Biochemical Pharmacology, Vol. 46, No. 5, pp. 813–819, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

DIFFERENTIAL EFFECTS OF CYCLOSPORIN A ON THE TRANSPORT OF BILE ACIDS BY HUMAN HEPATOCYTES*†

SAMY A. AZER and NEILL H. STACEY‡
National Institute of Occupational Health and Safety, The University of Sydney, Australia

(*Received* 25 *March* 1993; *accepted* 7 *June* 1993)

Abstract—Cyclosporin A (CsA) treatment has been reported to cause rises in serum bile acids both in humans and rats. It has also been shown to suppress bile flow *in situ* in rats and inhibit the transport of bile salts by rat hepatocytes. The purpose of this study was to examine the influence of CsA on uptake of radiolabelled cholate (CA), glycocholate (GC) and taurocholate (TC) by isolated human hepatocytes. CsA did not significantly change V_{max} for CA uptake [0.23 ± 0.01 vs 0.25 ± 0.02 nmol/mg protein/min for control and CsA (10 μM), respectively], but significantly increased K_m (37 ± 2 vs 86 ± 8 μM). Similarly, V_{max} for TC uptake was not affected (0.51 ± 0.02 vs 0.67 ± 0.05 nmol/mg protein/min) while K_m was significantly increased [46 ± 3 vs 109 ± 11 μM for control and CsA (10 μM), respectively]. On the other hand, neither V_{max} nor K_m for GC uptake was affected by CsA. The data indicate a competitive pattern of inhibition induced by CsA on CA and TC uptake. Furthermore, CsA was found to cause a dose-related inhibition of accumulation of both cholate and taurocholate, but not GC accumulation. None of the concentrations of CsA showed a significant effect on the integrity of the human hepatocytes as assessed by ALT (alanine aminotransferase), AST (aspartate aminotransferase) and LDH (lactate dehydrogenase) release. The findings, in human hepatocytes, are generally consistent with the observations reported from rodent studies. They strongly support the contention that serum bile acid increases in CsA-treated patients are due to interference with the hepatocellular transport and accumulation of particular bile acids.

Cyclosporin A (CsA§) is a lipophilic, cyclic endecapeptide which has revolutionized the field of organ transplantation [1–4] and is now being considered in the treatment of some autoimmune diseases [5, 6]. Its use, however, is associated with a number of side effects such as nephrotoxicity, neurotoxicity, hypertension and gingival hyperplasia [1]. CsA associated hepatic dysfunction has been reported in 86% of bone marrow transplantation [7] and in up to 20% of renal allograft transplant recipients [8, 9] and observed in heart transplant patients [10–12]. In all these cases, concurrent bacterial and viral infections, haemolysis, graft versus host disease, drug interactions and heart failure have been excluded. Patients treated with CsA for reasons other than organ transplantation also showed signs of hepatic dysfunction [13, 14] and similar observations have been reported from animal studies [15–17]. The major changes that characterized these hepatic effects were rises in total serum bile acids (TSBA) and serum bilirubin [15–19] with minor or unchanged activities of serum transaminases [8, 10, 13, 15, 16, 18]. Such changes were reported to be dose dependent and reversible when the dosage of CsA was discontinued or decreased [9, 19]. Furthermore, histological lesions were usually absent [20].

The mechanism of CsA-associated hepatic dysfunction in humans is still not fully understood although animal studies have related the changes to decrease in bile flow [21] and interference with the uptake of bile acids, using both *in vivo* [15] and *in vitro* treatment [22–24]. This inhibitory effect of CsA on hepatic uptake is not limited to bile salts as it is also observed with other cholephilic substances [24–27]. However, these studies used animals, particularly rats, to investigate this mechanism and there are no available data from humans. Using human hepatocytes to investigate this mechanism seems to be mandatory as this could provide confirmatory evidence for the animal studies and the actual mechanism responsible for these changes in CsA-treated patients. The need for human studies in this respect emerges from the fact that there are species differences in the percentage of primary and secondary bile acids and their conjugates [28, 29] and metabolism of drugs [30], other chemicals and cholephilic compounds [31–34]. Therefore, it was decided to study the effects of CsA on bile acid uptake by isolated human hepatocytes.

* Presented orally in part, at the 11th Annual Meeting of The Transplantation Society of Australia and New Zealand, Canberra, Australia, 1993.

† Disclaimer: The conclusions reached and scientific views expressed in this paper are solely those of the authors and they do not necessarily reflect the views and policies of the organization in which they work.

‡ Corresponding author: Dr Neill H. Stacey, NIOHS, GPO Box 58, Sydney, NSW 2001, Australia. Tel. (61)-2-5659297; FAX (61)-2-5659300.

§ Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA, cholic acid; CsA, cyclosporin A; DMSO, dimethyl sulphoxide; GC, glycocholic acid; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; LDH, lactate dehydrogenase; TC, taurocholic acid; TSBA, total serum bile acid(s); UW, University of Wisconsin.

MATERIALS AND METHODS

Chemicals and reagents. The following compounds were obtained from the indicated sources. Cholic acid (CA) [*carboxyl*-^{14}C], sodium salt (53.9 mCi/mmol) and glycocholic acid (GC) [*carboxyl*-^{14}C], sodium salt (50 mCi/mmol) from Amersham Australia Pty Ltd (Sydney) Australia. Taurocholic acid (TC) [^{3}H(G)] (2.1 Ci/mmol) from Du Pont, NEN Research products (NSW, Australia). Amphotericin B, solubilized; collagenase type IV (380 U/mg solid); ethylene glycol-bis (β-aminoethylether) *N, N, N', N'*-tetra acetic acid (EGTA); gentamicin sulphate (696 μg base per mg solid); streptokinase (132,000 U/mg protein) and trypsin inhibitor (Type II-L: lima bean-10,000 BAEE U/mg protein) from the Sigma Chemical Co. (St Louis, MO, U.S.A.). HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid] from Calbiochem (La Jolla, CA, U.S.A.). UW (University of Wisconsin) solution from Du Pont Critical Care (Wankegan, IL, U.S.A.). Percoll from Pharmacia LKB (Uppsala, Sweden). CsA was a gift from Sandoz Australia Pty Ltd (Sydney, Australia). All other chemicals used were of analytical grade and were obtained from commercial sources.

Isolation of hepatocyctes. This work has been carried out in cooperation with the Australian National Liver Transplantation Unit, Royal Prince Alfred Hospital. The studies were approved by both Central Sydney Health Service Ethics Review Committee, Royal Prince Alfred Hospital and the Human Ethics Committee, the University of Sydney. Postmortem human liver tissues were obtained from 10 liver donors aged 5–40 years. All pieces (50–200 g/piece) were supplied from a cut-down (usually from the right hepatic lobe) during liver transplant operations. Cut-down operations were performed in cases where the recipient's abdominal cavity is relatively small compared to the donor liver. The pieces were kept in UW solution (containing mmol/L: 100 K-lactobionate, 25 KH_2PO_4, 5 $MgSO_4$, 30 raffinose, 5 adenosine, 3 glutathione, 1 allopurinol, 50 g hydroxyl starch) for 1–4 hr until used. Neither insulin nor dexamethasone was added to UW solution since the addition of insulin showed deleterious effects on liver graft survival in rats [35] and the presence of dexamethasone can affect bile acid synthesis [36]. Hepatocytes were isolated by a two-step perfusion technique modified from that described for the isolation of rodent hepatocytes [37] with some modifications [38]. Briefly, the liver was perfused for 15 min with 3 L Ca^{2+}/Mg^{2+}-free buffer, pH 7.4, containing per L: 101.8 mmol NaCl, 5.6 mmol D-glucose anhydrous, 6.6 mmol KCl, 0.44 mmol KH_2PO_4, 0.34 mmol Na_2HPO_4, 49.9 mmol HEPES, 0.5 mmol EGTA, 15.4 mmol $NaHCO_3$, 100 mg gentamicin sulphate, 0.1 mg amphotericin B and 30,000 U streptokinase. It was then perfused with 500 mL HEPES buffer, pH 7.4, containing per L: 101.8 mmol NaCl, 5.6 mmol D-glucose anhydrous, 6.6 mmol KCl, 5.03 mmol $CaCl_2$-$2H_2O$, 49.9 mmol HEPES, 15.4 mmol $NaHCO_3$, 100 mg gentamicin sulphate, 0.1 mg amphotericin B, 15,000 U streptokinase, 100 mg trypsin inhibitor and 200,000 U of Type IV collagenase in a recirculation system. Non-viable cells were separated from viable cells by centrifugation in 10 mL of percoll solution (1 vol. of the 10 times concentrated Hank's buffer with 9 vol. of the stock percoll). Viability of hepatocytes was assessed by Trypan blue (0.4%) exclusion immediately after isolation and was always in the range of 84–88%. The yield of viable hepatocytes was variable ranging from 2×10^6 to 2×10^7 cells/g liver. Hepatocytes were then adjusted in Hanks-HEPES to a concentration of 1.4×10^6 cells/mL.

Uptake experiments. Bile acid uptake studies for CA, GC and TC were performed in separate 25-mL Erlenmeyer flasks (Schott, Duran, Germany) at 37°. For each bile acid used, aliquots (1.98 mL) of the freshly isolated hepatocyte suspensions in Hanks–HEPES buffer (1.4×10^6 cells/mL) were pre-incubated with shaking at 80 oscillations/min. Uptake for each bile acid was initiated (at zero time) by the addition of 10 μL of CsA in dimethyl sulphoxide (DMSO) or DMSO alone (as a control) followed by the addition of 10 μL of the radiolabelled bile acid substrate at 30 sec. The final concentration ranged from 10 to 100 μM and the radioactivity in each flask was about 50 nCi/mL for CA and GC experiments and 80 nCi/mL for TC. Sampling using the 400-μL polyethylene tube/silicone oil technique [39] was carried out at appropriate times. The polyethylene tubes (Stockwell Scientific, Walnut, CA, U.S.A.) were cut at the middle of the oil layer the next morning and the radioactivity in the pellet and supernatant was determined by a liquid scintillation counter (Packard Instruments Inc., Downers Grove, IL, U.S.A.). Protein concentration was measured [40] using bovine serum albumin as a standard. Determination of radiolabel and protein concentration of hepatocyte suspension allowed calculation of the uptake on a per milligram protein basis.

Cell viability. In addition to the Trypan blue exclusion test, cell membrane integrity was assessed by the measurement of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH). These assays were carried out on cell suspension supernatants using a CentrifiChem (model 400 distributed by Roche) and appropriate kits (Behring Diagnostic Inc., NJ, U.S.A.).

Data analysis and statistical procedures. Each data point represents the mean value obtained from at least three experiments. Statistical analysis was by analysis of variance and Duncan's test with a pre-set probability level of $P < 0.05$. For uptake experiments, initial rates of bile acid uptake were determined at all concentrations from 10 to 100 μM. The apparent kinetic parameters from the initial rates of uptake of each bile acid were calculated using Michaelis–Menten kinetics.

RESULTS

Cell viability

After an hour of incubation, Trypan blue exclusion did not show significant changes in hepatocyte viability compared to those tested immediately after

Table 1. *In vitro* effects of CsA at various concentrations on the release of cellular enzymes

Experimental groups	ALT (U/L)	AST (U/L)	LDH (U/L)
DMSO	67.0 ± 6.8	91.1 ± 8.1	187.9 ± 20.3
DMSO + CsA			
1 μM	71.8 ± 11.5	87.0 ± 13.0	201.3 ± 24.6
10 μM	77.5 ± 9.7	89.6 ± 9.8	235.5 ± 32.8
100 μM	76.3 ± 7.2	95.4 ± 14.9	199.3 ± 46.5

The values are expressed as means ± SEM of duplicate determinations each from four experiments. No significant difference was found in the protein concentrations of various experimental groups.

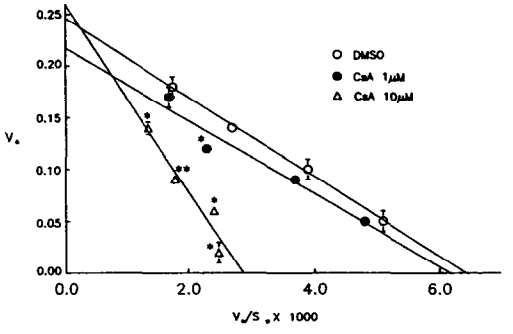

Fig. 1. Eadie–Hofstee plots of cholate uptake by human hepatocytes in the presence of varying concentrations of CsA or vehicle (DMSO). Initial uptake values (V_0) were determined over a 20–80 sec period of incubation. Each value represents the mean ± SEM from three experiments. * Significantly different from respective control values, ** significantly different from respective values of control and CsA 1 μM ($P < 0.05$).

preparation. Table 1 summarizes the effect of the various concentrations of CsA on viability of isolated hepatocytes. The data show that *in vitro* exposure of CsA did not cause any increase in leakage of ALT, AST and LDH compared to control.

Effects of CsA on bile acid uptake

Substrate uptake (CA, GC and TC) was linear with time (20–80 sec) at all the concentrations studied. The slope of the regression line was used to obtain the initial rate of uptake (V_0) which varied considerably among the different bile acids studied (Figs 1–3). The effects of various concentrations of CsA on the initial rates of uptake of CA, GC and TC and their apparent kinetic parameters can be obtained from Figs 1–3 and Tables 2 and 3. K_m for CA and TC increased significantly with no changes in V_{max} suggesting a competitive inhibition induced by CsA on these substrates. On the other hand, CsA did not show any inhibitory effect on the uptake of GC as both K_m and V_{max} for the various CsA concentrations did not show significant changes compared to control. CsA also caused a dose-related inhibition of both CA and TC accumulation (Fig. 4, A and C) but did not affect GC accumulation at any of the concentrations used (Fig. 4B).

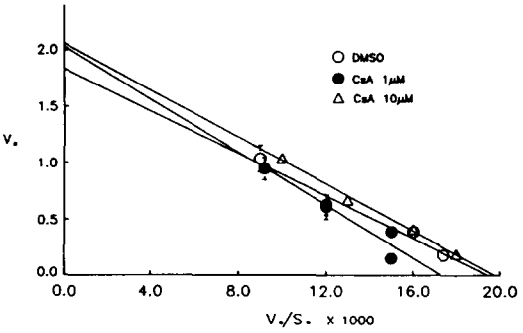

Fig. 2. Eadie–Hofstee plots of glycocholate uptakes by human hepatocytes in the presence of varying concentrations of CsA or vehicle (DMSO). Initial uptake values (V_0) were determined over a 20–80 sec period of incubation. Each value represents the mean ± SEM from three experiments. No values are statistically different from their respective control ($P < 0.05$).

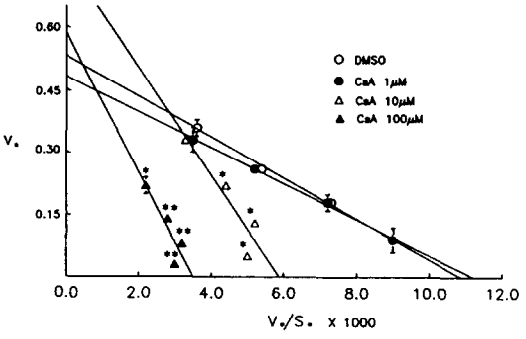

Fig. 3. Eadie–Hofstee plots of taurocholate uptake by human hepatocytes in the presence of varying concentrations of CsA or vehicle (DMSO). Initial uptake values (V_0) were determined over a 20–80 sec period of incubation. Each value represents the mean ± SEM from four experiments. * Significantly different from respective control values, ** significantly different from respective values of control and CsA 10 μM ($P < 0.05$).

DISCUSSION

The mechanism by which CsA induces a rise in serum bile acids on CsA treatment has been investigated in isolated perfused rat liver [21], *in vivo* treated rats and freshly isolated [15, 22, 24] and cultured rat hepatocytes [23] and rat plasma membrane vesicles [41, 42]. In the present study exposure of human hepatocytes to various CsA concentrations did not affect the integrity of hepatocytes as evidenced by a lack of ALT, AST and LDH leakage compared to control (Table 1). These findings are consistent with the reported observations from rat hepatocytes [23] and *in vivo* treated rats [15, 16]. CsA *in vivo* treatment has even been associated with lowered serum transaminases and alkaline phosphatase activity [43].

The use of human hepatocytes in toxicological

Table 2. Apparent kinetic parameters for cholate and glycocholate uptake by human hepatocytes in the presence of various concentrations of CsA

CsA concn (μM)	Cholate*		Glycocholate*	
	K_m	V_{max}	K_m	V_{max}
0	36.9 ± 2.0	0.23 ± 0.01	101.1 ± 6.8	1.92 ± 0.21
1	36.5 ± 1.8	0.22 ± 0.01	116.5 ± 16.5	2.06 ± 0.34
10	85.7 ± 8.2†	0.25 ± 0.02	95.7 ± 4.0	1.97 ± 0.11

* Values for K_m (μM) and V_{max} (nmol/mg protein/min) were estimated from the Eadie–Hofstee plot. Each value represents the mean ± SEM of three different cell preparations.
† Statistically different from respective control ($P < 0.05$).

Table 3. Apparent kinetic parameters for taurocholate uptake by human hepatocytes in the presence of various concentrations of CsA

CsA concn (μM)	K_m* (μM)	V_{max} (nmol/mg protein/min)
0	45.7 ± 3.1	0.51 ± 0.02
1	50.7 ± 0.6	0.53 ± 0.02
10	109.3 ± 10.7†	0.67 ± 0.05
100	194.2 ± 40.4†	0.65 ± 0.10

* Values for K_m and V_{max} were estimated from the Eadie–Hofstee plot. Each value represents the mean ± SEM of four different cell preparations.
† Significantly different from control ($P < 0.05$).

studies has provided a useful tool for investigating the hepatotoxic effects of drugs and chemicals particularly in situations where species differences need to be explored [33, 34]. However, obtaining a sufficient number of human liver pieces for perfusion and the yield of viable cells are the major reported disadvantages [38]. Our data show that the viability of isolated hepatocytes after the percoll step was in the range of 84–88% which is reasonable for uptake studies compared to recently reported studies [44, 45]. However, the major problem which we faced during this work was the relatively low numbers of liver cut-down operations (10 liver pieces in 18 months).

Our data show the ability of CsA to interfere with human hepatocellular uptake of two bile acids, CA and TC. The inhibition was rapid and competitive in nature as evidenced by the increase in K_m indicating a decrease in receptor affinity for substrate. V_{max} for both bile acids, however, was not affected (Tables 2 and 3). CsA also caused a dose-related inhibition of the accumulation of these two bile acids (Fig. 4 A and C). These results are consistent with the observations reported from freshly isolated rat hepatocytes [22, 24] and cultured rat hepatocytes [23], although some authors reported a non-competitive inhibitory effect of CsA on CA uptake in rats [24]. This could be related to differences in the experimental design (composition of buffers, for example, could possibly affect the nature of bile acid uptake).

It may be of interest to compare the reported kinetic parameters for TC uptake by *in vitro* treated rat hepatocytes [22] with our present results using human liver cells. In rats, K_m values for TC uptake were 32 ± 9 and 131 ± 7 μM for control (DMSO) and CsA (10 μM) in DMSO, respectively, while V_{max} values were 2.9 ± 0.4 and 3.7 ± 0.4 nmol/mg protein/min. The data from the present work for the same bile acid (Table 3) show K_m values of 46 ± 3 and 109 ± 11 μM for control and CsA (10 μM), respectively, and V_{max} values of 0.51 ± 0.02 and 0.67 ± 0.05 nmol/mg protein/min. It is clear that the K_m values for TC uptake in both species are quite similar. V_{max} values, however, were relatively low for human hepatocytes. This may be related to the species differences in the percentage of conjugated bile acids [29].

GC uptake was not inhibited by the presence of CsA and its accumulation was not affected by the various concentrations of CsA. These results suggest a substrate-specificity for the effects of CsA. A specific effect for CsA on the uptake of other substrates by rat hepatocytes has been reported [22, 46] and CsA has been shown to have selective hepatocellular membrane effects [47]. For example, it has been reported that the hepatocellular uptake of α-aminoisobutyric acid [22] and galactose [46] were not inhibited by the presence of CsA. The uptake of the former substrate is known to be linked to energy-dependent systems while the latter is associated with carrier-mediated transport [48]. Thus, while CsA interferes with the hepatocellular uptake of carrier-mediated substrates such as CA and TC [15, 22–24], CsA did not show any effect on the hepatocellular uptake of other substrates transported by similar processes. These observations are not inconsistent with the lack of inhibitory effect of CsA on GC as demonstrated in this study and suggest that CsA has a substrate-specific rather than a general effect on hepatocellular transport. Interestingly, however, these data suggest differences in transport mechanisms among bile acids that are deserving of further investigation. Substances like CsA would seem likely to be valuable experimental tools in further unravelling the nature of specific transport of bile acids.

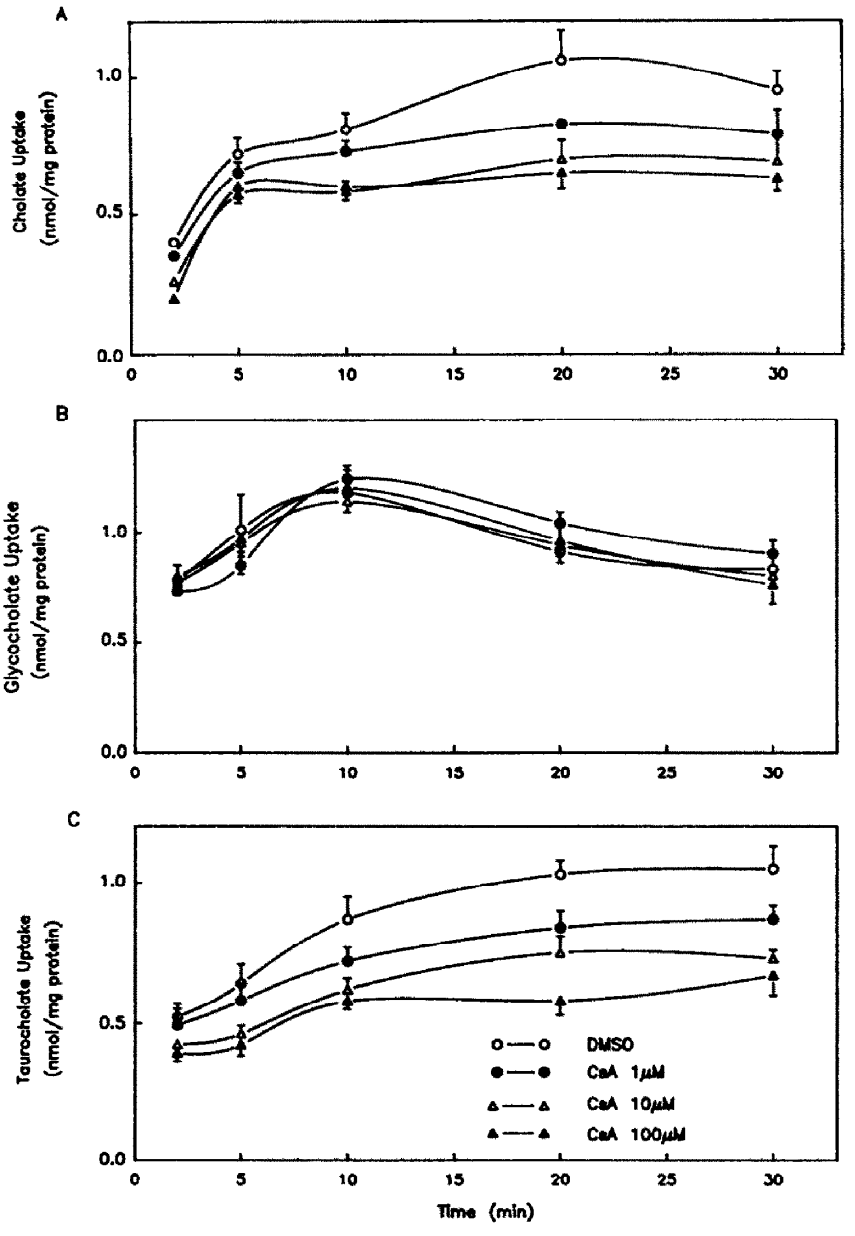

Fig. 4. Substrate accumulation by isolated human hepatocytes treated *in vitro* with DMSO and varying concentrations of CsA. Values represent the mean ± SEM (N = 3). (A) Cholate concentration (25 μM). All data points for the higher concentrations of CsA (10 and 100 μM) are significantly different to control at 2, 10, 20 and 30 min ($P < 0.05$). (B) Glycocholate concentration (25 μM). No data points for the various concentrations of CsA are significantly different to control at 2–30 min ($P < 0.05$). (C) Taurocholate concentration (25 μM). All data points for the higher CsA concentrations (10 and 100 μM) are significantly different to control at 10–30 min and at 5 min for the highest concentration ($P < 0.05$).

In conclusion, *in vitro* treatment of isolated human hepatocytes with CsA inhibited the initial uptake velocity of CA and TC but not GC. The inhibition was rapid and competitive in nature. CsA also induced a dose-related inhibition of both CA and TC accumulation. On the other hand, GC accumulation was not affected by various concentrations of CsA (1–100 μM). The findings, in human hepatocytes, are generally consistent with the observations reported from rodent studies. They strongly support the contention that serum bile acid increases in CsA-treated patients are due to interference with the hepatocellular transport and accumulation of particular bile acids.

Acknowledgements—This work was supported by a grant from the National Health and Medical Research Council, Australia. The authors would like to thank Associate Professor Geoffrey W. McCaughan and Mr G. Kyd, of the A.W. Morrow Gastroenterology and Liver Centre, Liver Transplant Unit, Royal Prince Alfred Hospital for their support of this work.

REFERENCES

1. Kahan BD, Cyclosporine. *N Engl J Med* **321**: 1725–1738, 1989.
2. Haverich A, Cyclosporine in heart transplantation. *Transplant Proc* **24**: 82–84, 1992.
3. Beveridge T, Clinical transplantation overview. *Prog Allergy* **38**: 269–292, 1986.
4. Beveridge T, Clinical development of cyclosporine. *Transplant Proc* **24**: 64–66, 1992.
5. Feutren G, Clinical experience with Sandimmune (Cyclosporine) in autoimmune diseases. *Transplant Proc* **24**: 55–60, 1992.
6. Fatham CG and Myers BD, Cyclosporine therapy for autoimmune disease. *N Engl J Med* **326**: 1693–1694, 1992.
7. Atkinson K, Biggs J, Dodds A and Concannon A, Cyclosporine-associated hepatotoxicity after allogeneic marrow transplantation in man: differentiation from other causes of posttransplant liver disease. *Transplant Proc* **15**: 2761–2767, 1983.
8. Rodger RSC, Turney JH, Haines I, Michael J, Adu D and McMaster P, Cyclosporine and liver function in renal allograft recipients. *Transplant Proc* **15**: 2754–2756, 1983.
9. Klintmalm GBG, Iwatsuki S and Starzl TE, Cyclosporin A hepatotoxicity in 66 renal allograft recipients. *Transplantation* **32**: 488–489, 1981.
10. Schade RR, Guglielmi A, Van Thiel DH, Thompson ME, Warty V, Griffith B, Sanghvi A, Bahnson H and Hardesty R, Cholestasis in heart transplant recipients treated with cyclosporine. *Transplant Proc* **15**: 2757–2760, 1983.
11. Welz A, Reichart B, Uberfuhr P, Kemkes B and Klinner W, Cyclosporine as the main immunosuppressant in clinical heart transplantation: correlation of hepatotoxicity and nephrotoxocity. *Transplant Proc* **16**: 1212–1213, 1984.
12. Gulbis B, Adler M, Ooms HA, Desmet JM, Leclerc JL and Primo G, Liver-function studies in heart-transplant recipients treated with cyclosporin A. *Clin Chem* **34**: 1772–1774, 1988.
13. Ellis CN, Gorsulowsky DC, Hamilton TA, Billings JK, Brown MD, Headington JT, Cooper KD, Baadsgaard O, Duell EA, Annesley TM, Turcotte JG and Voorhees JJ, Cyclosporine improves psoriasis in a double-blind study. *JAMA* **256**: 3110–3116, 1986.
14. Kassianides C, Nussenblatt R, Palestine AG, Mellow SD and Hoofnagle JH, Liver injury from cyclosporine A. *Dig Dis Sci* **35**: 693–697, 1990.
15. Azer SA and Stacey NH, Effect of cyclosporin A *in vivo* on taurocholate uptake by rat hepatocytes. *Biochem Pharmacol* **42**: 2053–2057, 1991.
16. Stone BG, Udani M, Sanghvi A, Warty V, Plocki K, Bendetti CD and Van Thiel DH, Cyclosporin A induced cholestasis: the mechanism in a rat model. *Gastroenterology* **93**: 344–351, 1987.
17. Le Thai B, Dumont M, Michel A, Erlinger S and Houssin D, Cholestatic effect of cyclosporine in the rat. An inhibition of bile acid secretion. *Transplantation* **46**: 510–512, 1988.
18. Farthing MJG, Clark ML, Pendry A, Sloane J and Alexander P, Nature of the toxicity of cyclosporin A in the rat. *Biochem Pharmacol* **30**: 3311–3316, 1981.
19. Cohen DJ, Loertscher R, Rubin MF, Tilney NL, Carpenter CB and Strom TB, Cyclosporine: A new immuno-suppressive agent for organ transplantation. *Ann Intern Med* **101**: 667–682, 1984.
20. Mason J, Pharmacology of cyclosporine (Sandimmune) VII. Pathophysiology and toxicology of cyclosporine in humans and animals. *Pharmacol Rev* **42**: 423–434, 1989.
21. Rotolo FS, Branum GD, Bowers BA and Meyers WC, Effect of cyclosporine on bile secretion in rats. *Am J Surg* **151**: 35–40, 1986.
22. Stacey NH and Kotecka B, Inhibition of taurocholate and ouabain transport in isolated rat hepatocytes by cyclosporin A. *Gastroenterology* **95**: 780–786, 1988.
23. Kukongviriyapan V and Stacey NH, Inhibition of taurocholate transport by cyclosporin A in cultured rat hepatocytes. *J Pharmacol Exp Ther* **247**: 685–689, 1988.
24. Ziegler K and Frimmer M, Cyclosporin A and a diaziridine derivative inhibit the hepatocellular uptake of cholate, phalloidin and rifampicin. *Biochim Biophys Acta* **855**: 136–142, 1986.
25. Cadranel JF, Dumont M, Mesa VA, Degott C, Touchard D and Erlinger S, Effect of chronic administration of cyclosporin A on hepatic uptake and biliary secretion of Bromosulfophthalein in rat. *Dig Dis Sci* **36**: 221–224, 1991.
26. Nassuato G, Strazzabosco M, Muraca M, Passera D, Fragasso A, Orlando R, Iemmolo RM, Lirussi F and Okolicsanyi L, Effect of cyclosporin A on hepatic bilirubin transport. *J Hepatol* **7**: S63, 1988.
27. Galan AI, Zapata AJ, Roman ID, Murioz ME, Muriel C, Ganzalez J and Jimenez R, Impairment of maximal bilirubin secretion by cyclosporin A in the rat. *Arch Int Physiol Biochim* **99**: 373–376, 1991.
28. Centre SA, Baldwin BH, Erb HN and Tennant BC, Bile acid concentrations in the diagnosis of hepatobiliary disease in the dog. *J Am Vet Med Assoc* **187**: 935–940, 1985.
29. Elliott WH, Metabolism of bile acids in liver and extrahepatic tissues. In: *Sterols and Bile Acids* (Eds. Danielsson H and Sjovall J), pp. 303–329. Elsevier, New York, 1985.
30. Meuldermans W, Hurkmans R, Swysen E, Hendrickx J, Thijssen J, Lauwers W and Heykants J, Excretion and metabolism of lorcainide in rats, dogs and man. *Eur J Drug Metab Pharmacokinet* **8**: 335–349, 1983.
31. Klaassen CD and Stacey NH, Use of isolated hepatocytes in toxicity assessment. In: *Toxicology of the Liver* (Eds. Plaa G and Hewitt WR), pp. 147–179. Raven Press, New York, 1982.
32. Green CE, Le Valley SE and Tyson CA, Comparison of amphetamine metabolism using isolated hepatocytes from five species including human. *J Pharmacol Exp Ther* **237**: 931–936, 1986.
33. Fabre G, Combalbert J, Berger Y and Cano J-P, Human hepatocytes as a key *in vitro* model to improve preclinical drug development. *Eur J Drug Metab Pharmacokinet* **15**: 165–171, 1990.
34. Timbrell JA, *Principles of Biochemical Toxicology*. Taylor and Francis, London, 1991.
35. Bitter-Suermann H, Yu W, Coddington D and MaDonald AS, Is insulin a useful component of UW solution in liver transplantation? *Clin Transplantation* **4**: 247, 1991.
36. Princes HMG, Meijer P and Hofstee B, Dexamethasone regulates bile acid synthesis in monolayer cultures of rat hepatocytes by induction of cholesterol 7α-hydroxylase. *Biochem J* **262**: 341–348, 1989.
37. Berry MN and Friend DS, High yield preparation of isolated rat liver parenchymal cells; a biochemical and fine structural study. *J Cell Biol* **43**: 506–520, 1969.
38. Berry MN, Edwards AM and Barritt GJ, *Isolated*

Hepatocytes Preparation, Properties and Applications. Elsevier, Amsterdam, 1991.
39. Eaton DL and Klaassen CD, Carrier-mediated transport of ouabain in isolated hypatocytes. *J Pharmacol Exp Ther* **205**: 480–488, 1978.
40. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
41. Moseley RH, Johnson, TR and Morrissette JM, Inhibition of bile acid transport by cylosporine A in rat liver plasma membrane vesicles. *J Pharmacol Exp Ther* **253**: 974–980, 1990.
42. Roman ID, Monte MJ, Gonzalez-Buitrago JM, Esteller A and Jimenez R, Inhibition of hepatocytary vesicular transport by cyclosporin A in the rat: relationship with cholestasis and hyperbilirubinemia. *Hepatology* **12**: 83–91, 1990.
43. Thomson AW, Whiting PH, Cameron ID, Lessels SE and Simpson JG, A toxicological study in rats receiving immunotherapeutic doses of cyclosporin A. *Transplantation* **31**: 121–124, 1981.
44. Hsu IC, Lipsky MM, Cole KE, Su CH and Trump BF, Isolation and culture of hepatocytes from human liver of immediate autopsy. *In Vitro Cell Dev Biol* **21**: 154–160, 1985.
45. Maekuba H, Ozaki S, Mitmaker OB and Kalant N, Preparation of human hepatocytes for primary culture. *In Vitro Cell Dev Biol* **18**: 483–491, 1982.
46. Kukongviriyapan V, Kotecka B and Stacey NH, Substrate transport and cyclosporin A in isolated rat hepatocytes. *Biochem Pharmacol* **42**: 193–195, 1991.
47. Whitington PF, Dudeja P, Hecht JR, Whitington SH and Brasitus TA, Lipid alterations in the hepatocyte basolateral sinusoidal membrane (BLM) domain may explain cyclosporine (CsA) induced cholestasis in the rat. *Hepatology* (abstract) **8**: 1363, 1988.
48. Baur H and Heldt HW, Transport of hexose across the liver-cell membrane. *Eur J Biochem* **74**: 397–403, 1977.