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Effect of bile on cyclosporin absorption in liver transplant patients

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1 We quantitated the effect of biliary diversion on cyclosporin (CyA) absorption in liver transplant patients. Multiple blood samples were obtained over one dosing interval following oral CyA administration in eight liver transplant patients before and after T-tube clamping.

2 Cyclosporin concentrations were measured by a high pressure liquid chromatographic method.

3 The mean (± s.d.) dose-normalized area under the blood concentration *vs* time curve (AUC) over a dosing interval was 5.23 (± 3.22) ng ml^{-1} h during the pre clamping period and 15.79 ± 7.92 ng ml^{-1} h during the post clamping period. A significant ($P < 0.05$) increase (276%) in the dose normalized AUC was observed following the T-tube clamping.

4 Analysis of bile revealed less than 1 mg (< 1% of dose) of unchanged CyA to be eliminated in bile over 12 h. Therefore the increased CyA AUC/dose following T-tube ligation cannot be explained by enterohepatic recirculation.

5 Increased bile flow secondary to clamping of the T-tube most likely increased the absorption of CyA. Increase in CyA absorption may be due to a bile mediated increase in CyA solubility or gastrointestinal membrane permeability, or increased residence time at the site of absorption.

6 Independent of the mechanism involved, our results indicate that adjustments in CyA dosage must be made whenever external bile diversion is instituted or discontinued.

Keywords cyclosporin absorption T-tube ligation bile

Introduction

Orthotopic liver transplantation has become a life saving procedure for patients with liver diseases such as biliary atresia, sclerosing cholangitis, alcoholic cirrhosis, primary biliary cirrhosis and certain metabolic disorders such as α_1-antitrypsin deficiency (Starzl *et al.*, 1982). The success of this procedure is partially due to the use of cyclosporin (CyA) in combination with low dose steroids to prevent the rejection of the transplanted liver. Cyclosporin is a highly lipophilic polypeptide of fungal origin. The absorption of orally administered CyA is often poor following liver transplantation (Burckart *et al.*, 1986; Venkataramanan *et al.*, 1985a). In order to optimize the postoperative care of the transplant patients all the factors affecting CyA absorption must be completely characterized.

Liver transplant patients often have a T-tube inserted in their common bile duct during surgery. Recently, Andrews *et al.* (1985) reported a significant increase in the trough CyA blood levels in liver transplant patients following T-tube clamping. Improved enterohepatic recirculation of CyA could not account for such an observation

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since only a very small amount of CyA is excreted unchanged in the bile (Venkataramanan *et al.*, 1985c). Since CyA is highly lipophilic, its absorption may be dependent on the availability of bile salts that are removed by biliary diversion. Clamping of the T-tube increases the bile flow into the gut and therefore may increase CyA absorption. In the present study we have quantitated the effect of external bile drainage on CyA absorption in liver transplant patients.

Methods

Eight adult orthotopic liver transplant recipients (two males and six females) with external bile drainage participated in the study. Written informed consent was obtained from each patient prior to the study. Routine biochemical parameters and medication information were obtained from the patients' medical records. Each patient was studied on two separate occasions. The first phase was conducted while the patient's bile was being drained through the T-tube. In this phase of the study, patients were studied between days 16 and 30 after the transplant surgery. The second phase was conducted after clamping the T-tube. All but one study was conducted between days 1 and 6 after T-tube clamping. Patient 6 was studied 68 days after T-tube clamping. Patients received oral CyA every 12 h in doses ranging from 500 to 1800 mg day^{-1} as part of their postoperative care. On both study days blood samples were collected in heparinized tubes prior to (0 h) and at 1, 2, 3, 4, 6, 8, 10, and 12 h following oral CyA (Sandimmune® oral solution, Sandoz) administration. Total bile output through the T-tube was also collected over the 12 h period during the first phase. Bile samples were centrifuged in an Eppendorf centrifuge (model 5412 Brinkman, Westbury, NY) for 5 min at 1500 *g* to separate any particulate matter and the clear supernatant was diluted 10 fold prior to analysis. Bile and whole blood samples were analysed for unchanged CyA by a high pressure liquid chromatographic (h.p.l.c.) method (Ptachcinski *et al.*, 1985a, b). The coefficient of variation (CV%) of the assay was 6% for blood and 4% for bile.

The disposition rate constant (λ_z) was estimated from the terminal linear segment of log CyA blood concentration *vs* time profile. The area under the CyA blood concentration *vs* time curve during the dosing interval (AUC(0–12)) was calculated by trapezoidal method. The area under CyA blood concentration *vs* time curve from time 0 to infinity (AUC(0–12)) was calculated for the study dose using the principle of reverse superposition (Bauer & Gibaldi, 1983). Dose normalized AUCs obtained from the two phases of the study were compared using a paired *t*-test at an α of 0.05.

Table 1 Biochemical parameters in patients before and after clamping of the T-tube

Patient	*BUN* ($mg\ dl^{-1}$)	*SCR* (*mg%*)	*HCT*	*Bili (T)* ($mg\ dl^{-1}$)	*Bili (D)* ($mg\ dl^{-1}$)	*SGOT* ($iu\ l^{-1}$)	*SGPT* ($iu\ l^{-1}$)
1 Pre	50	1.3	32.4	3.5	2.1	30	53
Post	71	1.1	30.9	1.2	0.6	17	20
2 Pre	62	2.0	34.4	4.4	2.9	33	101
Post	27	1.4	26.3	1.6	0.9	27	49
3 Pre	56	2.1	31.8	2.0	1.2	58	174
Post	73	1.9	33.2	1.0	0.5	17	53
4 Pre	67	2.4	33.3	5.3	3.3	24	68
Post	47	1.4	28.7	3.0	1.7	24	49
5 Pre	77	1.4	34.3	2.7	1.3	22	48
Post	20	1.6	27.8	0.4	0.1	22	25
6 Pre	79	2.2	30.1	1.7	1.1	44	161
Post	60	2.0	29.9	1.5	0.9	48	153
7 Pre	57	2.2	25.3	2.0	1.7	31	57
Post	54	1.7	26.9	2.5	1.5	18	62
8 Pre	44	1.4	32.2	3.0	1.7	17	17
Post	85	1.8	30.4	2.1	1.2	10	12

BUN blood urea nitrogen
HCT haematocrit
Bili (T) total bilirubin
Bili (D) direct bilirubin

Results

The biochemical parameters of the patients before and after T-tube clamping are presented in Table 1. There were no significant differences in transaminases (SGOT AST; SGPT ALT), blood urea nitrogen (BUN), serum creatinine, haematocrit and bilirubin direct (Bili D) concentrations in the patients studied during the two phases. Drug therapy in patients included CyA, prednisone, mycostatin, and hydralazine during both study periods. In addition some of the patients received frusemide, ranitidine, spironolactone, propranolol, polymyxin, neomycin or murine monoclonal antibody or OKT3 (orthoclone, Ortho Labs, NJ).

Figure 1 shows the CyA blood concentration *vs* time profile in patient 4. The pre-clamping and post-clamping doses were the same in this patient. The blood concentration profile during phase 1 demonstrated only small differences in the minimum and the maximum blood concentrations. Following T-tube clamping, the CyA blood concentration increased significantly during the dosing interval with well defined maximum and minimum blood concentrations. In 63% of the studies, the CyA concentration at time 0 and at 12 h differed by less than 20% indicating the attainment of steady state. The mean (± s.d.) disposition half-life of CyA during the phase 1 was 6.6 h (± 2.8) and was not significantly different from the half-life (5.2 ± 2.2 h) obtained during phase 2. Table 2 lists CyA dose, AUC(0–∞) and AUC(0–12) per mg of the dose in these patients during the two study phases.

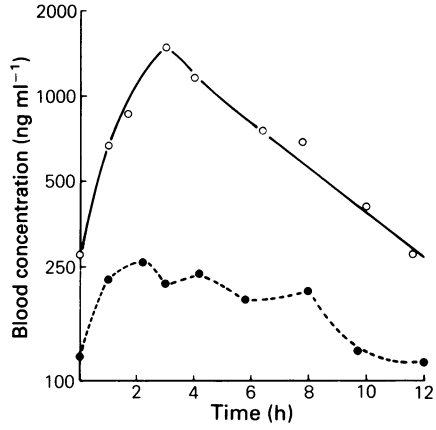

Figure 1 Cyclosporin blood concentration *vs* time profile in patient 4 receiving the same dose during the T-tube open (●) and T-tube closed (○) periods.

The mean (± s.d.) AUC(0–12) per mg dose during phase 1 was 5.23 ng ml^{-1}. This was significantly lower ($P < 0.50$) than the value of 15.79 ng ml^{-1} estimated during phase 2. However, the extent of increase in the AUC varied widely between patients.

Table 3 shows CyA concentrations in the bile obtained during the first phase. Bile could not be obtained from patient 6. Compared with blood, higher concentrations of CyA were observed in the bile samples. However, since the total bile output was small (105 to 670 ml), the amount of CyA excreted unchanged in the bile was less than 1% of the administered dose in each patient.

Table 2 Effect of T-tube clamping on cyclosporin absorption

	Pre clamping period			*Post clamping period*				
Patient	*Dose (mg)*	*AUC/mg dose (ng ml^{-1} h)*		*Dose (mg)*	*AUC/mg dose (ng ml^{-1} h)*		*% Increase in AUC*	
		A	*B*		*A*	*B*	*A*	*B*
1	600	11.16	11.58	300	26.51	23.63	138	104
2	800	2.63	3.02	900	10.03	10.08	281	234
3	600	4.61	2.65	300	26.74	27.21	480	927
4	350	2.58	2.58	350	13.22	7.48	412	190
5	600	7.56	7.56	250	17.42	17.42	130	130
6	600	5.91	3.60	600	6.30	4.89	7	36
7	800	1.17	0.64	800	7.73	10.63	561	1561
8	700	6.24	6.24	600	18.40	18.40	195	195
Mean		5.23	4.73		15.79[a]	14.97[a]	276	422
±		±	±		±	±	±	±
s.d.		3.22	3.53		7.92	7.96	193	538

A = based on $(AUC_0^{12})_{ss}$
B = based on $(AUC)_0^{\infty}$

[a] Significantly different from preclamping period $P < 0.005$.

Table 3 Biliary excretion of cyclosporin

Patient	*Bile volume (ml)*	*CyA bile concentration (μg ml^{-1})*	*Amount of CyA in bile (μg)*	*% Dose administered*
1	145	4.63	671.4	0.11
2	219	0.75	164.3	0.02
3	105	1.25	131.3	0.02
4	670	0.07	46.9	0.01
5	243	1.79	435.0	0.07
7	433	0.38	164.5	0.02

Discussion

Cyclosporin inhibits the immune system selectively. The selective immunosuppression makes it a useful drug for preventing the rejection of liver (Starzl *et al.*, 1981a), kidney (Starzl *et al.*, 1981b), heart (Griffith *et al.*, 1982), and bone marrow (Tutschka *et al.*, 1983) in transplant patients. The oral absorption of CyA in transplant patients is erratic and incomplete as indicated by its poor bioavailability ranging from 5 to 60% with a mean of 30% (Ptachcinski *et al.*, 1986). In order to optimize CyA dosing it is essential to characterize factors affecting its absorption.

Recently, Andrews *et al.* (1985) reported that clamping or removal of the T-tube leads to significantly higher CyA trough levels in liver transplant patients. Similar observations have been made with digoxin and vitamin B_{12} (Carruthers & Dujovne, 1978; Teo *et al.*, 1980). Blood concentrations of drugs which are extensively excreted unchanged in the bile will increase following T-tube clamping due to increased enterohepatic recycling of the drug. Previous studies in our laboratory (Venkataramanan *et al.*, 1985c) and the results of the present study indicate that very little CyA is excreted unchanged in the bile. Furthermore there is no evidence to suggest the presence of a conjugate of CyA in the bile that could be reconverted to CyA and subjected to enterohepatic recirculation (Maurer *et al.*, 1984). Therefore, enterohepatic recirculation of CyA does not contribute significantly to the increase in CyA trough concentration following T-tube clamping.

Bile salts have also been shown to increase gastrointestinal absorption of lipophilic drugs by enhancing their dissolution, by alteration of the gastrointestinal membrane permeability or by increasing the residence time of the drug at the absorption site. *In vitro* studies indicate increased solubility of CyA in various bile salts (unpublished observation). Bile salts significantly improve the absorption of vitamins A (Bernhard *et al.*, 1952), K (Greaves, 1939), and D (Greaves & Schmidt, 1933) in rats, vitamin D in dogs (Taylor *et al.*, 1935) and phenolphthalein in cats (Pekanmaki & Salmi, 1961). In man the absorption of fat soluble vitamins (A, D, E, and K) is significantly impaired in the absence of bile (Forsgren, 1969). Diversion of the bile into the gut significantly increased the absorption of vitamin B_{12} (Teo *et al.*, 1980) and tetracycline (Aukee *et al.*, 1975) in patients with a T-tube in their common bile duct. Administration of bile salts results in a 50–80% increase in the urinary recovery of riboflavin in man, indicating improved absorption (Mayersohn *et al.*, 1969). Increased bile flow following T-tube clamping could have increased the absorption of CyA.

Venkataramanan *et al.* (1985b) have observed a significant negative correlation between bilirubin level and CyA bioavailability. Significant impairment in CyA absorption was observed only in patients with a total bilirubin of greater than 10 mg dl^{-1}. In this study, the highest bili T value recorded was 5.3 mg dl^{-1} in patient 4 during phase 1. There was a poor correlation ($r = -0.05$) between the bilirubin levels and the CyA AUC values in this study. Therefore, the differing bilirubin values in some of our patients is not expected to account for the differences in the dose normalized AUCs observed between the two phases.

Recently, Ptachcinski *et al.* (1985) have reported increased CyA absorption in some kidney transplant patients when lower divided doses were administered as compared with a single large dose. In the present study three of the patients received the same dose during both phases. One patient received a higher dose during the second phase but still showed a significant increase in the AUC value after T-tube clamping. Four patients received a lower dose during the post clamping period. Consequently, dose dependent absorption could partially contribute to

the observed increase in the AUC in these four patients.

Cyclosporin is distributed in the red blood cell in a concentration dependent manner. Robinson *et al.* (1983) have attributed the concentration dependent distribution of CyA in the red blood cell to be responsible for the nonlinear relationship between dose and AUC of blood concentration *vs* time curve. Concentration dependent distribution of CyA between blood cells and plasma is seen only above 1000 ng ml^{-1} (Niederberger *et al.*, 1983). In this study less than 1000 ng ml^{-1} of CyA was present in most (94%) of the blood samples. Moreover, concentration dependent CyA distribution would only result in an underestimation of the effect of bile on CyA absorption since the concentrations were higher during the phase when bile was emptied completely into the gut.

We also evaluated any possible effect of co-administered drugs on the observed results by comparing the patient medication during both phases of the study. Four patients received identical medication during both phases of the study which ruled out drug interaction as a cause of an increased AUC. Two patients received OKT_3 during only one phase of the study. No information exists to suggest that OKT_3 will alter CyA metabolism. Drugs such as frusemide and prednisone which are shown to interact with CyA were given on a chronic basis to these patients before and during the study period and hence are not expected to affect the outcome of this study.

From the above discussion it is apparent that pharmacokinetic factors such as distribution, elimination, drug interaction and physiological factors such as hepatic and renal function did not contribute to the observed increase in the dose normalized CyA AUC following T-tube clamping. Enterohepatic circulation of CyA does not play a role in bile-mediated increase in CyA absorption. Bile increases CyA solubility significantly but this may be only one of the mechanisms by which bile increases CyA absorption. Co-administration of bile salts and CyA may prove to be beneficial in patients with poor CyA absorption or in patients with external biliary drainage. Studies are currently underway to determine the effect of chenodeoxycholic acid on CyA absorption.

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