Effect of T-Tube Clamping on the Pharmacokinetics of Mycophenolic Acid in Liver Transplant Patients on Oral Therapy of Mycophenolate Mofetil

Ashokkumar B. Jain, Imad Hamad,* Sheila Zuckerman,* Shimin Zhang, Vijay S. Warty,† John J. Fung,* and Raman Venkataramanan†‡*

The aim of the study was to evaluate the effect of t-tube clamping on the pharmacokinetics of mycophenolic acid (MPA) after oral administration of mycophenolate mofetil (MMF) in primary liver transplant recipients treated with tacrolimus as the primary immunosuppressive drug. We evaluated the pharmacokinetics of MPA and its primary metabolite, mycophenolic acid glucuronide (MPAG), before and after clamping the t-tube in 8 primary liver transplant recipients treated with oral MMF and tacrolimus. The concentration of MPA and MPAG in plasma, bile, and urine samples obtained over one dosing interval was measured by high-pressure liquid chromatography. Pharmacokinetic parameters of MPA estimated before and after clamping the t-tube were compared to evaluate any significant differences at a *P* of .05 or less. There were no significant differences in the time to reach peak plasma concentration (1.8 ± 1.7 *v* 1.0 ± 0.5 hours), trough plasma concentration of MPA (1.1 ± 1.4 *v* 1.4 ± 1.1 μg/mL), peak plasma concentration of MPA (10.6 ± 7.5 *v* 11.1 ± 4.6 μg/mL), area under the plasma concentration-versus-time curve (AUC) (40.1 ± 31.9 *v* 43.2 ± 21.1 μg/mL/h) of MPA, or the percentage of MPA that is free or unbound in the plasma (3.9% ± 1.6% *v* 4.1% ± 3.0%). There was also no significant difference in the ratio of the AUC of MPAG to MPA. These observations suggest that t-tube clamping does not affect the kinetics of MPA or MPAG and that no dosing alterations of MMF are required when the t-tube is clamped in liver transplant recipients.

Mycophenolate mofetil (MMF) has recently been approved for use along with cyclosporine for the prevention of rejection in kidney transplant recipients. MMF appears to be well absorbed in healthy volunteers after oral administration.[1] It is rapidly converted to mycophenolic acid (MPA), which is the active moiety.[2] MPA is converted to a glucuronide conjugate, mycophenolic acid glucuronide (MPAG), which is present in high concentrations in plasma and is excreted in the urine and bile.[2,3] After surgery, liver transplant recipients often have a t-tube placed over the choledochocholedochostomy that drains the bile externally. When liver function is stable, the tube is clamped and the bile is allowed to return to the gut. T-tube clamping can increase the blood/plasma concentration of certain drugs, either because of increased availability of bile for dissolution of the drugs that are very lipid soluble and require bile for dissolution or because of improved enterohepatic recycling of the drugs.[4-6] The blood concentration of cyclosporine increases significantly after t-tube clamping because of the increased availability of bile in the gut, leading to improved solubility and absorption of cyclosporine from the conventional formulation.[5] Conversely, clamping the t-tube does not affect the blood concentration of tacrolimus.[7,8] It is not known whether clamping the t-tube has any effect on plasma concentrations of MPA after the oral administration of MMF. The present study was performed to evaluate the pharmacokinetics of MPA after oral administration of MMF in liver transplant recipients with open and clamped t-tubes to determine whether bile is essential for the absorption of MMF and to determine the significance of the enterohepatic recycling of MPA in these patients.

*From the *Thomas E. Starzl Transplantation Institute, School of Medicine, †Department of Pharmaceutical Sciences, School of Pharmacy, and the ‡Department of Pathology, School of Medicine, The University of Pittsburgh, Pittsburgh, PA.*

Address reprint requests to Raman Venkataramanan, PhD, FCP, 718 Salk Hall, University of Pittsburgh, Pittsburgh, PA 15261.

1074-3022/99/0502-0003$3.00/0

Materials and Methods

Adult primary liver transplant recipients who had a t-tube over the choledochocholedochostomy and who were on a regimen of tacrolimus, MMF, and low-dose steroids were recruited for this study. These patients were part of a protocol approved by the biomedical institutional review board to evaluate the safety and efficacy of the addition of MMF to patients on tacrolimus and low-dose steroid therapy. Prior informed consent was obtained from all patients who participated in the study to evaluate the kinetics of MPA. All patients received tacrolimus, steroid, and 1 g of MMF twice daily. Tacrolimus and MMF were administered at the same time in all these patients.

Patients were studied at least 6 days after the surgery to prevent any impact of gut motility on MMF absorption. Patients were studied during the daytime on two separate occasions, once when the t-tube was open and again when the t-tube was clamped for at least 24 hours. During both phases, a study nurse monitored the intake of MMF and obtained multiple blood samples from the patients just before the dose (0 hours) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, and 12 hours after administration of the morning dose of MMF. The exact time of collection of each of the samples was recorded. Plasma was obtained by centrifugation at room temperature and was frozen at −20°C until analysis of MPA and MPAG by high-pressure liquid chromatography (HPLC). Bile samples and urine samples were also collected over a dosing interval (12 hours) and analyzed for MPA and MPAG by HPLC. Plasma protein binding of MPA was evaluated by ultrafiltration, and HPLC analysis of the plasma samples obtained in each patient after the addition of 20 µg/mL of MPA. Details of the analytic procedures have been reported elsewhere.[9]

Whole-blood concentrations of tacrolimus in these patients were also measured by microparticulate enzyme immunoassay (MEIA-IMx; Abbott, Abbott Park, IL). A paired *t*-test was used to test for significance of any differences in various parameters measured or estimated at a P of .05 or less.

Results

The demographics of the patients enrolled onto this study are listed in Table 1. Five men and 3 women aged 21 to 67 years participated in this study. The patients were administered a dosage of 4 to 24 mg of tacrolimus per day before clamping and 2 to 30 mg of tacrolimus per day after clamping of the t-tube. Figures 1 and 2 show the plasma concentration-versus-time profile of MPA and MPAG in 2 patients, 1 with normal liver and kidney function (Fig. 1) and 1 with impaired kidney function (Fig. 2). The plasma concentrations of MPAG were greater in all patients at all time points compared with the plasma concentrations of MPA. Patients with impaired kidney function had significantly greater concentrations of MPAG compared with that of the patient with normal kidney function. Table 2 lists the pharmacokinetic parameters of MPA before and after t-tube clamping. There were no significant differences in the (mean ± standard deviation [SD]) predose plasma concentration (1.1 ± 1.4 *v* 1.4 ± 1.1 µg/mL), the maximum plasma concentration (10.6 ± 7.5 *v* 11.1 ± 4.6 µg/mL), the time to reach maximum plasma concentration (1.8 ± 1.7 hours *v* 1.0 ± 0.5 µg/mL), the area under the plasma concentration-versus-time curve (AUC) for MPA (40.1 ± 31.9 *v*

Table 1. Demographics of Patients

Patient No.	Sex	Age (yr)	Body Weight (kg)	Total Bilirubin (mg/dL)		Serum Creatinine (mg/dL)		Total Daily Tacrolimus Dose (mg)		Tacrolimus Blood Levels (ng/mL)		Day After Transplantation	
				Open	Closed*	Open	Closed	Open	Closed	Open	Closed	Open	Closed
1	F	60	131	1.3	0.5	4.2	3.4	8	2	14.7	7	6	20
2	F	31	53	1.8	1.7	0.9	1.0	10	14	10.8	9.9	11	12
3	M	66	56	1.6	1.1	0.7	0.7	16	16	9.5	14.5	12	14
4	M	21	66	2.4	0.8	0.8	0.8	24	30	9.9	13.6	18	31
5	M	53	81	1.3	0.5	2.1	1.7	4	4	9.4	—	28	43
6	F	67	68	2.3	1.6	0.7	0.7	18	22	5.6	18	8	9
7	M	53	66	0.4	0.3	3.3	1.9	6	20	7.3	12.7	11	18
8	M	42	84	1.4	0.9	4.3	3.9	8	6	<5.0	5.0	30	34

*Refers to the status of the t-tube.

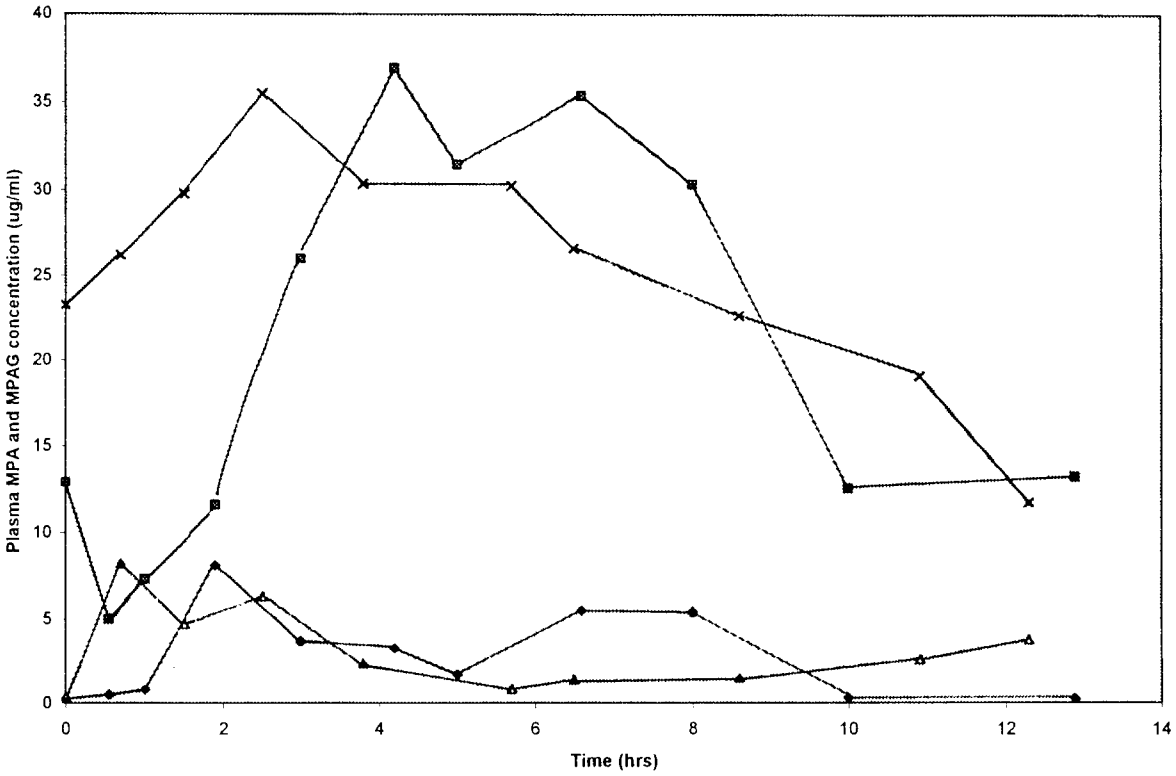

Figure 1. Plasma concentrations of MPA with open (◆) and closed (△) t-tubes. Plasma concentrations of MPAG with open (■) and closed (X) t-tubes in patient with normal liver and kidney function (patient no. 3).

43.2 ± 21.1 µg/mL/hr), and the percent of MPA in the plasma that is not bound to plasma proteins (percent unbound of MPA, 3.9% ± 1.6%; 4.1% ± 3.0%) with an open and closed t-tube, respectively.

Table 3 provides a summary of the AUC for MPA and MPAG and the ratio of MPAG to MPA before and after clamping of the t-tube. This ratio ranged from 6 to 249 before clamping to 9 to 280 after clamping. The mean ratios before (64 ± 84) and after (48 ± 94) clamping were not significantly different ($P = .334$). Higher ratios were observed in patients with increased serum creatinine values.

The mean ± SD volume of bile collected when the t-tube was open was 316 ± 67 mL (range, 226 to 425 mL). The mean concentration of MPA in the bile was 1.0 ± 0.4 µg/mL (range, 0.4 to 1.5 µg/mL). The mean ± SD amount of MPA excreted in the bile was 0.34 ± 0.19 mg (range, 0.1 to 0.7 mg). The mean ± SD amount of MPAG in bile expressed in terms of the MPA equivalent was 196 ± 200 mg. This indicates that less than 1% of the dose of MMF was excreted in the bile as MPA and nearly 29% of the dose was excreted as MPAG in the bile.

Discussion

We measured the plasma concentration of MPA and MPAG in liver transplant recipients over one dosing interval with open and closed t-tubes. The AUC of MPA in the liver transplant recipients over a dosing interval was generally greater than that published regarding kidney transplant recipients on cyclosporine therapy[2] or in healthy volunteers.[3] Consistent with the published information, plasma MPAG concentrations and the AUC of MPAG were greater than that of MPA.[1-3] The plasma MPAG AUC to MPA AUC was greater in patients with impaired renal function in comparison to patients with near-normal renal function, as measured by serum creatinine level. Similar observations have been reported in renal transplant recipients.[10] The mild impairment in liver function observed in 2 patients during the study (determined by serum bilirubin level >2 mg/dL) is not likely to have

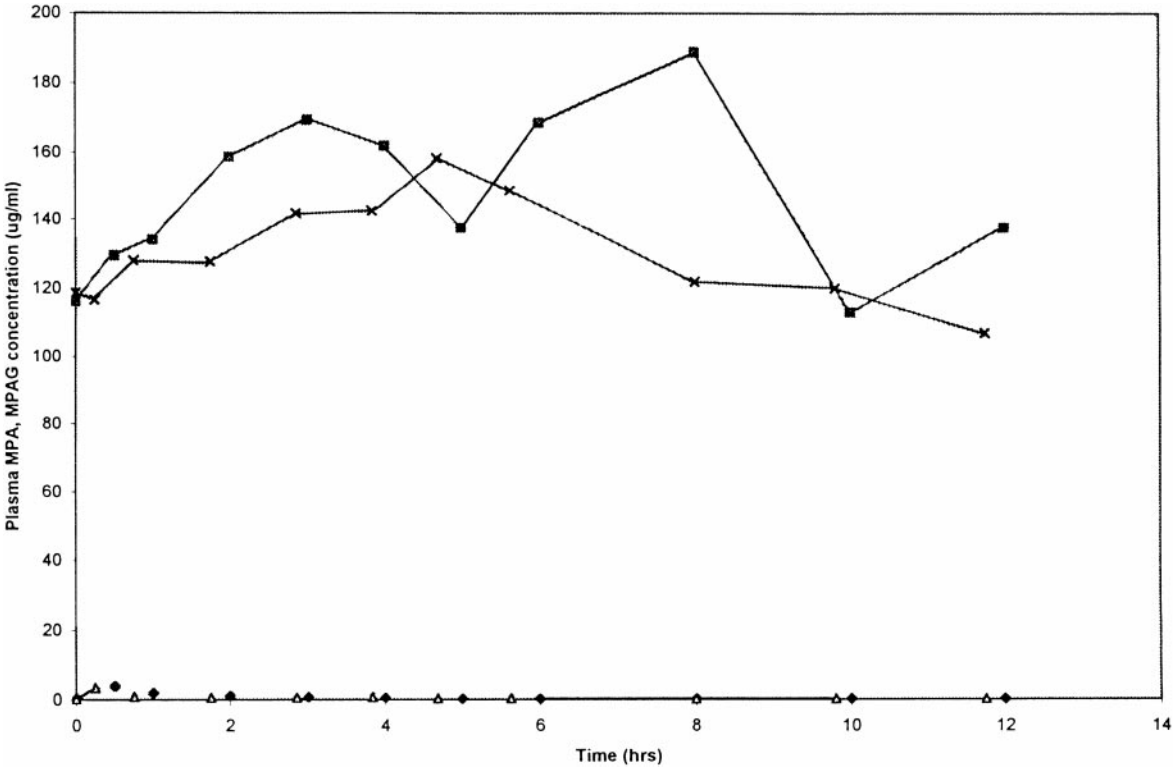

Figure 2. Plasma concentrations of MPA with open (◆) and closed (△) t-tubes. Plasma concentrations of MPAG with open (■) and closed (X) t-tubes in patient with normal liver but impaired kidney function (patient no. 8).

altered MPA kinetics, as reported in the literature.[3]

MPA is highly bound to plasma proteins.[11] Consistent with this report, the free fraction of MPA in the study patients ranged from 1.5 to 9.5, indicating that 90.5% to 98.5% of MPA is bound to plasma proteins. There were no significant differences in the free fraction of MPA with open or

Table 2. Pharmacokinetic Parameters of MPA

Patient No.	t_{peak} (hr)		C_{min} (µg/mL)		C_{max} (µg/mL)		AUC (µg/mL/h)		Free Fraction (%)	
	Open	Closed*	Open	Closed	Open	Closed	Open	Closed	Open	Closed
1	3.0	0.6	0.6	0.6	6.0	12.6	24.5	44.4	2.9	4.3
2	1.0	1.0	0.6	3.0	21.5	7.7	46.6	40.3	2.0	1.5
3	1.9	0.7	<0.3	0.4	8.1	8.2	37.3	35.7	5.2	9.5
4	0.5	1.0	4.4	0.9	19.7	16.4	102.3	56.4	2.2	1.7
5	2.0	1.0	0.3	2.6	4.2	17.2	12.7	81.0	5.2	3.2
6	0.5	2.0	0.3	1.6	17.1	10.5	62.6	37.4	2.8	3.0
7	5.2	1.0	1.7	2.1	4.3	12.5	27.1	45.0	4.4	1.7
8	0.5	0.5	0.4	0.3	3.9	3.4	7.3	5.5	6.3	7.9
Mean	1.8	1.0	1.1	1.4	10.6	11.1	40.1	43.2	3.9	4.1
SD	1.7	0.5	1.4	1.1	7.5	4.6	31.9	21.1	1.6	3.0
P	.172		.570		.884		.814		.782	

Abbreviations: t_{peak}, time to reach maximum plasma concentration; C_{min}, predose plasma concentration; C_{max}, maximum plasma concentration.
*Refers to the status of the t-tube.

Table 3. Pharmacokinetics of MPA and MPAG

Patient No.	MPA AUC		MPAG AUC		MPAG/MPA Ratio	
	Open	Closed*	Open	Closed	Open	Closed
1	24.5	44.4	1580	680	64.5	15.3
2	46.6	40.3	443	568	9.5	14.1
3	37.3	35.7	283	317	7.6	8.9
4	102.3	56.4	610	574	6.0	10.2
5	12.7	81.0	1443	823	114	10.2
6	62.6	37.4	1102	1055	17.6	28.2
7	27.1	45.0	1107	733	41.2	16.3
8	7.3	5.5	1816	1540	249	280
Mean	40.1	43.2	1048	786	64	48
SD	31.9	21.1	558	372	84	94

*Refers to the status of the t-tube.

closed t-tubes. This suggests that the distribution of MPA is not likely to be different during the two-study period.

In patients with a t-tube in the bile duct, clamping of the t-tube can increase the plasma concentration of certain drugs, either because of increased availability of bile for dissolution of the drugs that are very lipid soluble and require bile for dissolution or because of improved enterohepatic recycling of the drugs.[4-6] The increased concentration of digoxin after t-tube clamping has been ascribed to improved enterohepatic recycling of digoxin.[6] The plasma concentration-versus-time profile for MPA shows secondary peaks between 6 and 12 hours after MMF dosing.[2] This observation, along with the facts that MPA is primarily converted to a glucuronide conjugate that is significantly excreted in the bile and that the plasma MPA AUC decreases by 37% when cholestyramine is administered to patients,[2] suggests that MPA undergoes enterohepatic recirculation. MPAG excreted in the bile can be hydrolyzed to MPA and reabsorbed from the intestine. Such an enterohepatic recycling process is likely to be interrupted in patients with external biliary drainage of bile, and subsequent clamping of the t-tube (returning bile back to the bowel) is expected to increase the enterohepatic recycling and the systemic exposure of the drug.

Because nearly 30% of the dose of MMF is reportedly excreted in the bile as MPAG, which can be converted to MPA and reabsorbed, it was anticipated that the plasma concentrations of MPA would increase after clamping of the t-tube. In our patients, nearly 29% of the dose of MMF was recovered as MPAG in the bile during one dosing interval. There was, however, no significant increase in the AUC of MPA after clamping the t-tube, suggesting no significant improvement in the enterohepatic recycling of MPA with t-tube clamping.

MMF is reportedly absorbed completely, and the absorption of MMF is nearly 1.5 times greater than that of MPA[12] in primates. Given that, on average, 29% of the dose of MMF is excreted in the bile as MPAG and two thirds of this is potentially available for absorption, nearly 19% of the MMF dose is likely to be reabsorbed after t-tube clamping. From our results, it appears that t-tube clamping did not significantly increase the AUC of MPA or, therefore, the amount of MPA that was reabsorbed in liver transplant recipients. This suggests that MPAG is perhaps not completely hydrolyzed to MPA in the gut, or the MPA generated from MPAG is not absorbed well in liver transplant recipients.

Our results indicate that there is no significant increase in the AUC of MPA after clamping of the t-tube and that there is no need to alter the dose of MMF in liver transplant recipients when the t-tube is closed. These observations are, however, different from what is known for conventional cyclosporine formulation (Sandimmune, Novartis, Hanover, NJ) but are similar to what is known for tacrolimus and the newer formulation of cyclosporine (Neoral, Novartis, Hanover, NJ).

References

1. Bullingham R, Monroe S, Nicholls A, Hale M. Pharmacokinetics and bioavailability of mycophenolate mofetil in

healthy subjects after single-dose oral and intravenous administration. J Clin Pharmacol 1996;36:315-324.

2. Bullingham RES, Nicholls A, Hale M. Pharmacokinetics of mycophenolate mofetil (RS61443): A short review. Transplant Proc 1996;28:925-929.
3. Parker G, Bullingham R, Kamm B, Hale M. Pharmacokinetics of oral mycophenolate mofetil in volunteer subjects with varying degrees of hepatic oxidative impairment. J Clin Pharmacol 1996;36:332-344.
4. Aukee S, Venho VMK, Jussila J, Karjalaainen P. Drug absorption in patients with t-tube after cholecystectomy. Ann Clin Res 1975;7:42-46.
5. Mehta MU, Venkataramanan R, Burckart GJ, Ptachcinski RJ, Delamos B, VanThiel DH, et al. Effect of bile on cyclosporine absorption in liver transplant patients. Br J Clin Pharmacol Ther 1988;25:579-584.
6. Carruthers SG, Dujovne CA. Digoxin therapy during t-tube biliary drainage in man. JAMA 1978;240:2756-2757.
7. Venkataramanan R, Prasad TNV, Swaminathan A, Warty VS, Burckart G. FK506: Clinical pharmacokinetics. Clin Pharmacokinet 1995;26:404-430.
8. Jain A, Venkataramanan R, Cadoff E, Fung JJ, Warty VS, Todo S, et al. Effect of hepatic dysfunction and t-tube clamping on FK506 pharmacokinetics and trough concentrations. Transplant Proc 1990;22(suppl 1): 57-59.
9. Jain AS, Venkataramanan R, Fung JJ, Hamad I, Lever J, Zuckerman S, et al. The pharmacokinetics of mycophenolate mofetil in liver transplant patients treated with tacrolimus. J Clin Pharmacol (in press).
10. Shaw LM, Mick R, Nowak I, Korecka M, Bryman KL. Pharmacokinetics of mycophenolic acid in renal transplant patients with delayed graft function. J Clin Pharmacol 1998;38:268-275.
11. Nowak I, Shaw L. Mycophenolic acid binding to human serum albumin: Characterization and relation to pharmacodynamics. Clin Chem 1995;41:1011-1017.
12. Lee WA, Gu L, Miksztal AR, Chu N, Leung K, Nelson PH. Bioavailability improvement of mycophenolic acid through aminoester derivatization. Pharm Res 1990;7: 161-166.