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ORIGINAL ARTICLE

Mycophenolic acid clinical pharmacokinetics influenced by a cyclosporine C2 based immunosuppressive regimen in renal allograft recipients

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Summary

Therapeutic drug monitoring of mycophenolic acid (MPA) in combination with cyclosporine 2-h concentration (CsA C2, $n = 68$) or tacrolimus trough concentration ($n = 10$) was investigated by repeated measurements of MPA and MPA-glucuronide (MPAG) trough concentrations in renal allograft recipients during the first 3 months post-transplant. The acute rejection rate was lower (19% vs. 43%; $P < 0.05$) in patients achieving CsA C2 target range during the first week (1600–2000 μg/l), $n = 26$, compared with those who did not, $n = 42$. Median MPA concentration was 0.9 and 1.2 μg/ml in patients within or below C2 range, respectively ($P = 0.19$). CsA C2 correlated with MPAG-to-MPA ratio ($P < 0.01$, $r = 0.91$) and gamma-glutamyl-transpeptidase (GGT, $P < 0.01$, $r = 0.86$). Total MPA concentration increased during the 3 months, but not in patients on tacrolimus. High CsA C2 lowered the acute rejection rate and plasma MPA. High CsA C2 is associated with elevated GGT, probably because of cholestatic effects, which explain the increased MPAG-to-MPA ratio. Increasing MPA concentration is ascribed to per-protocol CsA C2 reductions. In conclusion, CsA may confound the relationship between MPA and the incidence of rejection, and contribute to the difficulty of obtaining a therapeutic range for MPA in clinical practice.

Introduction

Therapeutic drug monitoring is mandatory in order to optimize the immunosuppressive treatment in clinical transplantation. Even after three decades with cyclosporine (CsA), efforts are continuing to improve CsA monitoring [1]. Recently, monitoring by CsA 2-h concentration (CsA C2) proved superior to monitoring based on CsA C0 concentration in predicting the acute rejection rate [2]. Mycophenolate mofetil (MMF) has been used in combination with CsA since its approval in 1995. The active moiety of MMF, mycophenolic acid (MPA) has been under intensive investigation because of its highly variable pharmacokinetics. Reported protein bindings for MPA and its main metabolite, the inactive MPA-glucuronide (MPAG) are 97% and 82%, respectively [3]. Post-transplant changes in renal function, hepatic metabolism, enterohepatic recycling, albumin levels, concomitant medication as well as variations in hepatic uridinediphosphate glucuronosyl transferase (UGT) expression lead to the highly variable pharmacokinetics reported in a number of studies [4–8]. Several studies have addressed the correlation between MPA concentrations and clinical outcome in renal transplantation and shown that MPA concentrations correlate with acute rejections [9–11]. However, trough MPA has shown to be of poorer predictive value than MPA AUC [11,12]. In recent years different sampling strategies have also been suggested [13,14]. However, monitoring based on trough concentrations is widespread and more practical on the routine basis. Based

on AUC measurements, predose total MPA concentration between 1 and 3.5 μg/ml is anticipated as a reasonable target [8]. Sudden changes in the clinical situation and frequent changes in drug regimens during the immediate post-transplant phase may influence MPA pharmacokinetics and complicate interpretation of predose MPA. In some situations the relationship between free and total MPA concentrations may be altered. The purpose of the present study was to explore the relationship between MPA pharmacokinetics and CsA C2 in a cohort of consecutive renal allograft recipients starting on a CsA C2 monitored immunosuppressive regimen including MMF. Patients in whom contraindications or adverse effects prompted the use of tacrolimus, were included to explore the impact of tacrolimus on the clinical pharmacokinetics of MPA. For this purpose free and total MPA and MPAG concentrations were examined by analyzing repeated trough samples during the first 3 months post-transplant.

Patients and methods

Patients

Eighty-six adult kidney recipients were recruited consecutively for this prospective study. Eight patients were excluded; two due to postponed transplantation and six due to incomplete data, leaving 78 patients included in the study (Table 1). The immunosuppressive regimen after transplantation combined MMF with steroids and CsA. Patients in whom CsA was contraindicated received a combination MMF with tacrolimus and steroids (Table 1). Among 10 patients starting on tacrolimus, six had combined kidney–pancreas transplantation. CsA was administered in accordance with the standard immunosuppressive protocol to obtain target CsA C2 concentrations 1600–2000, 1400–1600, 1000–1200 ng/ml during the first, second and third month post-transplant, respectively. Samples for CsA C2 were drawn 2 h postdose, accepting deviations of ±10 min. For tacrolimus the target trough values were 10–15, 8–12 and 5–10 ng/ml during the first 3 months, respectively. According to the standard immunosuppressive protocol, at least five CsA or tacrolimus concentrations were measured during the first week post-transplant. For the rest of the study period these concentrations were measured at least three times per week. Steroids were given as methylprednisolone i.v. peroperatively followed by peroral prednisolone starting at 80 mg/day tapered by 10 mg/day to 20 mg/day and maintained at 15 mg/day during the second month and 10 mg/day during the third month respectively.

Table 1. Characteristics of renal allograft recipients included in the study.

Age, years	54 (19–77)
Gender, M/F	57/21
Bodyweight, kg	74 (49–139)
Donor	47 CD/31 LD
Re-transplants	10
DR mismatch (0/1/2)	22/54/2
Observation period, days (MPA analysis)	83 (30–90)
Immunosuppression	
Cyclosporine/MMF/steroids*	68
Tacrolimus/MMF/steroids	10

CD, cadaveric; LD, living; MPA, mycophenolic acid; MMF, mycophenolate mofetil.

*Eleven patients switched from CsA to tacrolimus during the study period. Another five patients switched to other immunosuppressants.

Mycophenolate mofetil was started at doses of 1 gram twice daily ($n = 72$), except in combined kidney plus pancreas transplantations in which 1 g was given three times per day ($n = 6$). Trough levels of free and total MPA and MPAG concentrations were measured in repeated samples from the first MMF dose (initiated at the day of transplantation) until 3 months post-transplant. The blood samples were collected predose in the morning two to three times a week during the first four weeks post-transplant and then 1–2 times weekly yielding a total of 17 ± 4 blood samples from each patient for MPA and MPAG analysis. Target concentrations for MPA were not identified in the standard immunosuppressive protocol; rather the reported concentrations were used on an individual basis to support decisions of dose reductions when adverse reactions were suspected. The MMF dose was not increased based on MPA concentrations.

Acute rejections were treated with i.v. doses of methylprednisolone (500 mg initially, reducing to 250 mg) for four consecutive days as first-line treatment. Steroid resistant rejections were treated with anti T-cell antibodies ATG or OKT3. Delayed graft function (DGF) was defined as need for hemodialysis during first week post-transplant.

In this descriptive study, blood samples drawn for routine biochemical and pharmacological analyses were used for extended measurements of MPA including free and total MPA and MPAG concentrations. According to the routine practice only the total MPA plasma concentrations were reported to the responsible nephrologists. As no interventions were made on the basis of the extended measurements and no extra blood samples were drawn, approval of the ethics committee was not required.

Methods

Determination of total and free MPA and MPAG concentrations in plasma was carried out by a previously published method [15]. Briefly, the assay is based on dialysis using ASTED (automated sequential trace enrichment of

dialysis) combined on-line with a LC system (Gilson, Villiers-le-Bel, France). Plasma samples were obtained from whole blood collected in EDTA vacutainer tubes, centrifuged at 2700 *g* and stored at −20 °C until analysis. Free concentrations are measured by dialysis of plasma against phosphate buffered saline solution and separation by HPLC on a Zorbax SB-Aq 5 μm, 4.6 × 150 mm column, Agilent Technologies, Palo Alto, CA, USA. For the measurement of total concentrations plasma proteins were precipitated and the supernatant was injected directly into the analytical column for separation. The chromatographic separation was performed at ambient temperature, in isocratic flow (2 ml/min) of a mobile phase consisting of acetonitrile 25% in 20 mmol/l phosphoric acid, final pH 3. The detection wavelength was set to 215 nm. Between series CV based on in house prepared controls was below 14% for the MPA and MPAG (free and total) assays. The analytical performance was also monitored using commercially available external controls (ASI Ltd, London, UK).

Calibrators for free MPA and MPAG concentrations were prepared from stock solutions (MPA 2 mg/ml in methanol, MPAG 2 mg/ml in water), diluted in protein free plasma to final concentrations of 0.005, 0.025, 0.05, 0.1, 0.2, 0.8 and 5, 20, 40 μg/ml, respectively. Calibrators for total MPA and MPAG concentrations were prepared from stock solutions, diluted in plasma to final concentrations of 0.25, 0.5, 1.0, 5.0, 20.0 and 5, 20, 40, 120, 240 μg/ml, respectively. Whole blood CsA C2 concentrations were measured using the CEDIA assay (Microgenics Corporation, Fremont, CA, USA). Whole blood tacrolimus trough concentrations were determined with a microparticulate enzyme immunoassay (Tacrolimus II MEIA/ImX analyzer, Abbott Laboratories, Abbott Park, IL, USA). Other data, such as albumin, urea, creatinine, alanine aminotransferase (ALAT), gamma-glutamyl-transpeptidase (GGT) and bilirubin were obtained from the routine laboratory.

Data analysis

Alterations in MPA pharmacokinetics were examined in the entire study population and by stratification according to concomitant immunosuppression, graft function, serum albumin and CsA C2 levels. Data are expressed as median (range), median (inter quartile range; IQR) or mean (SD) as specified. Data with skewed distribution were assessed using two-tailed Mann–Whitney or Wilcoxon Signed Ranks test. The chi-squared test was used for the difference in acute rejection rate. Statistical significance was set to $P < 0.05$. Two-tailed bivariate Spearman's-ρ correlations were performed using SPSS 11.0 for Windows (SPSS Inc., Chicago, IL, USA) and considered significant at $P < 0.01$.

Results

In this cohort of consecutive renal transplant recipients, substantial variations in predose MPA and MPAG concentrations were observed. This also held true when the concentrations were adjusted according to MMF dose and bodyweight (Table 2).

MPA pharmacokinetics in combination with CsA or tacrolimus

Stratification of the cohort according to concomitant immunosuppression revealed significant differences in MPA concentrations (Fig. 1a). Substantial variability in predose MPA concentration was observed in both the CsA and tacrolimus group. Compared with the CsA group, total MPA concentration in patients receiving tacrolimus as concomitant drug was significantly higher throughout the study period ($P < 0.05$). In contrast to tacrolimus, CsA induced consistent changes in the MPA pharmacokinetics (Fig. 1). In the CsA group the MPA concentration increased median 61% (IQR; −17 to 103%) from month 1

Table 2. Predose mycophenolic acid (MPA), MPA-glucuronide (MPAG) and corresponding free fractions in 78 renal allograft recipients. Data presented as median with corresponding range.

	Month 1	Month 2	Month 3	*P*-value*
MPA total, μg/ml	0.9 (0.3–6.1)	1.3 (0.2–8.9)	1.5 (0.2–6.5)	<0.05
MPA free, ng/ml	23 (7–102)	21 (6–107)	20 (8–84)	NS
MPA free fraction, %	2.1 (0.8–7.4)	1.6 (0.7–5.3)	1.4 (0.7–4.3)	<0.05
MPAG total, μg/ml	98 (24–341)	89 (23–273)	71 (13–320)	<0.05
MPAG free, μg/ml	33 (6–164)	23 (6–142)	18 (3–116)	<0.05
MPAG free fraction, %	32 (22–50)	27 (17–56)	25 (18–69)	<0.05
Adjusted by dose/body weight				
MPA total, ng/ml/(mg/kg)	36 (9–227)	54 (17–406)	60 (19–453)	
MPA free, ng/ml/(mg/kg)	0.8 (0.3–4.5)	0.8 (0.2–6.5)	0.8 (0.2–7.0)	
MPAG total, μg/ml/(mg/kg)	3.6 (0.8–14.1)	3.4 (0.8–11.1)	2.9 (0.7–11.3)	
MPAG free, μg/ml/(mg/kg)	1.2 (0.2–6.6)	0.9 (0.2–3.8)	0.8 (0.2–4.0)	

*Month 1 versus month 3.

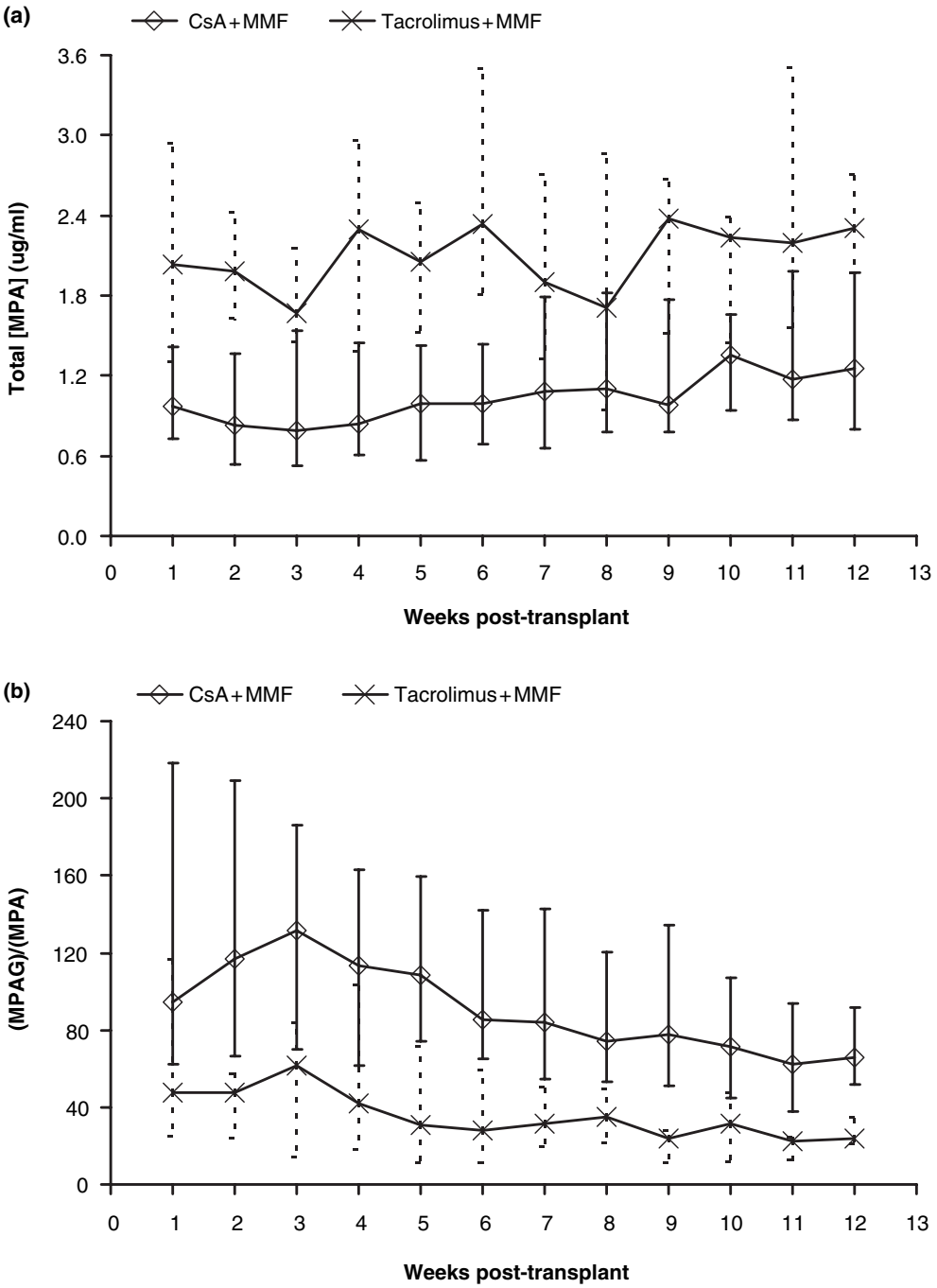

Figure 1 Predose mycophenolic acid (MPA) concentration (a) and MPA-glucuronide-to-MPA ratio (b) in subgroups receiving a combination of mycophenolate mofetil with cyclosporine (CsA) ($n = 52$) or tacrolimus ($n = 10$) throughout the 3-month study period. Only patients continuously on CsA or tacrolimus, respectively, during the study period are included. Data are presented as medians (per week) and interquartile ranges.

to month 3 ($P < 0.05$). Following the maximum of CsA C2 concentration at two weeks post-transplant, a steady decrease (because of per-protocol decrease in CsA dose) in CsA C2 until the end of the observation period was noted (Fig. 2b). This was accompanied by increasing MPA concentration. Fig. 1b displays the ratio between MPAG and MPA concentrations in patients combining MMF with either CsA or tacrolimus. This ratio was significantly

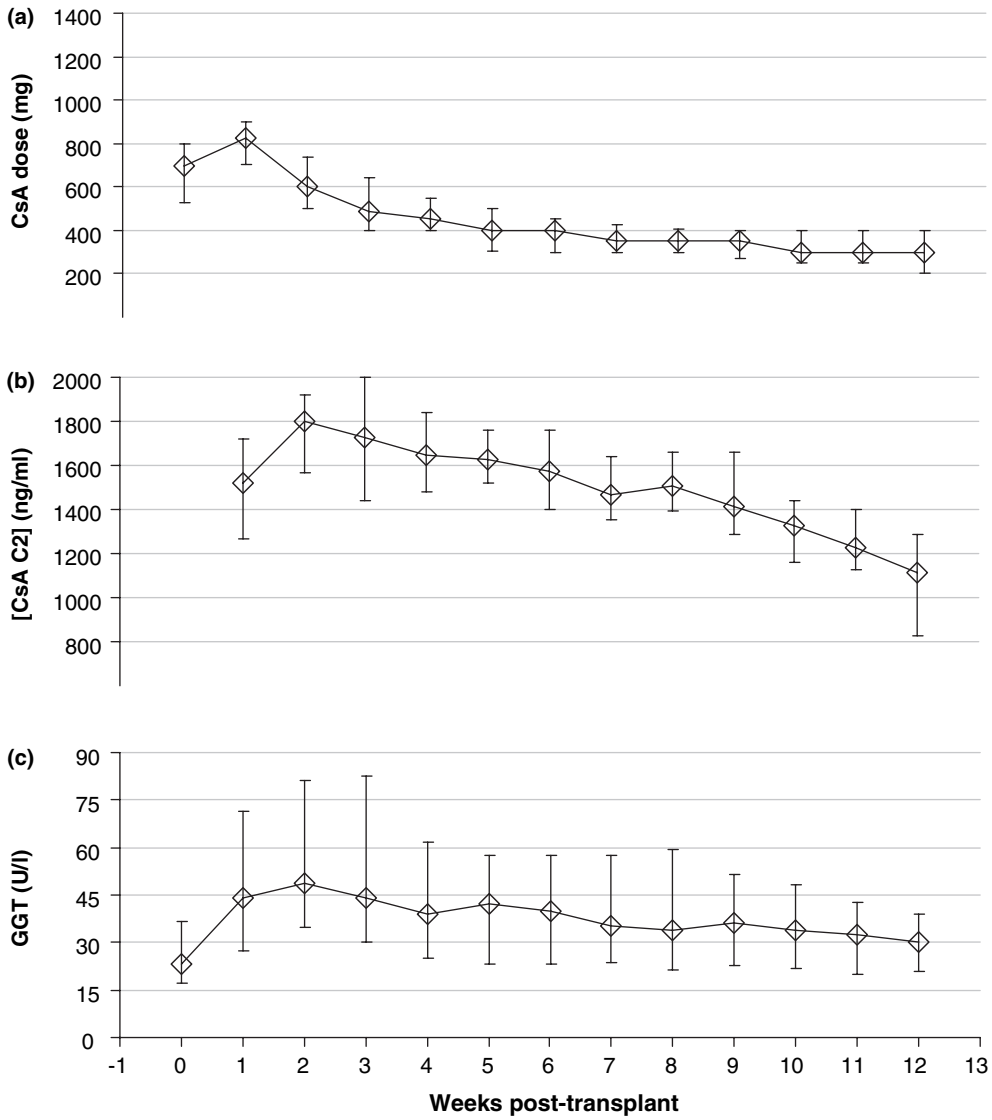

Figure 2 Cyclosporine (CsA) dose (a), cyclosporine 2-h concentration (b) and gamma-glutamyl-transpeptidase (c) in patients combining mycophenolate mofetil with CsA throughout the 3-month study period ($n = 52$). Only patients continuously on CsA during the study period are included. Data presented as medians (per week) and interquartile ranges.

higher in patients co-treated with CsA than with tacrolimus ($P < 0.05$). During the 3-month study period creatinine was median 142 (range: 64–480) μmol/l and 101 (range: 81–310) μmol/l in patients receiving CsA and tacrolimus ($P < 0.05$), respectively. The daily MMF dose was slightly higher in the tacrolimus (2.2 ± 0.5 g; $n = 10$) versus CsA group (2.0 ± 0.1 g; $n = 52$), although it should be noted that for a statistical comparison the number of patients were too few in the former group.

Among the 16 patients experiencing change of immunosuppression during the study period, the largest group comprised those converted from CsA to tacrolimus ($n = 11$, of which seven post acute rejection). Mycophenolate related adverse events occurring after the switch from CsA to tacrolimus prompted MMF dose reductions in five patients. Median dose/BW normalized MPA concentration 4 (range: 2–17) days before and 3 (range: 2–5) days after the switch from CsA to tacrolimus was 36 (range: 18–142) and 92 (range: 32–222) ng/ml/(mg/kg) ($P < 0.05$), respectively. Although not statistical significant, a tendency in the opposite direction was observed for dose/BW normalized MPAG concentrations: 4.6 (2.0–12.1) before and 3.0 (1.0–17.9) μg/ml/(mg/kg) after the switch ($P = 0.09$). In the remaining five patients, a

detailed analysis of the relationship between MPA and concomitant immunosuppression was precluded by low numbers in each subgroup, multiple changes in the immunosuppressive regimen during relative short period, ongoing dialysis because of oxalosis and MMF discontinuation.

Immunosuppressants and liver function tests

Cyclosporine induced significant changes in the liver function test GGT (Fig. 2), similar profiles were observed for ALAT and bilirubin (not shown). In patients combining CsA and MMF ($n = 52$) throughout the study period, significant correlations were observed between CsA C2 and GGT levels ($P < 0.01$, correlation coefficient: 0.86) and between CsA C2 and bilirubin ($P < 0.01$, correlation coefficient: 0.91). In parallel, CsA C2 correlated with MPAG-to-MPA ratio ($P < 0.01$, correlation coefficient: 0.91) (Fig. 3) and an inverse correlation was observed between CsA C2 and total MPA concentration ($P < 0.01$; correlation coefficient: −0.80).

The GGT levels in patients combining tacrolimus and MMF ($n = 10$) were median 82 (IQR: 28–298), 116 (IQR: 34–256), 87 (IQR: 33–201) U/l during the first 3 months post-transplant. There was no significant correlation between the tacrolimus concentration and GGT ($P = 0.51$, correlation coefficient: 0.21), bilirubin ($P = 0.41$, correlation coefficient: 0.26), MPAG-to-MPA ratio ($P = 0.09$, correlation coefficient: 0.52) or total MPA concentration ($P = 0.48$, correlation coefficient: −0.21). The above calculations are based on patients maintaining their primary immunosuppressive treatment throughout the study period and not experiencing hypoalbuminemia or DGF.

Clinical outcome

Twenty-four recipients (31%) experienced at least one clinical acute rejection during the 3-month study period, occurring median 19 (range: 4–46) days post-transplant. Twenty-three of the rejections were biopsy proven, the remaining one was supported by a combination of >20% increase in serum creatinine, graft swelling, increased body temperature and oligouria. Among the 24 patients experiencing rejection, 23 received CsA as primary calcineurin inhibitor in combination with MMF and one patient combined MMF with tacrolimus.

In 68 patients MMF was combined with CsA from the start. Stratification according to CsA C2 revealed that 42 patients had median CsA C2 below the C2 target range of 1600–2000 ng/ml during the first week post-transplant. The acute rejection rate in this group was significantly higher compared with patients with CsA C2 within this range during the first week post-transplant (Table 3). Although not significant, median MPA concentration in the first week post-transplant was slightly higher in patients below the CsA C2 target range in the first week post-transplant.

The MPA plasma concentration did not correlate with acute rejection episodes. A median MPA C0 above the

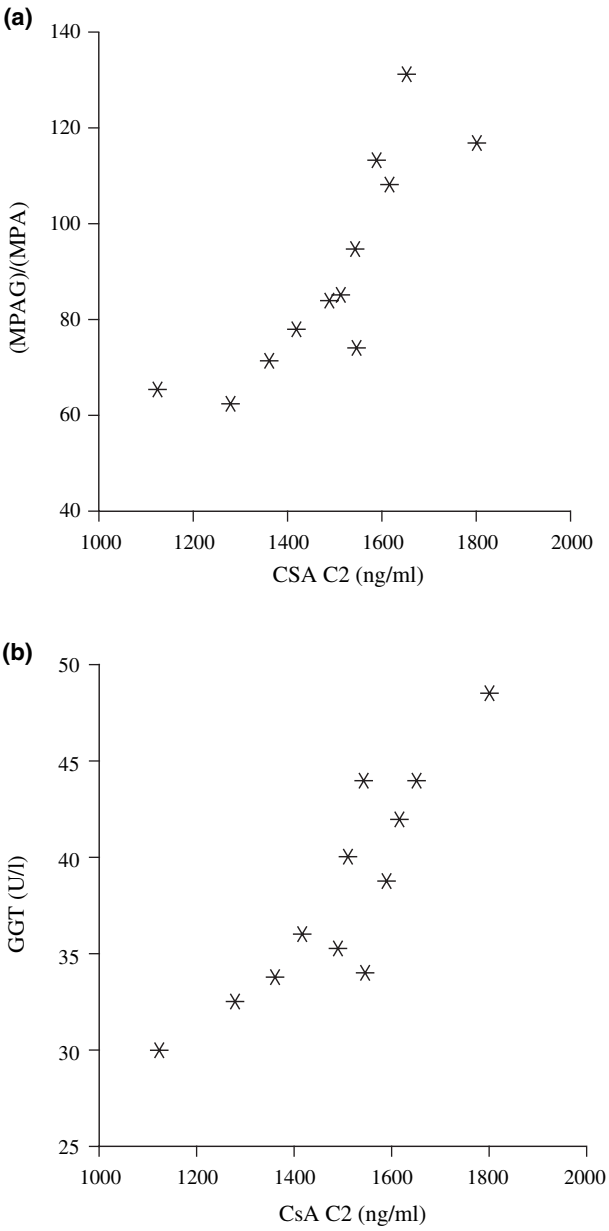

Figure 3 Correlations between cyclosporine 2-h concentration (CsA C2) and mycophenolic acid (MPA)-glucuronide-to-MPA ratio (a) and between CsA C2 and gamma-glutamyl-transpeptidase (b) in patients combining mycophenolate mofetil with CsA throughout the 3-month study period. Only patients continuously on CsA during the study period were included ($n = 52$).

Table 3. Median cyclosporine 2-h concentration (CsA C2) and predose mycophenolic acid (MPA) in patients within and below CsA C2 target range during the first week post-transplant and incidence of acute rejection in these subgroups.

	CsA C2 within target first week post-transplantation ($n = 26$)	CsA C2 below target first week post-transplantation ($n = 42$)	*P*-value
CsA C2, ng/ml	1760 (1610–2220)	1364 (740–1583)	
CsA dose, mg	741 ± 156	930 ± 235	<0.05
MPA total, μg/ml	0.9 (0.2–5.2)	1.2 (0.2–3.6)	=0.19
MPA dose, g	2.0 ± 0.0	2.0 ± 0.0	NS
Rejections	5 (19%)	18 (43%)	<0.05

suggested minimum therapeutic level of 1.0 μg/ml was achieved by 27 patients treated with CsA and MMF while 41 patients remained below this target during the first month post-transplant. The acute rejection rate was 44% and 27% respectively, i.e. higher with higher MPA concentration, as discussed below.

Free mycophenolate concentration

Seven patients experienced hypoalbuminemia with median serum albumin consistently below 35 g/l throughout the study period. The free fraction of MPA was higher and more variable in patients with persistent hypoalbuminemia compared with patients with normal serum albumin (Fig. 4). Total MPA concentration remained low with medians 0.9 (range: 0.7–1.8), 0.6 (range: 0.5–1.3) and 0.7 (range: 0.2–1.9) μg/ml throughout the 3-month study period in the hypoalbuminemia group, while MMF dose was reduced from 2.1 ± 0.5 g to 1.6 ± 0.4 g from month 1 to 3, respectively. During the first month, median free MPA concentrations were 50 (18–61) and 21 (8–102) ng/ml in patients with hypoalbuminemia and normal albumin ($P < 0.05$), respectively. Towards the end of the study period, the free fraction and the free concentration of MPA declined in the group of patients with persistent hypoalbuminemia, approaching the values in the patients with normal albumin.

Eight patients experienced delayed graft function requiring hemodialysis from the first week post-transplant. In this group the free fraction of MPA was higher compared with patients with initial graft function (Fig. 4) yielding free MPA concentration of 62 (range: 22–97) compared with 25 (range: 8–147) ng/ml during the first month, respectively. Median CsA C2 in 6 CsA-treated patients experiencing DGF was 1568 (range: 1085–1860) ng/ml during the first month post-transplant. Obviously, creatinine [month 1:587 (447–864) μmol/l] and urea [month 1:30 (range: 17–37 mmol/l)] were elevated in the initial phase, improving gradually through months 2 and 3.

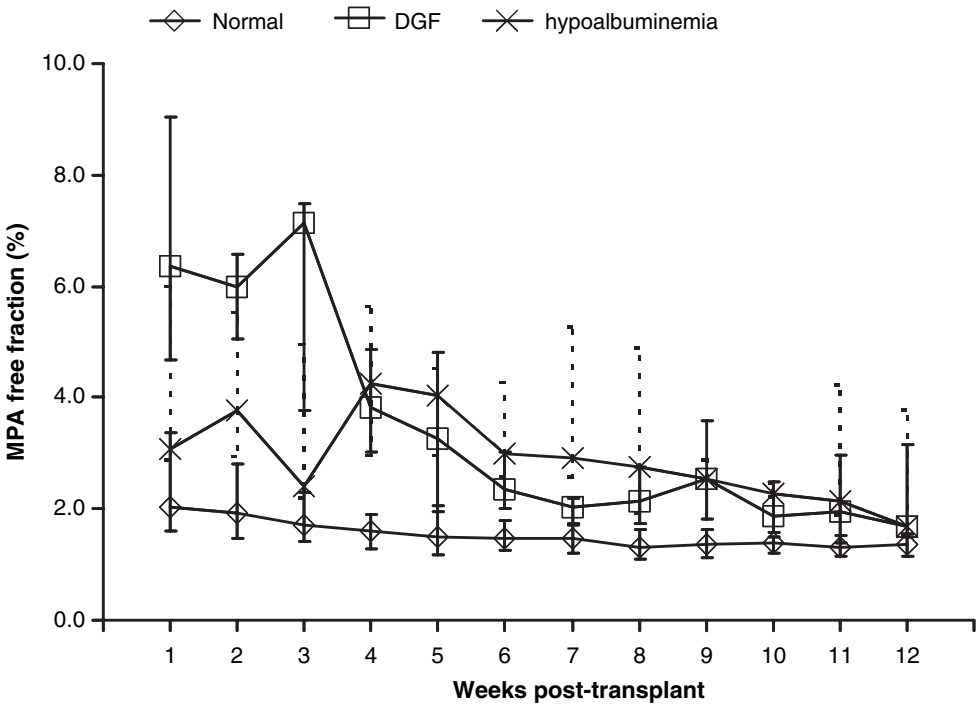

Figure 4 Mycophenolic acid free fraction (%) in patients experiencing hypoalbuminemia or delayed graft function compared to patients with normal albumin and immediate graft function. Data presented as medians (per week) and interquartile ranges.

MMF dose was reduced from 2.2 ± 0.3 g to 1.6 ± 0.5 g from month 1 to 3, respectively. Among patients requiring hemodialysis from the first week post-transplant, one experienced an acute rejection episode during the study period.

Discussion

This is a single center descriptive study of consecutive renal allograft recipients mainly on an immunosuppressive regimen based on C2-monitoring of CsA including MMF and steroids. The regimen implies a rather high average CsA dosage during the first days post-transplant in order to obtain the CsA target range within 3–5 days, followed also by a significant reduction of the average dose by the end of the first week. Therefore the impact of CsA on MPA may be more obvious than in a regimen employing gradual adjustment of CsA dosage according to C0 measurements [16].

Mycophenolic acid was under strong influence of CsA. Attempts to relate MPA concentration to the incidence of rejections in this cohort of patients revealed CsA C2 as a major confounding factor. This was demonstrated by the 'higher' MPA concentration and a 43% rejection rate in patients with median CsA C2 below the target range within the first week post-transplant, in contrast to 19% in patients actually reaching this CsA range, paralleled by a 'lower' MPA exposure (Table 3). Although the difference in MPA plasma concentration between the groups did not reach statistical significance, a significant inverse correlation between CsA C2 and MPA concentration was demonstrated in the group as a whole. This inverse relation between CsA C2 and MPA concentration may complicate any comparison between different immunosuppressive regimens that combine MMF and CsA, as the impact on MPA varies markedly with the CsA C2 levels. Indeed, the attempt to relate MPA trough concentrations to acute rejection episodes turned out with a higher rejection rate in patients within the suggested target range of MPA trough concentration (1.0–3.5 μg/ml) compared with patients below the target range. This paradox may be solely attributed to the inverse relation between CsA C2 and MPA C0.

The relationship between high CsA C2 and low MPA exposure was further demonstrated by the significant correlation between CsA C2 concentrations and MPAG-to-MPA ratio. Inhibition of MPAG secretion into the bile by CsA could explain elevated MPAG in plasma and consequently lowered MPA, probably because of less enterohepatic recirculation [17]. CsA is reported to induce cholestasis by inhibition of multidrug resistance-associated protein 2 (MRP2) thus impairing the bilirubin glucuronide transport into the bile [18]. In response to impaired or inhibited MRP2, up regulation of multidrug resistance-associated protein 3 (MRP3) was recently observed in another study [19], possibly reverting MPAG to the sinusoidal blood in patients combining CsA with MPA [20]. Almost all MPAG is excreted into the urine [3]. MRP2 is also expressed in the apical brush border of the renal proximal tubule [21] and involvement of renal MRP2 in excretion of MPAG to urine is likely. Thus, one would suspect that inhibition of hepatic MRP2 by CsA leads to lower enterohepatic recirculation of MPA while inhibition of renal MRP2 may be responsible for elevations in MPAG leading to higher MPAG-to-MPA ratio in patients combining MMF with CsA. These effects may be more obvious in the present study because of reinforced CsA co-therapy in the early post-transplant phase. The mechanisms also explain the observed lower MPA exposure and higher MPAG-to-MPA ratio in CsA-treated patients compared with the group combining MMF with tacrolimus (Fig. 1b). Another major factor, which in part may have contributed to the difference in MPAG-to-MPA ratio, is poorer renal function in patients receiving CsA than in tacrolimus treated patients. These results should be interpreted with caution as the number of patients in the tacrolimus group were few and in contrast to CsA, tacrolimus was monitored by trough concentrations.

The above mentioned mechanisms may be responsible for the observed increase in MPA concentrations paralleled by MPAG decrease in patients switching from CsA to tacrolimus during the study period. The marked MPA increase experienced by patients switching from CsA to tacrolimus highlights the clinical relevance of this interaction, as this prompted MMF dose reduction in a significant proportion of patients.

Our observation of significantly higher CsA doses in patients not reaching C2 target during the first week demonstrates the rather aggressive approach in order to achieve the target CsA C2 range (Table 3). The slight increase in total MPA concentration from week three post-transplant is probably caused by the reduction of CsA dose in order to adhere to a gradual decline in CsA concentrations as described in the immunosuppressive protocol. As CsA C2 start decreasing from week 2, CsA renders less inhibition on the enterohepatic recirculation, leading to a consistent rise in MPA plasma concentration. A similar relationship was not observed between tacrolimus and MPA trough concentrations. The somewhat delayed CsA C2 maximum compared with the CsA dose maximum (Fig. 2) may be related to the improved CsA absorption reported during the immediate post-transplant period [1].

Another aspect of the CsA C2 protocol is the accompanying subclinical but significant rise in the liver parameters. GGT is mainly located in the microsomes of the

hepatocytes that line the biliary canaliculi, and its plasma level will rise in response to cholestasis [22]. Although the cholestatic effect of CsA is frequently commented in the literature, data on the magnitude are sparse. One study reported elevated GGT levels in response to CsA treatment in heart transplant recipients [23]. Recently, another study reported the relationship between increased liver parameters and lower MPA exposure in renal allograft recipients presumably on C0 monitored CsA or tacrolimus [24]. Our results suggest that the actual mechanism is related to the cholestatic effects of CsA, leading to reduced MPA exposure and in parallel increasing the liver parameters GGT, ALAT and bilirubin. The majority of patients combining MMF with tacrolimus (6 of 10 patients) underwent combined kidney–pancreas transplantation, and in these patients there was no correlation between the concentration of tacrolimus and the liver parameters. The difference in MPAG-to-MPA ratios between tacrolimus and CsA treated patients demonstrates that in contrast to tacrolimus, CsA has a significant impact on the MPA pharmacokinetics (Fig. 1). It should be noted that this comparison was performed between two groups with different representation of diabetic patients. A recent study indicated that this comparison may still be valid, as no difference in MPA pharmacokinetics was demonstrated between diabetic and non-diabetic renal allograft recipients [25].

The findings in the present study highlights the challenge in the interpretation of MPA plasma concentrations in combined immunosuppression therapy, especially in studies exploring the efficacy of MMF in combination with the frequently used calcineurin inhibitors, CsA and tacrolimus. There is a significant difference in MPA levels obtained when MMF is combined with CsA or tacrolimus, respectively. Moreover, the MPA concentrations tend to vary according to CsA C2, which precludes the direct comparison of different immunosuppressive regimens. These findings question the value of MPA monitoring based on predose measurements when MMF is combined with CsA. On the contrary, MPA AUC has been shown to correlate with acute rejection episodes [10,11]. Obtaining a full or even abbreviated AUC is more complicated in the routine setting, therefore the search for a single measurement should continue in order to simplify MPA monitoring. Measurements of MPAG are normally not indicated in renal transplant recipients as this main metabolite does not have any immunosuppressive effect. However, measuring MPAG provides insight into MPA metabolism, which may prove useful in investigations of MPA pharmacokinetics.

In patients experiencing DGF or persistent hypoalbuminemia, the free MPA concentration and the free fraction were consistently higher than in patients with normal graft function. In addition to reduced binding capacity with low albumin, displacement of MPA by elevated MPAG and urea probably increased the free MPA concentrations. Higher free concentration enhance the rate of elimination, leading to lower total MPA levels observed in patients with hypoalbuminemia.

Although the long-term graft survival is reported to be poor in patients experiencing DGF [26], elevated free MPA may contribute some protection from acute rejection episodes. With the reservation of few observations, the incidence of acute rejections in patients experiencing DGF was somewhat lower than the total rejection rate in this study. Browne *et al.* [27] found no difference in short-term outcome related to DGF in patients on triple drug regimen including CsA, MMF and steroids. All patients were able to reach the predefined CsA target of 650 ng/ml (average utilizing 2 and 6 h postdose levels) and MMF dose was maintained at 1 g b.i.d. throughout the first 3 months post-transplant. However, MPA pharmacokinetics was not performed and outcome was related to the maintenance of the overall immunosuppressive treatment [27]. In the present study, patients experiencing DGF and hypoalbuminemia constituted 19% of the population. Because of the discordance between total and free MPA concentrations in this subgroup, free MPA monitoring could be helpful in maintaining an adequate level of immunosuppression.

In conclusion, the dual effects of a CsA C2 monitored regimen, namely a reduced rejection rate with high concentrations and simultaneously a C2 concentration-dependent lowering of MPA exposure, explain the lack of association between predose MPA plasma concentrations and the acute rejection rate. Moreover, this strong confounding effect of CsA represents an obstacle to the efforts toward identification of a therapeutic range for predose MPA concentrations. The cholestatic effects of CsA are probably a major source of the interaction with MPA, demonstrated by significant correlations between CsA C2 and MPAG-to-MPA ratio as well as GGT levels. A similar relationship was not observed in patients treated with tacrolimus. Given the magnitude and clinical relevance of the interaction between CsA and MPA, it is mandatory to include this aspect in any trial or comparison where MPA is a part of the immunosuppressive regimen.

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