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Biliary Excretion of Cyclosporine in Liver Transplant Patients

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Cyclosporine (CyA) is a novel cyclic polypeptide with potent immunosuppressive properties.[1,2] CyA is presently used with steroids to prevent the rejection of transplanted kidneys,[3] livers,[4] and hearts.[5,6] This drug is primarily eliminated from the body by hepatic metabolism, and biliary excretion is the major route of CyA elimination in animals.[7] In humans, enterohepatic recycling of CyA has been suggested because of the presence of a second peak in CyT (CyA plus metabolites measured by radioimmunoassay [RIA]) plasma concentrations and the appearance of the drug in the ileostomy drainage of one patient.[8] We have observed increases in CyA blood levels (parent compound measured by high-pressure liquid chromatography [HPLC]) following T-tube clamping in liver transplant patients, which is also indicative of possible enterohepatic recycling of CyA. In order for CyA to undergo any significant enterohepatic recirculation, large amounts of CyA must be excreted in the bile. In the present study, we quantitated the biliary excretion of CyA and CyT in adult liver transplant patients in order to determine whether CyA undergoes significant enterohepatic recycling.

MATERIALS AND METHODS

Eighteen biliary excretion studies were performed in 13 patients (12 liver transplant recipients and one liver disease patient) with a T-tube in their common bile ducts. CyA was administered either as an intravenous (IV) infusion (130 to 150 mg; n = 7), as a combination of IV infusion and oral dose (70 to 150 mg IV, 300 to 700 mg orally; n = 3), or as an oral dose (400 to 600 mg; n = 8). Prior to drug administration, the T-tube was drained and the total bile output over the study period of eight to 24 hours was collected at one- to four-hour intervals. The total bile volume in each collection was measured. Aliquots of the bile samples were kept frozen at −20 °C until being analyzed for CyA and CyT. HPLC was used to analyze CyA in samples from all of the patients. Samples from four patients were also analyzed for CyT by RIA.

Bile samples (0.1 to 1.0 mL) were extracted and assayed following minor modification of the HPLC procedure of Sawchuk and Cartier.[9] RIA was performed using a kit provided by Sandoz, Inc (East Hanover, NJ) according to a procedure described by Donatsch et al, [10] following 1:20 and 1:50 dilution of the bile samples. Standards were prepared for both methods in blank bile.

Biliary excretion studies were performed in patients with varying degrees of liver function, as indicated by total serum bilirubin concentrations. Patients with a total bilirubin concentration of 1 mg/dL or less were considered to have normal liver function. Patients with a total serum bilirubin concentration of greater than 1 mg/dL were considered to have

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poor liver function. Three studies were performed in patients with normal liver function, and the remaining studies were carried out in patients whose liver function was poor. Bile output, bile CyA and CyT concentrations, and the total amounts of CyA and CyT excreted in bile was compared between patient groups with normal and poor liver function, using a Student's *t* test, assuming a level of significance of $P \leq .05$.

RESULTS

Analysis of blank bile by HPLC confirmed the absence of any interfering materials in the bile. The standard curve for HPLC analysis of CyA in bile was run from 25 to 4,000 ng/mL. The coefficient of variation (n = 9) at 887 ng/mL was 6.5%. For the RIA analysis of CyT, the standard curve was linear from 78 to 2,500 ng/mL. The coefficient of variation (n = 6) at 611 ng/mL was 17.8%. The bile samples were diluted to ensure that the results of the analysis were in the linear range of the standard curve for both HPLC and RIA.

The total volume of bile collected over one dosing interval ranged from 13.5 to 417.5 ml (Table 1). Patients with normal liver function had higher bile output than patients with poor liver function (24.1 mL/h *v* 7.6 mL/h; $P < .05$).

The maximum bile concentration of CyA ranged from 32 ng/mL in a patient with severe liver disease to 5,212 ng/mL in a liver transplant recipient with normal hepatic function. The total amount of CyA excreted in the bile over a dosing interval ranged from 0.003 to 0.814 mg. There was a positive linear correlation between the bile output and the total amount of cyclosporine excreted in the bile ($r = .686$; $P < .01$). The amount of cyclosporine excreted in the bile in patients with normal liver function was greater than that excreted in patients whose liver function was poor at the time of the study (789 μg *v* 98 μg; $P < .01$). The patient with severe liver disease who was studied pretransplant had the lowest maximum bile concentration of CyA and excreted the smallest amount of CyA (3.7 μg) in the bile. A negative linear correlation was observed between serum bilirubin and the total amount of CyA excreted in the bile ($r = -.597$; $P < .01$). The total amount of CyA excreted in the bile over one dosing interval represents less than 1% of the administered dose in all of the patients studied (Table 2).

The concentration of CyT in bile ranged from 9,280 to 61,300 ng/mL, as measured by RIA. The maximum bile CyT concentration measured by RIA was 18 to 36 times higher than the CyA concentration by HPLC measurements. The total amount of CyT excreted in the bile when measured by RIA ranged from 0.980 to 6.900 mg, with a mean of 3.018 mg. The ratio of the total amount of CyT to CyA excreted in bile ranged from 23:1 to 39:1, with a mean of 30:1.

DISCUSSION

Cyclosporine and steroids are the primary immunosuppressants used in heart, liver, and kidney transplant patients. Very little information is available on CyA kinetics in transplant recipients. Urinary excretion of CyA and CyT is a minor elimination pathway in dogs, rats, and humans. In dogs, most of the orally administered radioactive CyA is eliminated in the feces.[7] More than 50% of the intravenously administered radioactive CyA is excreted in rat bile.[7] In humans, indirect evidence for the involvement of bile in CyT elimination has been obtained from the analysis of ileostomy drainage.[8] This drainage accounted for 61.5% (measured as CyT) of the orally administered dose of CyA in one patient. As the drug was administered orally, unabsorbed drug may account partially for this observation. Moreover, RIA was used to analyze the ileostomy drainage. At present, no data on the amount of intact drug excreted in the bile are available.

In our study, we obtained a direct estimate of the biliary excretion of CyA and CyT in adult liver transplant patients with a T-tube. The concentration of CyA and CyT in bile was greater than the concentrations in blood. However, less than 1% of the administered dose of CyA was excreted in the bile during one dosing interval. The amount of CyA excreted in bile was highly variable and was partially dependent on the funtional status of the liver as well as the volume of bile. Patients with poor liver function had a low bile output and a low concentration of drug in their bile. Patients with normal liver function had the highest bile output and higher drug concentrations in their bile. One patient with liver disease who was studied pretransplant excreted the least amount of CyA in his bile. This may be due to a combination of poor absorption (bioavailability was 3%) and poor excretory capacity of the diseased liver in this patient.

There was no significant difference in the percentage of the dose excreted in the bile following oral and IV administration of CyA in patients with poor liver function. Increases in the blood CyA and CyT concentrations following T-tube clamping could be rationalized on the basis of a possible increase in the absorption of CyA due to increased amounts of bile being available to facilitate the absorption of fat-soluble cyclosporine.

Analysis of bile by RIA revealed the presence of large amounts of CyT. The CyT:CyA concentration ratio of 18–36:1 in the bile as compared to a ratio of 2–5: 1 in blood indicates that relatively greater amounts of CyA metabolites are excreted in the bile. The amount of CyT excreted in the bile as determined by RIA is 30 times greater than that measured by HPLC. RIA measurements of bile and blood are an underestimate of the total amount of CyA and its metabolites, as various CyA metabolites do not interact with the antibodies used in the RIA to the same extent that the intact parent CyA does.

In our patients with normal liver function, bile collection represented more than half of the estimated normal bile output. Using a normal bile output of 500 mL in a 12-hour dosing interval and a mean CyA bile concentration of 2.73 μg/mL (as observed in patients with normal liver function), one can estimate that approximately 1.37 mg of CyA is excreted in the bile. This represents 0.29% of the orally administered dose (mean oral dose in patients with normal liver function, 467 mg). CyA is incompletely absorbed following oral administration. Assuming a bioavailability of 20%, one can calculate the percentage of the dose absorbed that is excreted in the bile as 1.46. Therefore, a patient receiving 500 mg of oral CyA with a bioavailability of 20% would excrete approximately 1.46 mg of CyA in the bile. The amount of CyT excreted would be nearly 30 times greater and thus, a total of 43.8 mg of CyT will be excreted in the bile.

The low amount of parent CyA excreted in bile indicates that intact CyA is not likely to undergo any significant enterohepatic recycling. These results differ from an earlier report that suggested the possibility of significant enterohepatic recycling of CyA in humans. This discrepancy may be explained partially because of the use of RIA assay to measure CyA in plasma and ileostomy drainage. CyA metabolites may, however, undergo enterohepatic recycling and hence produce secondary plasma CyT peaks. Secondary blood peaks have also been observed in a few of our patients when CyA was measured by HPLC. From our present results, it appears that mechanisms other than enterohepatic recirculation may be responsible for such observations.

In conclusion, very little intact CyA is excreted in the bile of liver transplant recipients. In contrast, a large amount of CyT (CyA plus metabolites) is excreted in the bile. Intact CyA is not likely to undergo any significant enterohepatic recycling in patients following liver transplantation.

SUMMARY

The biliary excretion of CyA was studied in 13 patients with externalized bile flow. Analysis of bile by HPLC revealed that less than 1% of an administered dose of CyA is excreted in the bile. RIA analysis of bile revealed that large amounts of CyT are excreted in the bile. Enterohepatic recycling of CyA is unlikely in liver transplant patients, but CyA metabolites may be subject to this process.

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Table 1

Biliary Excretion of Cyclosporine

	Dose (mg)					Total Amount of Cyclosporine in Bile (mg)	
Study No.	IV	Oral	Total Bilirubin (mg/dL)	Duration of Bile Collection (h)	Total Bile Volume (mL)	HPLC (CyA)	RIA (CyT)
1	150	–	25.8	12	17.0	0.031	–
2	150	–	–	12	13.5	0.025	–
3	150		–	12	23.0	0.028	–
4	130		24.6	8	53.5	0.208	–
5	140		13.7	8	54.5	0.100	2.990
6	150		4.3	13	59.0	0.097	–
7	150	300	24.0	8	14.5	0.013	–
8	100	700	14.8	12	50.5	0.035	0.980
9	70	300	2.5	12	143.0	0.177	6.900
10		600	2.5	12	280.0	0.222	–
11		400	7.9	13	40.2	0.053	1.200
12		550	5.8	13	239.0	0.092	–
13		600	2.5	12	168.0	0.195	–
14		500	0.5	12	417.5	1.052	–
15		500	1.0	12	350.5	0.501	–
16		400	1.0	12	97.7	0.814	–
17*		730	18.4	24	129.0	0.003	–
18*	144	–	18.4	23	83.0	0.005	–

*Pretransplant, liver disease patient.

Table 2

Effect of Liver Function and Route of Administration on Cyclosporine Biliary Excretion

Status of Subjects	No. of Studies*	Route of CyA Administration	Bile Drainage		Amount of CyA Excreted (mg)	Dose Excreted in Bile as CyA (%)	Absorbed Dose Excreted in Bile as CyA (%)
			mL/Dosing Interval	mL/h			
Poor liver function	6	IV	37	3.75	0.082	0.059	0.059
Poor liver function	3	IV and PO	69	6.0	0.075	0.018	0.053†
Poor liver function	4	PO	182	14.7	0.141	0.025	0.124†
Normal liver function	3	PO	289	24.1	0.789	0.170	0.850†

All values reported are the means for each group. PO, per orally.

*Excluding the pretransplant studies.

†Based on an assumed oral bioavailability of 20%.