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Mycophenolate mofetil pharmacokinetics in renal transplant recipients on peritoneal dialysis

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Abstract We prospectively studied the impact of peritoneal dialysis (PD) on the pharmacokinetics of mycophenolic acid (MPA) in five patients following renal transplantation. Three patients had a glomerular filtration rate (GFR) of less than 10 ml/min and two had a GFR of more than 40 ml/min. Pharmacokinetics of MPA and of its main metabolite, mycophenolic acid glucuronide (MPAG), were studied during two consecutive 12-h periods (with and without PD). After initiation of PD in patients with severe renal impairment (GFR < 10 ml/min), MPA-area-under-the-concentration-curve (AUC) decreased up to 59 % and MPAG-AUC decreased up to 26 %. We did not observe any substantial changes in the MPA-AUC or MPAG-AUC of either patient with a GFR above 40 ml/min. Patients with a reduced GFR had much higher MPAG values than patients with a GFR above 40 ml/l; yet, we did not observe any differences in the MPA values. We found a significant inverse correlation between GFR and MPA-AUC ($r = 0.81$, $P < 0.05$) and between GFR and MPAG-AUC ($r = 0.94$, $P < 0.01$). While MPA was found only in traces in the peritoneal ultrafiltrate, the cumulative amount of MPAG removed by PD reached up to 2 g/12 h, representing 1.2 g of MPA. This is the first report describing a reduction in MPA-AUC and MPAG-AUC during PD. Further studies are needed to completely understand the pharmacokinetics of mycophenolate mofetil during PD.

Key words Mycophenolate mofetil, kidney transplantation · Kidney transplantation, mycophenolate mofetil · Peritoneal dialysis, mycophenolate mofetil

Introduction

Mycophenolate mofetil (MMF) is the morpholinoethylester pro-drug of the new immunosuppressant mycophenolic acid (MPA). MMF is completely absorbed after oral administration [2] and gets rapidly converted to its active metabolite (MPA) by plasma esterases [7]. MPA is a noncompetitive, reversible inhibitor of eukaryotic inosine 5′monophosphate dehydrogenase (IMPDH), the rate-controlling enzyme in de novo biosynthesis of guanosine triphosphate [5]. It selectively decreases intracellular guanine nucleotide pools and specifically suppresses purine de novo biosynthesis [12]. The mean serum half-life of MPA is about 18 h [2]. Metabolization of MPA occurs in the liver, where it gets glucuronidated to mycophenolic acid glucuronide (MPAG). MPAG is pharmacologically inactive [1], but due to enterohepatic recirculation, it plays an important role in the maintenance of steady-state plasma MPA levels [2]. Residential bacterial flora in the intestinal mucosa lead to cleavage of glucoronic acid from hepatic secreted MPAG, yielding MPA that gets rapidly reabsorbed. Elimination of MPA occurs primarily through the urine (93 %), mostly as MPAG (97 %) [2].

Table 1 Main clinical parameters of the patients under investigation. Peritoneal filtration refers to the accumulative amount of ultrafiltrate gained by three 2000-ml dwells (*DN* diabetic nephropathy, *TIN* tubulointerstitial nephropathy, *FSGN* focal segmental glomerulonephritis, *SLE* systemic lupus erythematosus, *ADPKD* autosomal dominant polycystic kidney disease)

	Sex	Age (years)	Weight (kg)	Height (cm)	Renal disease	Days after NTP	Creatinine (mmol/l)	GFR (ml/min)	Urine (ml/24 h)	Peritoneal filtration (ml/12 h)	Peritoneal protein loss (g/l)
Patient 1	F	60	71.3	160	TIN	35	900	< 10	1800	800	2.7
Patient 2	F	25	60	168	FSGN	28	990	< 10	2800	650	1.3
Patient 3	F	37	52.3	159	SLE	19	1050	< 10	200	1300	0.7
Patient 4	F	43	77.5	168	DN	30	240	40	1200	750	0.8
Patient 5	M	55	70	178	ADPKD	23	126	60	2500	300	1.4

In the early post-transplantation period, a significant proportion of renal allografts have delayed graft function. Although renal impairment is usually transient, it often necessitates dialysis for volume and/or uremia control. Studies on the impact of hemodialysis on the pharmacokinetics of MMF have shown no influence on the MPA levels due to its high protein binding; however, MPAG accumulates three- to sixfold in patients with a GFR below 25 ml/min [8].

To date, no data concerning the effect of peritoneal dialysis (PD) on MPA and MPAG levels are available. As PD becomes more and more attractive, the proportion of these patients receiving renal grafts is increasing. Blood purification by PD depends on the permselective ultrafiltration capacity of the peritoneal membrane. In contrast to hemodialysis, in which membrane pore size almost completely restricts passage of molecules above a certain size, in the peritoneal system there is some transport of larger molecules, including proteins. The peritoneal membrane as a biological membrane is different from an artificial dialysis membrane; thus, different MMF pharmacokinetics are to be expected. In this study we prospectively analyzed the effect of PD on MMF pharmacokinetics in five renal transplant recipients.

Materials and methods

Patients

MMF pharmacokinetics were studied in five patients. The study was approved by the ethics commitee. Informed consent was obtained from all patients. All patients were in good clinical condition with normal liver function, normal serum albumin, and normal serum protein. The main patient characteristics are summarized in Table 1. All patients were in the early post-transplant period. Three patients had delayed graft function and, due to volume and/or uremia control, were on PD (group 1). The glomerular filtration rate (GFR) in this group was below 10 ml/min. The GFR was calculated using the formula developed by Cockcroft and Gault [3] for creatinine clearance. In addition, two patients with recovering renal function (GFR 40 and 60 ml/min; group 2) were studied as controls. Both patients were on PD before transplantation and were asked to perform a 12-h PD dwell. The immunosuppressive regimen in all patients consisted of methylprednisolone (12–20 mg/day), cyclosporin (adjusted to a monoclonal trough level between 120 and 140 ng/ml; Emid assay, Roche, Basel, Switzerland)[1] and MMF (2 × 1 g/day; 12-h period). Intravenous antibiotics (oxacillin and azlocillin) were given for 10 days, starting with the day of transplantion. Concomitant medication did not change during the study period.

Peritoneal dialysis and study protocol

A dialysate with a glucose concentration of 2.27 % (Baxter, Ettlingen, Germany) was used in all patients. Peritoneal transport characteristics were assessed according to Twardowski by a standardized peritoneal equilibration test [11]. All patients were classified as average transporters according to the peritoneal capacity of eliminating blood urea nitrogen. On day 1, PD was started immediately after MMF intake. It was performed for 12 h in a standardized regimen consisting of three 4-h dwells with a filling volume of 2000 ml each. On day 2, PD was suspended for 12 h, immediately prior to MMF intake, leaving a dry peritoneal cavity. EDTA blood and dialysate specimens were collected before dosing and at 0.2, 0.4, 1.25, 2, 5, 8, 11, and 12 h after MMF intake. The linear trapezoidal rule was then applied to the concentration-time curve consisting of the observed concentration and times, providing the estimate of AUC.

Biochemical analysis

MPA samples were prepared by liquid phase extraction. One hundred microliters of plasma was mixed with 100 µl of 10 % potassium phosphate buffer, pH 4.6, and 400 µl dichlormethane (ICN Biomedicals, Eschwege, Germany) containing 1 µg of internal standard I (2,6-Bis-[bis-(β-hydroxyethyl)-amino]-4,8-dipiperidino-pyrimido[5, 4-d]pyrimidine; Arzneimittelwerk Dresden, Dresden, Germany). After 2 min of centrifugation at 10000 *g*, the upper phase was removed and the organic phase was evaporated with nitrogen at 50 °C. The sediment was resolved in 50 µl acetonitril/50 mM potassium phosphate buffer (45/55 v/v, pH 2.3). MPAG was extracted using a solid phase extraction column, Chromabond C_{18} (Machery-Nagel, Düren, Germany) equilibrated with 3 ml methanol and 3 ml water. One hundred microliters of plasma was mixed with 2 ml 4,5 N Hcl containing 100 µg internal standard II, and samples were loaded on the extraction column. After washing with 1 ml H_2O, samples were eluted with 1 ml methanol/100 mM sodium acetate buffer (80/20 v/v, pH 4). The liquid on the column was allowed to pass through by gravity flow until the column dripped dry. Finally, 50 µl of MPA and MPAG extracts were mixed and analyzed using a semiautomated HPLC method. The HPLC

Table 2 MPA-C_{max}, -t_{max} and -AUC values during peritoneal dialysis (CAPD) compared to a 12-h dwell-free-period with a dry peritoneal cavity

MPA							
		CAPD			Dry peritoneal cavity		
		C_{max} (μg/ml)	t_{max} (h)	AUC (μg · h/ml)	C_{max} (μg/ml)	t_{max} (h)	AUC (μg · h/ml)
Group 1	Patient 1	5.0	0.5	20.75	4.0	2.0	42.01
	Patient 2	3.7	2.0	22.81	11.2	0.25	27.08
	Patient 3	2.7	1.33	16.43	4.7	5.0	39.56
Group 2	Patient 4	7.15	2.0	27.65	3.6	1.3	16.42
	Patient 5	11.6	2.0	33.65	6.7	2.0	24.98

Table 3 MPAG-C_{max}, -t_{max} and -AUC values during peritoneal dialysis (CAPD) compared to a 12-h dwell-free period with a dry peritoneal cavity

MPAG							
		CAPD			Dry peritoneal cavity		
		C_{max} (μg/ml)	t_{max} (h)	AUC (μg · h/ml)	C_{max} (μg/ml)	t_{max} (h)	AUC (μg · h/ml)
Group 1	Patient 1	440	0.5	4202	539	2.0	5422
	Patient 2	688	2.0	7435	743	0.5	8140
	Patient 3	477	5.0	5937	730	1.25	8017
Group 2	Patient 4	231	4.5	2457	249	4.5	2699
	Patient 5	172	2.0	1580	117	2.0	1107

system consisted of a Shimadzu LC-6A model, Shimadzu Auto Injector SIL-9A, UV-Vis detector Shimadzu SPD-6AV, and data system Shimadzu C-R4AX CHROMATOPAC (Shimadzu Europe, Düsseldorf, Germany) using a 3-μm column (VDS Optilab Hypersil C_{18}, 125 × 4,6 mm, Chromatographie-Technik, Berlin, Germany). HPLC-grade methanol and acetonitril were purchased from ICN Biomedicals (Eschwege, Germany); all other reagent grade chemicals were purchased from Merck (Darmstadt, Germany). Chromatography was carried out with 100 μl of sample extract at a flow rate of 0.7 ml/min using an isocratic mobile phase of acetonitril /50 mM potassium phosphate buffer (30/70 v/v, pH 2.3). The UV detector was set at 254 nm.

Calibration samples were generated by spiking drug-free plasma with a standard of MPA dissolved in methanol (Sigma, St. Louis. Mo., USA) to concentrations ranging from 0.1 up to 50 μg/ml or with a standard of MPAG dissolved in methanol (Roche, Basel, Switzerland) to concentrations ranging from 1 to 500 μg/ml. For both MPA and MPAG, calibration graphs of the ratio of peak areas of the analytic substance to the internal standards were plotted against the concentration of MPA or MPAG. The ratio of peak areas was used to determine the concentration of MPA or MPAG in patient samples from the calibration curves.

Statistics

Statistical analysis was performed using SPSS standard software (release 6.1). The Spearman test was used for testing. A P value below 0.05 was regarded as significant. Data are expressed as mean ± SD.

Results

In three patients with a GFR below 10 ml/min (group 1), a substantially lower MPA-AUC (between 15 %–59 %; Table 2) were observed during PD compared to the dwell-free period. In contrast, MPA-AUC was higher during PD in both patients with a GFR above 40 ml/min (group 2). Mean AUC values during PD were 20 ± 3 μg*h/ml for group 1 and 31 ± 4 μg*h/ml for group 2; values on the dwell-free day were 36 ± 8 and 21 ± 6 μg*h/ml, respectively. PD seemed to have no impact on the C_{max} or t_{max} values (Table 2). A significant inverse correlation between GFR and the MPA-AUC was noticed ($r = 0.81$; $P < 0.05$) on the dwell-free day.

Mean MPAG-AUC values during the dwell-free period were 7193 ± 1534 μg*h/ml in group 1 and 1903 ± 1125 μg*h/ml in group 2, and they were 5859 ± 1616 μg*h/ml versus 2018 ± 620μg*h/ml during PD (Table 3). Again, after initiation of PD in patients with a GFR below 10 ml/min, a marked decrease in the MPAG-AUC (9 %–26 %) was obvious. In patients with mild to moderate renal impairment, MPAG-AUC remained stable. Analysis of MPAG C_{max} and t_{max} did not show any marked differences between the 2 days. In patients with a severe reduction in GFR, substantially higher (up to twofold) MPAG-AUCs were noted. Again, a reduced GFR significantly correlated with ele-

Table 4 Maximum MPA and MPAG concentrations in the dialysate of a 4-h dwell

		Dialysate	
		MPA (μg/ml)	MPAG (μg/ml)
Group 1	Patient 1	0.19	124.8
	Patient 2	0.26	196.1
	Patient 3	0.19	306.9
Group 2	Patient 4	0.14	67.4
	Patient 5	0.19	40.2

vated MPAG-AUCs ($P < 0.01$). The correlation coefficient between GFR and MPAG-AUC, measured for the dwell-free period, was $r = 0.94$.

The mean ultrafiltration volume by PD was 760 ± 350 ml/12 h. The 12-h cumulative peritoneal drain volume was 6760 ± 350 ml (three 2000 ml dwells + ultrafiltrate). MPA elimination into the peritoneal cavity ranged between 0.14 μg/ml and 0.26 μg/ml, resulting in a cumulative loss of approximately 1000–1500 μg/12 h. We observed no marked differences between the two groups (Table 4). The MPAG concentrations in the dialysate are summarized in Table 4. Cumulative MPAG loss ranged between 0.3 g/12 h and 2.0 g/12 h (representing between 0.18 g/12 h and 1.2 g/12 h of MPA), depending upon the plasma MPAG concentration. A higher MPAG plasma concentration correlated with higher MPAG elimination ($r = 0.9$; $P < 0.05$). MPA and MPAG concentrations in the dialysate reached their maximum approximately 3 h after the dialysate had been instilled and remained stable thereafter.

Discussion

To date, no data are available about the pharmacokinetics of MPA and MPAG in patients on PD. In this study we investigated the pharmacokinetics of MPA during PD and during a 12-h dwell-free period in three patients with a GFR below 10 ml/min and in two patients with moderate to good renal function corresponding to a GFR above 40 ml/min.

The pharmacokinetic profile of MPA is characterized by a sharp absorption peak occurring 0.6–0.9 h after oral administration, followed by a rapid decline in MPA concentration. A second smaller peak is due to gastrointestinal reabsorption and results 6–12 h after oral administration [5]. Since peak plasma concentrations of MPA (C_{max}) are highly dependent upon food intake [6], neither C_{max} nor trough levels are considered adequate markers for drug monitoring [5, 6]. The area under the plasma concentration-time curve (AUC) gives more accurate information about effective drug levels as it is not food-dependent and generally proportional to dosage [10].

Hemodialysis, due to the high protein binding of MPA, has no effect on the MPA-AUC [4, 9]. In contrast, we observed a substantial reduction in the MPA-AUC during PD in patients with severely impaired renal function. In parallel, initiating PD led to a substantial decline in the MPAG-AUCs. As MPA and MPAG concentrations are in a steady-state balance, the decline under PD may be caused by an increased removal of MPA or MPAG. In all patients, a significant amount of MPA was removed in the diaylsate, almost completely in its glucuronidated form. Thus, the cumulative loss of MPAG, which corresponds to up to 1.2 g of MPA per 12 h, seems to be the main cause of reduction in MPA-AUC in these patients. In both patients with only moderately impaired renal function, we observed an increase in the MPA-AUC of about 25 % during PD, even though PD led to an additional removal of MPAG. Most likely this was a result of the additional water loss and relative hemoconcentration by PD. While in patients with severely impaired renal function hyperhydration was tolerated (these patients were approximately 2–3 kg above their original dry body weight), patients with only moderately impaired renal function were in an equilibrated hydration status. In addition, less MPAG was removed, due to lower blood concentrations of MPAG in these patients.

Other factors, such as uremic enteropathy or changes in residential intestinal flora associated with the antibiotic prophylaxis, may also contribute to the changes observed in our patients. These factors may alter MPA reabsoption or MPAG glucuronidation, resulting in changes in MPA and MPAG pharmacokinetics. Due to the small number of patients in our study, statistically significant differences cannot be estimated properly. Nevertheless, our findings suggest that the different dialysis techniques result in marked differences in the pharmacokinetic behavior of MPA in renal transplant recipients with delayed graft function. Whether this may lead to a reduced risk of toxicity or to the occurrence of relevant under-immunosuppression in patients on PD remains to be investigated.

In our experience, a reduction in the MPA dose in renal transplant recipients with delayed graft function was seldom necessary, but as there is no clinically feasible measure to predict over-dosing of MPA, in our institution the MPA dose is only reduced when clinical effects of over-dosing become apparent. With continuing efforts to achieve better adapted individual dosing, factors influencing MPA pharmacokinetics will become more important. Therefore, further studies including more patients are necessary to evaluate the impact of PD on the efficacy of the immunosuppressive protocol.

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