blood levels as a result of inhibition of metabolism (Castelao *et al.*, 1988; Wadhwa *et al.*, 1987).

The co-administration of Ca^{2+} channel antagonists with CsA in renal allograft recipients does have potential beneficial effects on early graft function (Kohlaw *et al.*, 1988). Thus diltiazem, despite causing elevated CsA levels, had a protective effect on posttransplant tubular necrosis (Wagner *et al.*, 1988). What is not clear at present is whether all the commonly used Ca^{2+} channel antagonists interfere with CsA metabolism in addition to exerting a protective effect on CsA nephrotoxicity. The only direct way to study inhibition of metabolism is *in vitro*. Hence we have investigated the effects of diltiazem, verapamil, nifedipine and nicardipine on CsA metabolism in human liver microsomes.

Methods

Histologically normal livers were obtained from kidney transplant donors (three males; one female; aged 27–46 years). Ethical approval for the study was granted and consent to removal of the liver was obtained from donor's relatives.

Washed microsomes (105,000 g pellets) were prepared using the classical differential sedimentation method as previously described (Purba *et al.*, 1987). Microsomal protein was assayed by the method of Lowry *et al.* (1951).

Microsomal incubations containing [^3H]-CsA (0.2 μCi ; specific activity of 17 Ci mmol^{-1} ; Amersham), CsA (40 μM), MgCl_2 (5 mM), EDTA (1 mM), KCl (1 mM), NADPH (1 mM), microsomal protein (3 mg) and 0.067 M phosphate buffer (pH 7.4) to a final volume of 2.5 ml were performed in Erlenmeyer flasks at 37°C with agitation for 30 min. In some incubations, diltiazem, verapamil, nifedipine and nicardipine (0–50 μM Sigma) were added. CsA and Ca^{2+} antagonists were dissolved in methanol which was evaporated to dryness before addition of other reaction constituents. The reaction was initiated by the addition of NADPH and terminated by extraction into ether (6 ml for 10 min). The ether layer was separated by centrifugation (2000 g for 10 min), removed and evaporated to dryness in a Buchler vortex evaporator. Extraction of total radioactivity was greater than 98%. The residue was redissolved in mobile phase (200 μl) and an aliquot (100 μl) analysed by high performance liquid chromatography using a slightly modified version of the method described by Sawchuk & Cartier (1981).

Separations were performed at 76°C on a Partisil ODS-3 column (25 cm $\times$ 0.46 cm) protected by an in-line guard column. The mobile phase used was acetonitrile:water (67:33) and the flow rate 1.5 ml min^{-1} . Metabolites were identified according to the retention times of the authentic standards (M17, M21; gifts from Sandoz, Basle) monitored by u.v. detection at 210 nm. CsA and metabolites were measured by determining the radioactivity in 0.5 min eluate fractions in 4 ml scintillant (Aqua Luma, LKB).

Results

Table 1 shows the effect of Ca^{2+} channel antagonists on the formation of M17 and M21. At 40 μM CsA, 12.1 $\pm$ 1.9% (mean $\pm$ s.d.; $n = 4$) was metabolized to M17 and 5.2 $\pm$ 0.9% to M21 (as judged by the elution time of co-chromatographed authentic metabolites). In incubations without NADPH, metabolite formation was less than 1%. Nicardipine produced marked inhibition of both M17 and M21 production. At 50 μM nicardipine M17 was reduced by 81% (to 2.3 $\pm$ 0.9%) and M21 by 79% (to 1.1 $\pm$ 0.3%). The IC_{50} values (i.e. the concentration producing 50% inhibition of metabolite formation) were 7.0 μM for both M17 and M21.

Table 1 The effect of Ca^{2+} channel antagonists on the formation of M17 and M21 in human liver microsomal incubations. Results are expressed as mean $\pm$ s.d. ($n = 4$)

	% metabolite formed	
	M17	M21
Control	12.1 $\pm$ 1.9	5.2 $\pm$ 0.9
Nicardipine (μM)		
5	7.1 $\pm$ 2.4	3.1 $\pm$ 0.9
10	5.6 $\pm$ 2.2	2.2 $\pm$ 0.6
20	4.0 $\pm$ 1.8	1.7 $\pm$ 0.6
50	2.3 $\pm$ 0.9	1.1 $\pm$ 0.3
Nifedipine (μM)		
5	12.2 $\pm$ 1.8	5.6 $\pm$ 0.9
10	12.6 $\pm$ 2.7	5.7 $\pm$ 1.3
20	11.1 $\pm$ 3.1	5.0 $\pm$ 1.4
50	10.0 $\pm$ 2.5	4.5 $\pm$ 1.1
Verapamil (μM)		
5	12.5 $\pm$ 2.8	5.5 $\pm$ 1.5
10	10.9 $\pm$ 3.0	5.0 $\pm$ 1.3
20	9.9 $\pm$ 3.1	4.6 $\pm$ 1.3
50	8.6 $\pm$ 2.6	3.8 $\pm$ 1.3
Diltiazem (μM)		
5	11.7 $\pm$ 2.1	5.2 $\pm$ 1.1
10	11.5 $\pm$ 2.2	5.2 $\pm$ 1.2
20	10.9 $\pm$ 2.5	5.0 $\pm$ 1.2
50	9.7 $\pm$ 2.5	4.4 $\pm$ 1.1

In contrast, nifedipine caused less than 20% inhibition of M21 and M17 even at the highest concentration (50 μM). Diltiazem data were comparable with those for nifedipine (at 50 μM , 21 and 16% inhibition of M17 and M21, respectively). Verapamil inhibited to a greater extent than either nifedipine or diltiazem but very much less than nicardipine. A mean histogram representation of the h.p.l.c. profiles for the nicardipine study is shown in Figure 1.

Discussion

The results of this study indicate differential inhibitory effects of the Ca^{2+} channel antagonists on CsA metabolism. Nicardipine had the greatest inhibitory effect and, therefore, these *in vitro* data help to explain the basis of the interaction (increased CsA blood concentrations) observed *in vivo* (Bourbigot *et al.*, 1986). We have observed IC_{50} values of approximately 7 μM for both M17 and M21 formation. Although nicardipine concentrations in plasma are unlikely to reach such values (Graham *et al.*, 1985; peak concentration 0.4–0.5 μM), concentrations in the hepatocytes may well be much higher than those measured in plasma.

Figure 1 Mean data from four h.p.l.c. radiochromatograms showing the effect of nicardipine (5, 10 and 50 μM) on CsA metabolism.

Diltiazem has been demonstrated to have a protective effect on posttransplant tubular necrosis in renal transplant patients despite significantly higher CsA whole blood concentrations (Wagner *et al.*, 1988). Grino *et al.* (1986) also reported high concentrations of CsA without nephrotoxicity after diltiazem administration. Based on the fact that diltiazem has previously been shown to inhibit the microsomal metabolism of aminopyrine in mice (Renton, 1985) and also antipyrine metabolism in human volunteers (Abernethy *et al.*, 1988), it has been assumed that diltiazem is an inhibitor of CsA metabolism (Castelao *et al.*, 1988; Kohlaw *et al.*, 1988). However, in the study of Wagner *et al.* (1988), not only did blood concentrations of CsA increase but those of M17 were also elevated. They suggested that an alteration of CsA metabolism by diltiazem was unlikely and that a decrease in apparent volume of distribution was a more probable explanation. Our findings with diltiazem also suggest that inhibition of CsA metabolism is unlikely given that peak diltiazem concentrations approximate to 1 μM (Montamat & Abernethy, 1987).

Verapamil has been reported in one clinical study to cause an increase in CsA blood concentrations, although since a radioimmunoassay using a polyclonal antiserum was employed there is no information on metabolite concentrations (Henricsson & Lindholm, 1988). Verapamil has been shown to inhibit aminopyrine *N*-demethylation in mice (Renton, 1985), to

decrease antipyrine and theophylline clearance in volunteers (Abernethy *et al.*, 1988) and to decrease quinidine clearance (Edwards *et al.*, 1987). However in a study on mephenytoin and sparteine metabolism in human liver microsomes, verapamil did not have any inhibitory effects (Inaba *et al.*, 1985). It is difficult, without additional evidence, to know if the *in vitro* inhibition of CsA by verapamil (approximately 30% at 50 μM) is likely to have clinical relevance.

There are at least two reports showing that nifedipine does not alter the pharmacokinetics of CsA (Bourbigot *et al.*, 1986; Henricsson & Lindholm, 1988); the *in vitro* data would be consistent with these findings. It is, however, of interest to note that Kronbach *et al.* (1988) have identified a cytochrome P-450, similar or identical to P-450_{NF} as the major isozyme in human liver catalysing the formation of M17, M21 and M1 from CsA. Why if this is so, we were unable to observe pronounced inhibition of CsA by nifedipine is as yet unclear.

In conclusion, although some Ca^{2+} channel antagonists have been demonstrated to exert a beneficial effect on early graft function in renal allograft recipients treated with CsA it is important to be aware that there are clear differences in the metabolic interaction potential of these drugs.

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