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Monitoring of mycophenolic acid and kidney function during combined immunosuppressive therapy

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Abstract

Background: Mycophenolic acid (MPA), a selective inhibitor of lymphocyte proliferation, has lately been used to improve renal function and prolong graft survival in renal transplanted patients. Still, there is no consensus considering the recommended dosing and the therapeutic range of MPA.
Methods: To estimate the safe therapeutic range of MPA, its plasma level and indicators of kidney function were measured in 216 patients (138 male, 78 female, age 46±12 years) 67±46 months after transplantation. Besides MPA, patients received cyclosporine (Group A, n=122) or tacrolimus (Group B, n=77). Seventeen patients (Group C) were treated with MPA in combination with everolimus or sirolimus. Plasma MPA was measured by enzyme inhibition assay.
Results: In the whole study group MPA level increased with the dose of MPA (p=0.013). MPA level was below the therapeutic range in 40% (Group A) and 45% (Group B) of patients, respectively. MPA was 1.9±1.56 mg/L in Group A, 2.4±1.69 mg/L in Group B. In Group A MPA level increased and cyclosporine decreased with the progress of renal disease.
Conclusions: Increasing MPA/cyclosporine ratio at more severe stages of chronic kidney disease was tolerable for the patients and rejection could be avoided. Tubular damage detected by urinary N-acetyl-β-D-glucosaminidase did not correlate with the MPA level.

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Introduction

Mycophenolic acid (MPA) is the pharmacologically active form of the immunosuppressant mycophenolate mofetil (MMF). By inhibiting inosine monophosphate dehydrogenase – the key enzyme in purine biosynthesis – MPA selectively inhibits the proliferation of human T and B lymphocytes, which depend on the de novo pathway for DNA synthesis instead of the alternative salvage pathway. According to current and experimental immunosuppressive drug protocols in kidney transplanted patients, MPA is used during pre- and post-adaptation maintenance, in various combinations with calcineurin inhibitors (cyclosporine or tacrolimus), sirolimus or prednisone (1). Insertion of MMF into the protocols has greatly contributed to the reduction of acute rejection (2, 3). Moreover, a recent meta-analysis found that the minimization or even elimination of calcineurin inhibitors in patients receiving MMF significantly improved renal function and prolonged graft survival (4).

Recently, a new EMIT (Enzyme Multiplied Immunoassay Test) with a strong correlation with HPLC (5–7) has been developed for the determination of plasma mycophenolic acid. In the liver MPA is transformed into its major glucuronide metabolite (MPAG). Among minor metabolites (at least three) acyl-glucuronide is pharmacologically active. The method we used is only capable of measuring total MPA concentration, it cannot distinguish between parent and daughter compounds.

Only free MPA is responsible for immunosuppressive activity, while protein binding can reach 99%. Therefore, conditions causing hypoalbuminemia can alter the pharmacodynamic effect of MPA by increasing the plasma concentration of the free drug (8). In addition to significant inter-individual variability, MPA also demonstrates time-dependent pharmacokinetics (9) and ethnic variability. At increasing MPA concentrations adverse effects may arise (gastrointestinal symptoms, leukopenia, anemia) resulting from MPA-induced inhibition of DNA synthesis and cell proliferation. Former studies showed a strong correlation between therapeutic plasma total MPA trough levels and clinical response (10), which points out the importance of accurate dosing.

According to the literature the personal dosage for mycophenolate mofetil (MMF) should be empirically determined. MMF dosage suggested in combination with cyclosporin A is 2 g/day, when combined with tacrolimus it is 1 g/day.

Although therapeutic drug monitoring (TDM) is not obligatory, patients often complain about side-effects of MMF (nausea, abdominal spasm, diarrhea, leukopenia, anemia and thrombopenia), which diminish as the dose is lowered.

The aim of this study was to investigate the relation between the dosage and blood level of MPA at different stages of chronic kidney disease (CKD). Since cyclosporine may have nephrotoxic side-effects, we estimated the MPA/cyclosporine ratio which is still safe and effective against kidney rejection. Finally, we investigated whether these doses of MPA-cyclosporine combination may cause tubular damage, anemia or leukopenia.

Materials and methods

We retrospectively surveyed the data of 216 patients (138 male, 78 female, age: 46±12 years (mean±SD)), they were studied 67±46 months (mean±SD) after kidney transplantation. During the observation period of 4 months (September 1st–December 31st 2008) no kidney rejection was registered.

Enrollment criteria

Adult patients, who were treated with MPA combined with another immunosuppressive drug after kidney transplantation and suffered from gastrointestinal side-effects and/or anemia (monitored since the 3rd post-operative day) were enrolled in the study.

Exclusion criteria

The exclusion criteria were lack of compliance during treatment, age under 18 years, pregnancy, lactation, history of hepatopathy, severe leukopenia (total leukocyte count <2 G/L) or thrombopenia (<50 G/L) at the time of enrollment, inability to be nurtured and treated orally. Simultaneously added immunosuppressive drugs were cyclosporin A (Group A, n=122), tacrolimus (Group B, n=77), other patients (Group C) received everolimus (n=5) or sirolimus (n=12) with MMF and prednisone. MMF (mycophenolate mofetil) dosage was 0.5–2 g/day depending on the individual patient's tolerance. The 10–15 mg/kg cyclosporin A administration was divided into two doses. Dose was raised when cyclosporine C_2 level decreased below the therapeutic range (400–1400 μg/L).

Tacrolimus was administered orally starting from 0.1 mg/kg every 12 h, to reach the blood trough level therapeutic range of 5–15 μg/L.

Everolimus had an initial dose regimen of 0.75 mg twice a day. Dose was adjusted after 4–5 day intervals until blood trough level reached 3.0–8.0 μg/L.

Initial Sirolimus dose was 2 mg/day; blood trough level therapeutic range was 5–10 μg/L.

For drug monitoring whole blood was collected in K_3EDTA anti-coagulated Becton Dickinson vacutainer tubes (BD, UK) and, if necessary, plasma was separated by centrifugation (15 min, 900 *g*). In the case of MPA sampling was performed before the daily intake of MPA. In Group A peak level of cyclosporine was measured 2 h after medication, if patients entered the outpatient center at the proper time. In some cases, when patients arrived late, the trough level of cyclosporine was checked instead, and therefore these cases were excluded from the statistical analysis of cyclosporin C_2. Trough concentration (C_0) of total plasma MPA was measured by enzyme inhibition assay (EIA, Cobas Integra 800, Roche Ltd). Tacrolimus and sirolimus were determined in K_3EDTA anti-coagulated blood samples (BD, UK) with microparticle enzyme immunoassay (MEIA-IMx, Abbott Laboratories, USA). Cyclosporine A and everolimus levels were measured in K_3EDTA anti-coagulated blood (BD, UK) by fluorescent polarization immunoassay (FPIA-TDx, Abbott Laboratories, USA).

Serum samples were separated by centrifugation (15 min, 900 *g*) from the blood samples collected in native BD tubes containing a gel separator. Renal function was characterized by serum creatinine (compensated Jaffe method for creatinine, Roche Modular P800) and estimated glomerular filtration rate (eGFR). GFR (mL/min/1.73 m^2) were calculated by the 4v-MDRD (four-variable Modified Diet for Renal Disease study group) formula (11–13), as the National Kidney Education Program recommended for the IDMS calibrated creatinine (SCr) method:

$$eGFR = 175 \cdot Age^{-0.203} \cdot (SCr\ 0.0113)^{-1.154} \cdot (0.742 \text{ if female}) \cdot (1.21 \text{ if black})$$

Tubular function was followed by N-acetyl-β-D-glucosaminidase activity (NAG) measured by colorimetric enzyme assay (PPR Diagnostics, London, UK) from the first morning urine collected in plastic native tubes (BD, UK). In order to compensate the diurnal variance in urinary excretion, NAG activity of the first morning urine was related to urinary creatinine (Jaffe kinetic assay, Cobas Integra-800, Roche Ltd, Germany) and their ratio was given as urinary NAG index (14, 15).

The white blood cell (WBC) count and hemoglobin levels were measured in venous blood anti-coagulated with K_3EDTA (Advia 2120, Siemens Ltd). Serum C-reactive protein was determined by immunoturbidimetric method (Modular P800), serum procalcitonin was measured by chemiluminescent immunoassay (Cobas e411, Roche Ltd, Mannheim, Germany). Serum samples were separated from the blood collected in native tube containing gel (BD, UK).

Normality of the drug levels distribution was checked by Kolmogorov-Smirnov test. Drug levels in different CKD stages were compared by Kruskal-Wallis test and ANOVA.

Results

In the whole study group (216 patients) MPA levels increased with the daily dose (Figure 1, Kruskal-Wallis test:

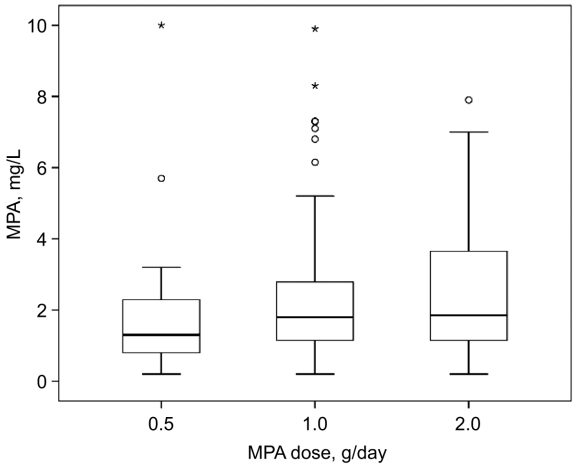

Figure 1 Relationship of MPA levels with daily dose.
In the whole study group (216 patients) MPA level slightly increased with the daily dose. Kruskal-Wallis test: p=0.013. Box and whiskers plot; circles and asterisks label outliers.

$p = 0.013$), and depended on several factors (kidney and liver function, concurrently used drugs, hypoalbuminemia). MPA levels were below the therapeutic range in 40% of the patients in Group A and in 45% of the patients in Group B.

Mean MPA was 1.9 ± 1.56 (SD) mg/L in Group A (target $C_0 > 1.3$ mg/L) and 2.4 ± 1.69 mg/L in Group B (target $C_0 > 1.9$ mg/L). In Group C average value of MPA was 1.78 ± 0.5 (SD) mg/L coadministered with everolimus ($n = 5$) and MPA was 3.6 ± 2.5 (SD) mg/L in patients treated with sirolimus ($n = 12$). The mean values of cyclosporine C_2 (598 ± 174 μg/L), tacrolimus (6.8 ± 2.92 μg/L) and everolimus (5.6 ± 3.7 μg/L) were in the therapeutic range; the only exception was sirolimus (3.7 ± 2.4 μg/L), which was under the therapeutic range (5–10 μg/L).

MPA levels of patients treated with a cyclosporine-MPA combination were different at various stages of CKD. In Group A trough level of MPA significantly increased (Kruskal-Wallis test: $p = 0.012$), while peak level of cyclosporine decreased (ANOVA: $p = 0.039$) in more severe CKD stages (Figure 2). In the case of renal function impairment cyclosporine dose was lowered and MPA dose was raised to avoid side-effects. Therefore, the MPA/cyclosporine ratio was significantly higher at the stages of CKD3 and CKD4, compared to CKD2 (Figure 3, Kruskal-Wallis test: $p = 0.015$ and 0.04). Increasing MPA/cyclosporine ratio in more severe stages of chronic renal disease was tolerable for the patients and rejection could be avoided.

The dose adjusted according to the glomerular function was tolerable for the patients and proved to be safe as no kidney rejection was detected during the study.

Potential cytotoxic side-effects of immunosuppressive drugs were also assessed by the WBC count and hemoglobin level. Mean WBC count was 8.43 ± 2.75 G/L in Group A and 8.68 ± 2.89 G/L in Group B (reference range: 4.8–10.8 G/L), slightly decreased WBC count was detected in 2% of these patients. In Group C, WBC count was normal in all patients with an average 7.60 ± 1.73 G/L value. In Group A, CRP was slightly elevated in 9% of the patients (3.8 ± 4.8 mg/L) and procalcitonin was in the reference range in 98% of the patients (0.3 ± 0.1 μg/L). Although individual hemoglobin values compared to the sex-dependent reference ranges (female: 120–160, male: 135–170 g/L) showed slight anemia in 49% of the patients (128 ± 21 g/L) in Group A, hemoglobin or the number of red blood cells did not correlate with MPA. So we concluded that mild anemia may be the consequence of kidney disease and independent of the MMF treatment. Although elevated urinary NAG index (> 0.7 μmol/min/mmol) occurred in 79% of patients in Group A, which indicated tubular damage, NAG indices did not correlate with MPA levels. We suppose that other factors may contribute to the tubular damage (complication of transplantation, side-effects of concurrently applied immunosuppressive drugs).

Discussion

On completion of kidney transplantation there are strict clinical regimens for immunosuppressive therapy. When the combined dosage of drugs is established or modified, the first aim is to avoid kidney rejection and to minimize kidney damage. MPA blood level depends on the dosage of the used co-drug that can modify the enterohepatic recirculation of MPA. Cyclosporine inhibits the recirculation of MPA, with the starting dose of 2×1.5 g/day the target MPA value is

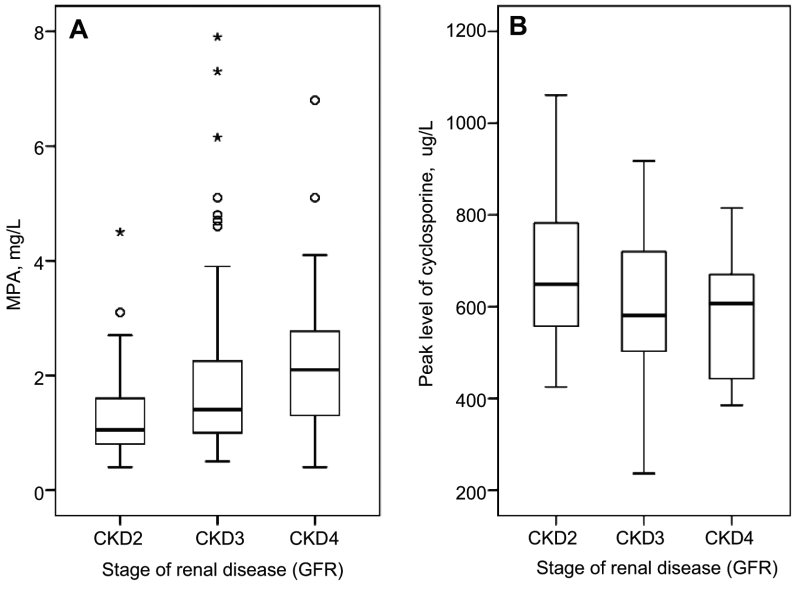

Figure 2 MPA level and peak level of cyclosporine in Group A.
MPA level significantly increased (Kruskal-Wallis test: $p = 0.012$), and peak level of cyclosporine significantly decreased (ANOVA: $p = 0.039$) with renal impairment in Group A. The stage of chronic kidney disease (CKD) was estimated on the base of GFR (4v-MDRD).

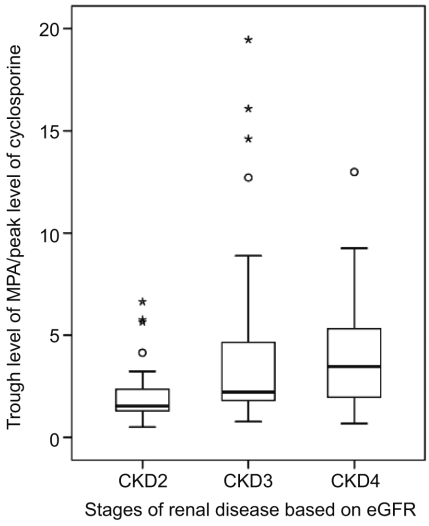

Figure 3 Ratio of MPA trough level/cyclosporine peak level in Group A.
Ratio of MPA trough level/cyclosporine peak level (units: μg/L for both) was significantly higher at the stages of CKD3 and CKD4 compared to CKD2 in Group A (Kruskal-Wallis test: $p=0.015$ and 0.04).

$C_0 > 1.3$ mg/L. As tacrolimus does not inhibit the recirculation of MPA (8) with the starting dose of 2×1 g/day the target value is higher, $C_0 > 1.9$ mg/L. Our results were in agreement with these requirements, the mean MPA level was lower in Group A (1.9 mg/L) than in Group B (2.4 mg/L). Although the mean MPA concentration (1.9 mg/L) was above the target value (1.3 mg/L) in Group A, 40% of the patients had MPA levels lower than required. The increase in MPA level and the decrease in cyclosporine with the progression of renal impairment can be the consequence of both the dosage adjustment according to CKD stages and the change in the renal elimination, as MPA is excreted mainly by the kidneys. The individual variations in MPA levels may be connected to the renal and liver function, body mass and genetic polymorphisms in the enzymes and other proteins responsible for drug metabolism and transport (16, 17). Since 2008, TDM of MPA is not suggested as a routine test after renal transplantation (8, 18, 19). The considerable individual variance in blood level observed in this study underlines the fact that MPA level depends on several factors. Our results suggest that the applied doses for immunosuppression were safe. Dosage adjustment according to the kidney function can be characterized by the ratio of MPA trough level/cyclosporine peak level. When the ratio increased from 1.53 (stage CKD 2, median value) to 3.48 (stage CKD 4), kidney rejection could still be prevented and the therapy was more tolerable because of the decrease in gastrointestinal and hematological side-effects. Although we observed a slight correlation between the dosage and MPA level, the wide range of blood levels necessitates individual therapy, taking kidney function and the patient's general clinical state into consideration (20). As a long-term strategy, side-effects and cost minimization should be evaluated together with the actual kidney function.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
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