Mycophenolic Acid Metabolite Levels in Pediatric Liver Transplantation: Correlation with a Limited Sampling Strategy

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KEY WORDS: pediatrics, liver transplantation, mycophenolate mofetil, metabolites

ABSRACT

Background: Although mycophenolate mofetil (MMF) has proven efficacy in preventing solid organ allograft rejection in adults, well-established dosing practices have yet to be established in children. This has led to the notion of developing therapeutic drug-monitoring techniques based on measuring the active mycophenolic acid (MPA) metabolite.

Aim: The aim of this study is to establish an effective measure of plasma MPA metabolite levels based on a limited sampling strategy in pediatric liver transplant recipients. Plasma MPA levels were also correlated with conventional MMF dosing practices and concomitant immunosuppression.

Methods: Plasma MPA metabolite levels were measured in 41 (23 female, 18 male) patients post (>7days) liver transplant from 2 major pediatric transplant centers by either a high performance liquid chromatographic or EMIT™ monitoring assay technique. The formal plasma MPA AUC was compared to an estimated MPA AUC by regression analysis.

Results: Plasma MPA $AUC_{(0\text{-}8\ h)}$ metabolite levels correlated well with a limited sampling strategy based on the following equation: $9.1 + 5.7 \times C_0 + 1.1 \times C_{40\ min} + 2.1 \times C_{2\ h}$ ($R = 0.74$). There was a wide inter-patient variability in plasma MPA AUC metabolite levels despite conventional drug dosing practices. Patients on concomitant cyclosporine required higher

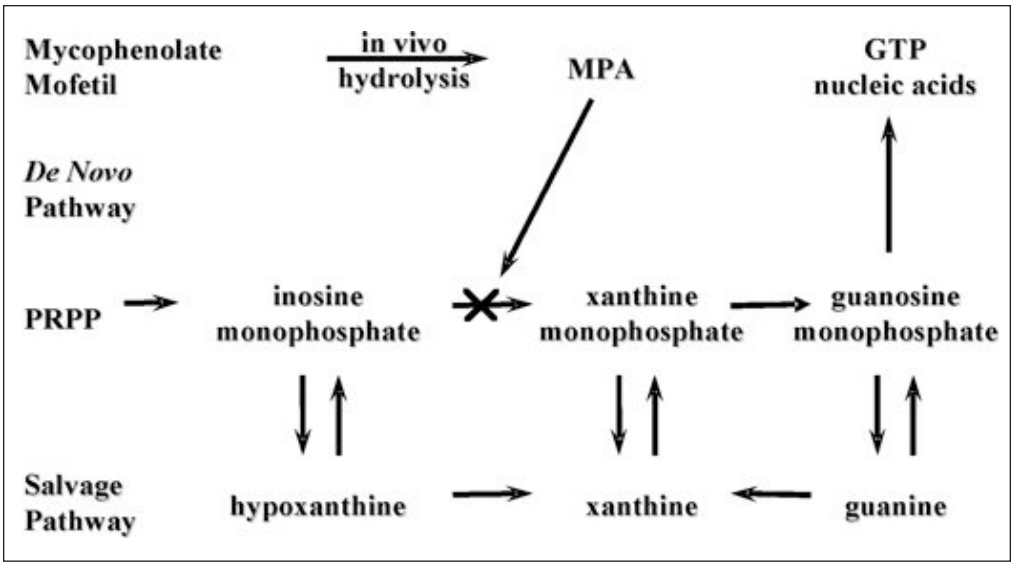

Figure 1. Mycophenolate mofetil (MMF) metabolism. Mycophenolate mofetil is converted to its active mycophenolic acid (MPA) metabolite. Mycophenolic acid inhibits inosine monophosphate dehydrogenase (IMPD), a key enzyme in the de novo synthesis of guanosine nucleotides (GTP).

mean (SEM) doses (548 [71] mg/day) of MMF compared to patients on tacrolimus (285 [71] mg/day) therapy ($P < 0.02$).

Conclusion: Plasma MPA metabolites can be monitored in children post liver transplantation based on a limited sampling strategy. Future studies are needed to determine whether MMF therapy can be effectively tailored to improve overall clinical response based on the notion of therapeutic MPA metabolite monitoring.

INTRODUCTION

Mycophenolate mofetil (MMF) is well-known for its immunosuppressive and lymphocytotoxic properties in the management of solid organ allograft transplant recipients.[1,2] In combination with cyclosporine and corticosteroids, MMF has been shown to prevent acute cellular rejection in patients post-kidney[2,3] and liver transplantation,[4] and in reversing allograft rejection in patients refractory to high-dose corticosteroids.[5] Furthermore, MMF has also been shown to avert the need for anti-CD3 therapy and spare cyclosporine use in both kidney and liver allograft recipients.[6-8] Although MMF has proven efficacy as an adjunct immunosuppressant in transplantation, some patients are susceptible to drug-induced toxicity, thereby raising concerns that inherent polymorphism in MMF metabolism may influence clinical responsiveness to therapy.[9,10]

Mycophenolate mofetil is the 2-morpholinoethyl ester of mycophenolic acid (MPA), a potent and selective noncompetitive inhibitor of inosine monophosphate dehydrogenase (IMPD), a key enzyme in the de novo synthesis of guanosine nucleotides. Since B and T lymphocytes cannot utilize the salvage pathway of purine biosynthesis, MPA is believed to provide a selective immunosuppressive effect on lymphocytes (Figure 1).[11] However, MMF-induced leucopenia is a noteworthy complication of maintenance therapy that may in part be dependent on inherent polymorphisms in IMPD enzyme activity. Moreover, inter-patient differences in drug metabolism has also classified MMF as a critically dosed immunosuppressant, thereby raising the notion of

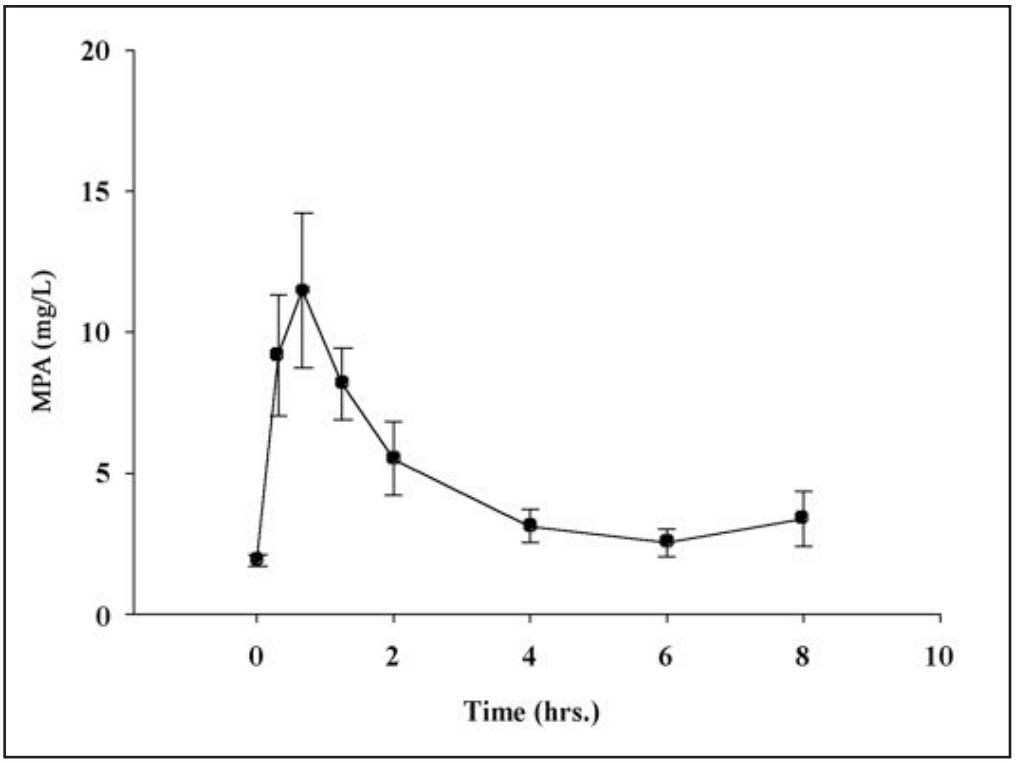

Figure 2. The pharmacokinetics of mycophenolic acid (MPA) in pediatric liver transplant recipients. Pediatric liver transplant recipients show a bimodal distribution of plasma MPA with a wide inter-patient variability (mg/L).

developing therapeutic drug monitoring in solid organ transplant recipients based on the measurement of plasma MPA metabolite level.[12-14]

Mycophenolate mofetil is almost completely absorbed when given orally and is rapidly transformed to MPA in the plasma. Mycophenolic acid is mainly bound to albumin and is metabolized by the liver into its inactive metabolite MPA glucuronide (MPAG).[14] Recent studies have shown that when combined with MMF, cyclosporine enhances the renal excretion of MPAG in kidney transplant recipients. In these patients, higher dosages of MMF are generally required to achieve optimal graft survival.[15] In comparison, concomitant cholestyramine use will interfere with the enterohepatic recirculation of MPAG thereby facilitating a 40% drop in plasma MPA metabolite levels in kidney transplant recipients.[16] Furthermore, drugs that bind albumin could potentially displace MPAG and increase free MPA metabolite levels and render patients at risk for toxicity.

Although experience in heart and kidney transplantation would support the use of MMF as an adjunct immunosuppressant in children, current dosing practices in pediatrics have been based on the adult experience and may contribute to the increased risk of MMF-induced leukopenia observed in clinical practice.[17] Furthermore, the increased risk of lymphoproliferative disease cannot be understated in Epstein-Barr virus-naïve patients.[18] In view of wide inter-patient variability in MMF metabolism, the impact of concurrent drug therapy on MMF metabolism, and the variability in dosing practices in pediatric transplantation, several institutions, including our

Table 1. Pediatric liver transplant recipients.

	Baltimore	King's College (London)
Patients, n	20	21
Median age, years (range)	4.0 (0.5-17.2)	6.2 (1.2-16.5)
Mean weight, kg (SEM)	26.7 (4.3)	33.6 (4.2)
Mean height, cm (SEM)	113.8 (7.1)	127.7 (5.5)
Mean surface area, m^2 (SEM)	0.9 (0.1)	1.1 (0.1)

own, have now adopted therapeutic drug monitoring in clinical practice.

On account of significant enterohepatic recirculation, and the fact that MPA excretion does not follow first-order kinetics, trough plasma MPA levels are inadequate to establish a therapeutic window of treatment efficacy and toxicity. In comparison, formal area-under-the curve (AUC) determinations are laborious and time consuming, and have led several investigators to develop a limited sampling strategy that would provide a practicable therapeutic monitoring approach in all transplant recipients, including children, on maintenance MMF therapy.[19] While this approach has been proposed and studied in pediatric renal transplant recipients, the impact of renal clearance on plasma MPA metabolite levels cannot be underscored. It is the purpose of this study to validate a limited sampling strategy in pediatric liver transplant recipients independent of renal function by comparing a formal MPA $AUC_{0\text{-}8\ h}$ with an extrapolated AUC using 2 well-established MPA measurement techniques adopted at The Johns Hopkins Children's Center in Baltimore and The King's College Pediatric Transplant Centre in London, England. Our secondary goals included a comparison of the effect of concurrent immunosuppressants, including tacrolimus and cyclosporine on MMF metabolism. The inherent differences in drug levels between patients may be explained by these polymorphisms, and should be the aim of future studies.

PATIENTS AND METHODS

Patients

As part of a multicentered open-labeled study into the pharmacokinetics of MMF metabolism in pediatric (age <18 years) patients post cadaveric or living related liver transplant, a total of 41 $AUC_{0\text{-}8\ h}$ were determined in 41 children on MMF in combination with stable doses (>7 days) of either cyclosporine or tacrolimus therapy. In total, 20 children (12 female, 18 male) with a median (range) age of 4.0 (0.5-17.2) years were recruited from The Johns Hopkins Children Transplantation Center, and 21 children (11 female, 10 male) with a median (range) age of 6.2 (1.2-16.5) years from the The King's College Pediatric Transplant Centre in London, England. There was no apparent statistical difference in mean patient weight, height, and surface area among each study population (Table 1). Each patient was enrolled after informed written consent was obtained by each patient's respective parent or guardian, and verbal assent from each age-appropriate child. Each respective institution's Ethical Review Board provided written approval for the study.

Patient demographic data, current medical history, including medications and dosage, past medical history, salient physical findings (height and weight), and biochemical parameters at the time of plasma MPA AUC measurement were all tallied for comparison. Each patient had blood drawn at specified time intervals (0, 20, 40, and 75 minutes

Table 2. The influence of adjunct immunosuppressive agents on plasma mycophenolic acid (MPA) metabolite levels.

	Baltimore	King's College (London)	
Adjunct Agent	FK506 (n = 20)	FK506 (n = 11)	CSA (n = 10)
Mean MMF dose, mg/day (SEM)	219 (40)†	285 (45)*	548 (71)*
		407 (59)†	
Median MPA levels, mg/L (range)	24 (18-50)	29 (18-50)	42 (22-54)

*$P < 0.02$, compares the mean doses between the FK506, and the CSA treatment groups at King's College.
†$P < 0.001$, compares the mean doses between the patients from Baltimore and all the patients from King's College.

and 2, 4, 6, and 8 hours) after the first morning dose of MMF. A urine sample was also obtained in order to provide an estimate of glomerular filtration rate (GFR) based on the Schwartz equation.[20] Since GFR is recognized to contribute to inter-patient variability in plasma MPA AUC in kidney transplantation recipients, all patients with a low (<50 mL/min/m^2) GFR for non-transplant related hepatic pathology were excluded from participating in the study.

Plasma MPA Metabolite Levels

Each patient had blood (0.5 mL) drawn in EDTA-treated tubes at 0, 20, 40, and 75 minutes, and 2, 4, 6, and 8 hours after their morning dose of MMF. Among the patients treated at The Johns Hopkins Children's Transplant Center, plasma MPA metabolite levels were measured by a modified high performance liquid chromatographic (HPLC) technique originally adapted by Li and coworkers.[21] In brief, an internal standard of MPA was obtained from Roche Pharmaceuticals (Palo Alto, California) and was used to develop the existing assay and to establish standardized plasma MPA concentration curves. Plasma was collected by centrifugation at 2000g for 10 minutes and stored at -70°C prior to analysis. To 0.5 mL of plasma, 100 mL of prednisone solution (50 pmole/L methanol) was added as an internal standard in combination with 2 mL of 0.06 mole/L HCL. The mixture was then vortexed for 15 seconds and loaded on to a C-18 solid phase extraction column. The sample was then washed with 1 mL of water and then eluted with 10 mL of elution reagent (80% v/v methanol in 0.1 mole/L acetate buffer, pH 4.0.) by vacuum extraction. A Waters HPLC system was used, including pumps, detector, column oven, auto injector, and computer interface. The column used was a C-18 Novopak HPLC column (4.6 mm × 250 mm). Chromatography was carried out at 40°C with a flow rate of 1 mL/min, and monitored with a UV wavelength of 254 nm. An isocratic mobile phase was composed of acetonitrile:tetrahydrofuran:H_2O (47.5:2.5:50%). The total run was 20 minutes. A calibration curve was plotted of the ratio of the peak height for MPA against the concentration of MPA. The slope was then used to determine the MPA concentration from our patients' plasma samples. Among the patients treated at The King's College Pediatric Transplant Centre, plasma MPA levels were measured by the EMIT-MPA assay, as described elsewhere.[22]

Plasma AUC Measurements

Plasma MPA AUC levels were calculated for each patient's pharmacokinetic profile (Table 2) using the trapezoid rule.[23]

Statistics

A formal MPA plasma AUC measurement was achieved through the well-established trapezoid method previously described. This formal calculation was

compared by simple regression with an extrapolated MPA AUC based on the equation AUC = 5.2 × 7.1 × C_0 + 1.0 × $C_{75\ min}$ + 5.4 × $C_{6\ h}$ previously described in pediatric renal transplant recipients on MMF therapy.[19] The extrapolated AUC was also compared with a second equation (AUC = 9.1 + 5.7 × C_0 + 1.1 × $C_{40\ min}$ + 2.1 × $C_{2\ h}$) as adopted elsewhere. Simple parametric *t*-tests were used to compare physical and biochemical parameters.

RESULTS

Patients

Among the 21 patient recruited from the The King's College Pediatric Transplant Centre, 11 were on primary tacrolimus and 10 were on cyclosporine-based immunosuppressive therapy. Patients on cyclosporine required significantly ($P < 0.02$) higher mean (SEM) doses of MMF (548 [71] mg/day) than patients on primary tacrolimus therapy (285 [71] mg/day). In comparison, patients treated at The Johns Hopkins Children's Transplant Center were on a significantly ($P < 0.001$) lower overall mean (SEM) dose of MMF (219 [40] mg/day) compared to the The King's College Pediatric Transplant Centre patients, despite no noted differences in mean population height, weight, and surface area (Table 1). The difference in MMF doses was shown to be attributed to the higher MMF dose required to treat liver transplant recipients on combination cyclosporine therapy. There was no apparent difference in MMF dose among the tacrolimus-treated patients in either pediatric patient population. All patients had normal GFR, as assessed by the Schwartz method.

Plasma MPA Metabolite Levels

A typical pharmacokinetic profile of plasma MPA metabolite levels is illustrated in Figure 2. Peak plasma MPA metabolite levels are achieved at 40 minutes post-MMF dose. Thereafter, there is a progressive decline in plasma MPA levels that follow first-order kinetics up and until the point of achieving the second peak at approximately 8 hours post-MMF dose. The second peak is representative of the enterohepatic recirculation of MPA.

There was significant inter-patient variability in plasma MPA AUC measurements in both patient populations and within each of the 2 separate drug-monitoring techniques. While the dose of MMF associated well with plasma MPA AUC metabolite levels, there was no significant differences in median (range) MPA AUC levels (Table 2).

No patient incurred MMF associated toxicity, including leucopenia.

Extrapolated Plasma MPA AUC Metabolite Levels

The formal plasma MPA AUC is compared with an extrapolated AUC:

AUC = 9.1 + 5.7 × C_0 + 1.1 × $C_{40\ min}$ + 2.1 × $C_{2\ h}$ (R = 0.74)
AUC = 5.2 + 7.1 × C_0 + 1.1 × $C_{75\ min}$ + 5.4 × $C_{6\ h}$ (R = 0.88)

DISCUSSION

This study further supports the notion that MMF is a critically dosed drug in pediatric liver transplantation. By using a multicentered approach, pediatric liver transplant recipients were shown to demonstrate an inherent variability in MMF metabolism that was independent of age, weight, and height. Furthermore, our study also underscored the value of maintaining an MMF dosing practice based on the adult experience. Indeed, inter-patient differences in plasma MPA metabolite levels were shown to be independent of patient surface area, thereby questioning the application of conventional drug dosing practices. Interestingly, despite obvious surface to volume differences, the very young (age

<1 year) transplant recipients were shown to achieve a lower overall plasma MPA AUC metabolite level compared to adolescents. Future controlled studies are needed to determine if an optimized treatment strategy based on the measurement of plasma MPA AUC metabolite levels can be used to optimize drug therapy in pediatric liver transplant recipients with the aim at improving overall graft survival and facilitate tacrolimus sparing.

The effect of concurrent immunosuppressants, including cyclosporine on MMF metabolism, was noteworthy and further supports the notion of drug monitoring in liver transplantation. This study would also support the role for therapeutic monitoring in establishing a therapeutic window of efficacy and toxicity based on the measurement of plasma MPA metabolite levels. Our patients on concomitant cyclosporine typically required higher overall doses of MMF in order to achieve the same plasma MPA levels measured in those patients on tacrolimus therapy. Although there were no complications noted among those patients on the higher doses of MMF, the potential risk of MMF-induced leukopenia cannot be underscored. The apparent lack of observable MMF-associated side effects in our patient population may have been secondary to a selection bias, since only those patients on stable doses of MMF were preferentially recruited for drug monitoring.

Mycophenolate mofetil-induced leukopenia represents the primary clinical indication for discontinuing MMF therapy among our pediatric liver transplant recipients (personal observation). Moreover, failure to achieve effective immunomodulation on standard doses of MMF would necessitate higher maintenance doses of cyclosporine. This would preclude the possibility of achieving a cyclosporine-sparing treatment strategy. Failure to do so may indeed increase the overall risk for lymphoproliferative disease in Epstein-Barr virus-naïve liver transplant recipients.

Our study has also shown that a limiting sampling strategy could be adopted in estimating plasma MPA AUC metabolite levels. An extrapolated plasma MPA AUC would be conducive to monitoring MPA levels in the very young (age <1 years) due to the limitation on the amount of blood that can be drawn at one time, as well as providing a means of monitoring children on an outpatient basis. Furthermore, our study has validated the limited sampling strategy by comparing the pharmacokinetics of MPA metabolism in 2 pediatric patient populations using 2 well-established MPA monitoring techniques. Future studies are required to apply these monitoring techniques into clinical practice.

In summary, inherent polymorphism in MMF metabolism influence plasma MPA metabolite levels in pediatric liver transplant recipients, and may impact on clinical responsiveness to conventional doses of MMF therapy. The influence of polypharmacy on MMF metabolism cannot be underscored. Future drug optimization studies are now in progress at our respective institutions in the hope of developing a therapeutic window of treatment efficacy and toxicity based on the measurement of plasma MPA metabolite levels.

ACKNOWLEDGMENTS

This study was supported in part by a research award by Roche Pharmaceuticals (CC).

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