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Cyclosporin metabolism by human gastrointestinal mucosal microsomes

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The *in vitro* metabolism of the immunosuppressant cyclosporin (CsA) by human gastrointestinal mucosal microsomes has been studied. Macroscopically normal intestinal (n = 4) and liver (n = 2) tissue was obtained from kidney transplant donors, and microsomes prepared. Intestinal metabolism was most extensive with duodenal protein (15% conversion to metabolites M1/M17 after 2 h incubation at 37°C; metabolite measurement by h.p.l.c). Western blotting confirmed the presence of P-4503A (enzyme subfamily responsible for CsA metabolism) in duodenum and ileum tissue, but not in colon tissue. The results of this study indicate that the gut wall may play a role in the first-pass metabolism of CsA, and could therefore be a contributory factor to the highly variable oral bioavailability of CsA.

Keywords cyclosporin A gut wall metabolism cytochrome P-4503A

Introduction

Cyclosporin A (Cyclosporine, CsA) is a widely used immunosuppressant agent with applications in anti-rejection therapy for allograft recipients, and increasing use in autoimmune diseases.

CsA is cleared from the body largely in the form of monohydroxylated (M1 and M17) and *N*-demethylated (M21) metabolites. These are the product of cytochrome P-450-mediated biotransformations. The major site of CsA metabolism is the endoplasmic reticulum of hepatocytes, where the largest amounts of P-450 enzymes are localised, although other organs may make a significant contribution to the total clearance.

One of the major difficulties of CsA therapy is the considerable inter- *and* intra-individual variation in bioavailability (mean F 0.3, inter-individual range 0.08–0.89; Kahan, 1985; McMillan, 1989; Ptachcinski *et al.*, 1986). This is largely due to differences in absorption between doses and rapid first-pass metabolism in the liver. However, in dogs greater concentrations of CsA metabolites have been found in hepatic portal venous blood following oral dosing compared with intravenous (Gridelli *et al.*, 1986), and we have previously demonstrated metabolism of CsA by human colonic mucosa (Tjia *et al.*, 1991). CsA metabolism has also been shown in liver transplant recipients during the anhepatic phase of surgery (Kolars *et al.*, 1991).

The aim of the present study was to examine the capability of the microsomal fraction (endoplasmic reticulum) of human intestinal mucosa to metabolise CsA, and compare this with immunoblotting data for cytochrome P-4503A, which is the enzyme sub-family known to mediate this conversion (Combalbert *et al.*, 1989; Kronbach *et al.*, 1988).

Methods

Materials

CsA and metabolites were a gift from Sandoz Pharmaceuticals, Basel, Switzerland. [^{3}H]-MeBmt-CsA (specific activity 17Ci mmol^{-1}) was obtained from Amersham Radiochemicals, Amersham, Bucks., U.K. H.p.l.c. solvents were obtained from Fisons plc, Loughborough, Leics., U.K. NADPH was obtained from Sigma Chemical Company, Poole, Dorset, U.K. Scintillation cocktail (Flo-Scint A) was obtained from Canberra-Packard, Pangbourne, Berks, U.K. All other chemicals and reagents were obtained from B.D.H., Poole, Dorset, U.K.

Methodology

The study was approved by the Mersey Regional Hospital Ethics Committee and the St Radboud Hospital Human Research Review Committee.

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Macroscopically normal human hepatic (n = 2) and intestinal (n = 4) tissues were obtained from kidney donors (for details see Table 1). None of the donors was known to be smokers, or to have received enzyme-inducing drugs. Intestinal mucosa was obtained by scraping the lumenal surface. Microsomes were prepared by the classical differential centrifugation technique (Peters & Kremers, 1989). Immunoblots were made using the method described previously (Peters & Jansen, 1988). A monoclonal antibody raised against P-450$_5$ was employed (Mab K03 13-7-10, Beaune *et al.* (1985) isozyme subsequently named P-4503A4 under the system of Nebert *et al.*, 1989). This antibody crossreacts with both the P-4503A3 and P-4503A4 isoenzymes. Immunoblots were stained as described (Peters & Jansen, 1988), and were subsequently scanned at 600 nm with a laser densitometer (LKB 2202 Ultrascan, LKB Bromma, Sweden). Densitometry data were expressed as absorbance units per mg microsomal protein.

Microsomal protein from various sections of the gastrointestinal tract (2–8 mg) or liver (0.5–1.5 mg) was incubated at 37°C with 5 μM CsA and 0.2 μCi [^{3}H]-CsA, in 0.067 M phosphate buffer pH 7.5, with 5 mM $MgCl_2$, 1 mM EDTA, and 1 mM KCl. NADPH 1 mM was added and the mixture was shaken for 2 h. Total incubation volume was 2.5 ml. The reaction was terminated by addition of 6 ml diethyl ether. The tubes were placed in a rotary mixer for 10 min, then centrifuged at 2000 *g* for 10 min. The aqueous layer was frozen by immersion in Cardice-chilled methanol, and the ether layer poured off into clean tubes. The ether was evaporated to dryness and samples were reconstituted in 300 μl far-u.v. grade acetonitrile. 100 μl was injected onto the h.p.l.c. system, as described previously (Tjia *et al.*, 1989). Radioactivity was monitored by an A-250 series Radiomatic Flo-One Beta on-line detector, with u.v. spectrophotometric measurement at 210 nm. Metabolites were measured by calculation of the peak area of radioactivity and tentatively identified according to the retention times of authentic standards (M1, 6 min; M17, 6 min; M21, 8 min; CsA, 12 min). M1 and M17 were not separated in this system.

Results

Figure 1 shows radiochromatograms from incubations with protein derived from different regions of the gastrointestinal tract of three patients and the liver of one

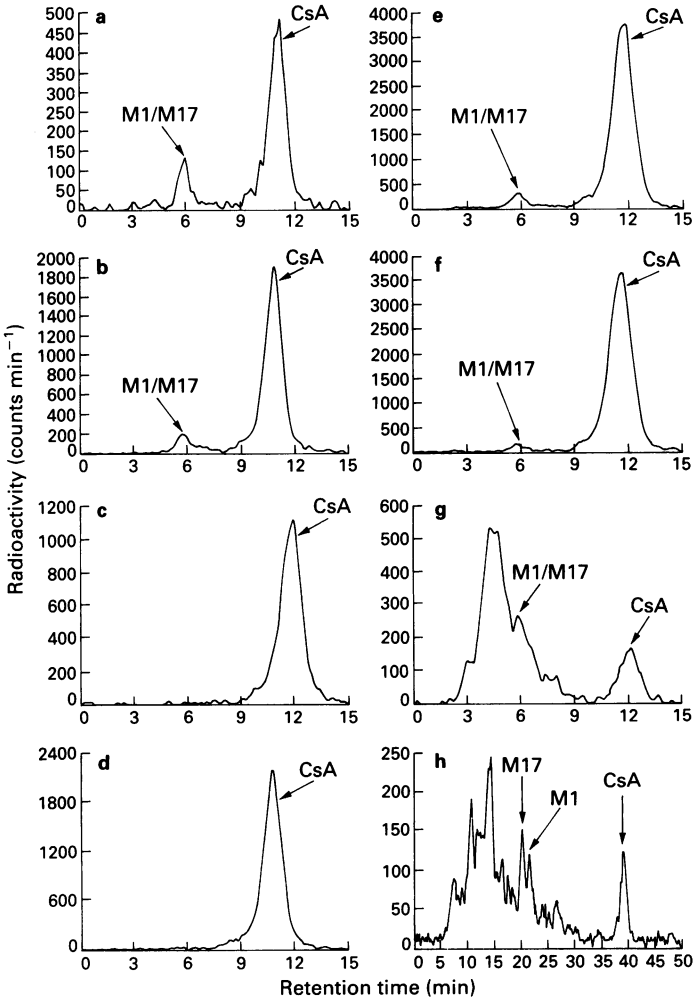

Figure 1 Radiochromatograms from incubations with intestinal and hepatic microsomal protein. All incubations contained 5 μM CsA and 0.2 μCi [^{3}H]-CsA, with 2 h incubation time at 37°C.
(a) Patient 2, duodenum, 6 mg protein (15% conversion to M1/M17); (b) Patient 2, ileum, 6 mg protein (8% conversion to M1/M17); (c) Patient 3, caecum, 7 mg protein (no metabolites detected); (d) Patient 2, colon, 6 mg protein (no metabolites detected); (e) Patient 4, duodenum, 6 mg protein (6% conversion to M1/M17); (f) Patient 4, ileum, 6 mg protein (3% conversion to M1/M17); (g) Patient 3, liver, 1.5 mg protein (80% conversion to metabolites); (h) Sample (g) run on gradient h.p.l.c. system over 50 min.

Table 1 Immunoquantitation of cytochrome P4503A in microsomes from duodenum, ileum, colon and liver

	Absorbance (units mg^{-1} microsomal protein)			
Protein source	*Patient 1* ♀, *3½ months*	*Patient 2* ♂, *18 years*	*Patient 3* ♂, *49 years*	*Patient 4* ♂, *18 years*
Duodenum	33.7 (0.28)	47.1 (0.58)	41.1 (0.72)	66.9 (1.93)
Ileum	40.6 (0.23)	45.8 (0.57)	32.1 (0.39)	68.8 (0.84)
Colon	*n.d.* (0.20)	*n.d.* (0.28)	*n.d.* (0.25)	*n.a.*
Liver	*n.a.*	*n.a.*	229.3	212.7

n.d. not detected; *n.a.* not available
Figures in parentheses denote yield of microsomal protein from intestinal tissue sections, expressed as mg protein cm^{-2}.
Duodenum and ileum were differentiated by distance from pylorus.
Total microsomal cytochrome P-450 has previously been quantified in tissue from patient 4, and was found to be two to three times greater in duodenum compared with ileum (Peters *et al.*, 1989).

patient. The extent of metabolism by microsomes from the duodenum and ileum of patient 1 (3½ month old girl) was very small (< 2.5% of CsA converted to metabolites) and data are not shown. Similarly, metabolism by duodenal and ileal microsomes of patient 3 was less than 3%. However, in this patient there was extensive liver metabolism (Figure 1g/1h).

We have previously shown that M17 and M21 are major metabolites generated by the human liver microsomal system with an incubation time of 15–20 min (Back *et al.*, 1989; Tjia *et al.*, 1989). In the present study, since the incubation time is much longer (2 h) a different metabolic profile was observed. A more polar peak prior to M17/M1 (Figure 1g) was presumed to represent secondary derivatives of M17, M1 and M21, including dihydroxylated and hydroxylated/*N*-demethylated metabolites. The profile obtained running this sample on a gradient h.p.l.c. system (initially acetonitrile: water 35:65, changing to 55:45 over the first 20 min) demonstrated the number of metabolites generated, with at least 10 discernible metabolite peaks (Figure 1h). Running samples from gut incubations on the same system yielded only two major metabolites (M17 and M1), with detection of small quantities of M21 on some chromatograms. No metabolism was observed in experiments with colon or caecum microsomal protein.

The results of immunoquantitation of P-4503A family proteins are illustrated in Table 1, which shows densitometric data and yield of microsomal protein from three regions of gut from each patient, and densitometric data from liver of two patients.

Discussion

The demonstration of metabolism of CsA by gut microsomes extends our previous studies using colonic mucosal sheets mounted in Ussing chambers (Tjia *et al.*, 1991). The inference from this work is that there is the potential for gut wall metabolism of CsA *in vivo*. A comparison of the studies raises the question as to why using the Ussing chamber the colonic tissue metabolises CsA and yet in the present study there was no evidence of either P-4503A or generated metabolites in microsomes from this region of the g-i tract? Part of the reason for the difference may be that the mucosal sheets have intact enterocytes, and that the drug is exposed to more protein in the incubation. Although we were unable to demonstrate the presence of P-4503A in colon (Table 1), de Waziers *et al.* (1990) have found P-4503A to be present in colon at 1.5% of liver-specific content.

Results from the present study indicate that the metabolism of CsA is greatest in the duodenum, with the ileum (at equivalent protein concentrations) producing less metabolite. The decrease in metabolism seen with microsomes from the ileum suggests that less P-4503A is present. However, reduced ileal P-4503A was not seen in all patients studied (immunoquantitation indicated similar levels of P-4503A in duodenum and ileum of patients 2 and 4; Table 1), in contrast to the study of de Waziers *et al.* (1990). It has been suggested that metabolism in the stomach may account for the variations in CsA bioavailability related to changes in gastric motility (Ueda *et al.*, 1984; Wadhwa *et al.*, 1987). Our results suggest metabolism in the small intestine may also significantly affect the bioavailability of CsA.

The significance of first pass metabolism in the variable bioavailability of CsA has recently been highlighted (Kolars *et al.*, 1991; Schwinghammer *et al.*, 1991; Tredger *et al.*, 1991). With variations in g.i. tract enzyme profiles between patients, and even within the same patient in response to changing drug treatments and disease states, gut wall metabolism may well be far more important than previously recognised.

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