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ORIGINAL ARTICLE

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Abbreviated mycophenolic acid AUC from C0, C1, C2, and C4 is preferable in children after renal transplantation on mycophenolate mofetil and tacrolimus therapy

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Abstract In order to allow a similar algorithm to be used for both adults and children on tacrolimus-based and mycophenolate mofetil [MMF, a pro-drug for mycophenolic acid (MPA)]-based immunosuppression, a limited sampling technique from the trough level (C0) and the levels 30 min (C0.5) and 2 h (C2) after intake was to be developed from MPA area under the time–concentration curves (AUC). We retrospectively analyzed 49 full ten-point pharmacokinetic (PK) profiles from 29 pediatric patients on MMF and tacrolimus. We used stepwise multiple regression analysis to calculate limited sampling approaches. Agreement with the AUC was tested by means of Bland and Altman analysis. The correlation between AUC and pre-dose trough concentration was $r^2 = 0.5188$ ($P < 0.0001$) and between AUC and post-dose trough concentration $r^2 = 0.6924$ ($P < 0.0001$). The next best correlations were with 2 h (C2, $r^2 = 0.6711$, $P < 0.0001$), 4 h (C4, $r^2 = 0.6411$, $P < 0.0001$), 1.5 h (C1.5, $r^2 = 0.6344$, $P < 0.0001$), and 6 h (C6, $r^2 = 0.6219$, $P < 0.0001$). Three-point estimates at C0, C0.5, and C2 resulted in an acceptable correlation between predicted AUC and AUC from the full profile when we used the formula AUC = 10.01391 + 3.94791×C0 + 3.24253 ×C0.5 + 1.0108×C2, Pearson's $r = 0.8996$, 95% confidence interval 0.8277–0.9424. However, even better results could be obtained when we used AUC = 8.217 + 3.163×C0 + 0.994 ×C1 + 1.334×C2 + 4.183 ×C4, Pearson's $r = 0.9456$, 95% confidence interval 0.9051–0.9691. Bland and Altman analysis revealed good agreement between AUC predicted from C0, C0.5, and C2 and AUC from the full profile, but was inferior to the four-point approach. Also, the previously reported formula derived for adults was not usable in these patients. A special formula must be used for children. The AUC of MPA can be predicted by limited sampling including C0, C0.5, and C2, while an approach using C0, C1, C2, and C4 is preferable.

Keywords Mycophenolate mofetil · Mycophenolic acid · Tacrolimus · Pharmacokinetics · Area under the curve · Children

Introduction

Over the last decade tacrolimus [1] has gradually been replacing cyclosporine. Similarly, mycophenolate mofetil (MMF), which acts by impairment of de novo purine synthesis [2] via inhibition of inosine monophosphate dehydrogenase (IMPDH) [3], has been replacing azathioprine. In fact, many pediatric centers now use this combination therapy, although pediatric dosing has not yet been fully established.

Pharmacokinetic (PK) monitoring is needed either in the case of a narrow therapeutic window (i.e., when the range between toxicity and sub-therapeutic levels is only small) or when drug levels are unpredictable in patients because of inter-individual or intra-individual variation. This applies for both tacrolimus and MMF. The area under the time–concentration curve (AUC) most closely resembles a patient's drug exposure, and, typically, pre-dose trough levels (C0) are measured because there is usually a good correlation between the AUC and C0. The need for PK monitoring of tacrolimus in children is well established, and there is reasonably good correlation between the AUC and trough level [4].

MMF has been shown to reduce the frequency of rejection in renal transplantation [5]. MMF is a pro-drug of mycophenolic acid (MPA) that can be measured by means of HPLC or the enzyme-mediated immunoassay technique (EMIT). Few data are available on the dosing of MMF in pediatric kidney transplantation, and the data are predominantly on MMF in combination with cyclosporine [6, 7]. Very little data exist on the interaction between tacrolimus and MMF in pediatric patients, although it has become clear that dosing might be influenced by concomitant medication and that there is substantial inter-individual variation [8, 9]. Because of drug interaction between cyclosporine and MMF, lower dosing of MMF is needed in combination with tacrolimus when compared to cyclosporine [10]. For these reasons, PK monitoring of MMF therapy in children is mandatory. Therapeutic drug monitoring (TDM) of MMF is not generally accepted for the treatment of adult patients; however, there is increasing evidence that TDM might help diminish both short-term and long-term side effects of MMF [11, 12]. Pharmacokinetic monitoring of MPA trough levels is unsatisfactory, and for PK assessment of an abbreviated AUC at least three time points are required [13]. The best parameters for the assessment of the MPA AUC appear to be C1, C2, and C6 [13]. More recently, an even better approach was derived from the use of C0, C1, C2, and C4 [14]. However, there has been no investigation to date to analyze whether an abbreviated AUC can also be derived from C0, C0.5, and C2 as has been shown to be accurate in adults [15]. We therefore embarked on this retrospective study.

Patients and methods

Patients

Twenty-nine pediatric patients receiving tacrolimus in combination with MMF were investigated. A total of 49 full ten-point PK profiles on tacrolimus with concomitant MMF were analyzed retrospectively. All PK profiles were obtained from pediatric renal transplant patients in steady state. Patients had a median of two full PK profiles (range 1–5). Eight patients had a primary therapy with this combination, and their first PK profile was performed a median 23 days after transplantation. Eight patients received the combination therapy for vascular rejection, and both drugs were started simultaneously. Eight patients had developed chronic cyclosporine toxicity on a cyclosporine- and MMF-based therapy, so the cyclosporine was replaced by tacrolimus, and, finally, two patients had developed rejection episodes on tacrolimus- and azathioprine-based immunosuppression, and MMF replaced the latter.

The mean age of the patients was 11.9 ± 4.9 years (range 1.8–19.7 years) at the time of the first PK profile; thus, the entire pediatric age range was covered in the study. The mean age of the patients at transplantation was 10.3 ± 4.9 years (range 1.2–18.2 years). Liver disease was absent and liver function tests were normal in all patients.

All patients underwent TDM after establishment of a stable trough concentration. All patients had at least one full pharmacokinetic profile after a median 192 days following transplantation. PK profile determination was standard care; thus, no written consent was obtained. The analysis of the data for this study was performed retrospectively.

Pharmacokinetic monitoring

Tacrolimus whole-blood concentration was measured by means of the Abbott tacrolimus II assay. MPA was measured by an automated EMIT assay (Dade Behring) [16]. Pharmacokinetic profiles were obtained after an intravenous cannula had been inserted and a baseline trough level at 7 a.m. had been obtained. The patients were then asked to take their usual morning dose of tacrolimus and MMF, and immediately thereafter they had a standard breakfast. The manufacturers generally recommend that the drugs be taken on an empty stomach at least 1 h before meals. However, this time interval does not reflect the everyday routine at home, especially in teenagers. The objective of the monitoring was to establish a setting that resembled the situation at home as closely as possible. Patients had free access to non-dairy product drinks during the day (except grapefruit and orange juice) and had a normal lunch at noon. In addition to the pre-dose trough concentration (C0), 2-ml EDTA whole-blood samples were taken for duplicate measurements of tacrolimus and MPA concentration at 0.5, 1, 1.5, 2, 3, 4, 6, 8, and 12 h, respectively, for a full ten-point, 12-h PK profile. No saline was injected into the cannula as it was sealed after each blood sampling with a sterile heparinized mandrin instead. The AUC was calculated according to the trapezoidal rule.

Statistics

Continuous data were tested for normal distribution by use of the Kolmogorov–Smirnov test. All data are presented as mean ± SD for normally distributed data and as median and range for not normally distributed data. Student's *t*-test was used for normally distributed variables, and the Mann–Whitney test for not normally distributed continuous variables. Standard correlation analysis and

linear regression analysis were also performed. All statistical analyses were performed with GraphPad Prism Software for Science Version 3.0 (San Diego, Calif., USA) or Medcalc, Version 6.14.000 (Mariakerke, Belgium). Agreement between methods was tested by means of Bland and Altman plot. The Bland and Altman plot is a statistical method used to compare two measurement techniques. In this graphical method the differences (or, alternatively, the ratios) between two techniques are plotted against the averages of the two techniques. Horizontal lines are drawn at the mean difference and at the mean difference ± 1.96×SD of the differences. If the differences within mean ± 1.96×SD are not clinically important, the two methods may be used interchangeably. To compare the Bland and Altman analysis plots derived from the two abbreviated methods, we used a mountain plot. A mountain plot (or "folded empirical cumulative distribution plot") is created by computation of a percentile for each ranked difference between a new method and a reference method. For a folded plot to be obtained, the following transformation is performed for all percentiles above 50: percentile = 100 percentile. These percentiles are then plotted against the differences between the two methods [17].

Results

We first investigated whether there was a difference when only one PK profile per patient was used in comparison to the pooling of all available 49 PK profiles, and there was no significant difference between the slope of the regression lines for the trough level ($P=0.427$ for slope, $P=0.98$ for elevation of the regression line) and the full AUC. Therefore, the data were pooled. Figure 1 shows the mean and 95% confidence intervals for the MPA concentrations after oral intake. The mean MPA AUC was 57.6 ± 28.8 mg×h/l, and the mean tacrolimus AUC was 124.2 ± 35.6 µg×h/l.

The correlation between AUC and pre-dose trough concentration was $r^2=0.5188$ ($P<0.0001$) and between AUC and post-dose trough concentration $r^2=0.6924$ ($P<0.0001$). The next best correlations were with 2 h (C2, $r^2=0.6711$, $P<0.0001$), 4 h (C4, $r2=0.6411$, $P<0.0001$), 1.5 h (C1.5, $r^2=0.6344$, $P<0.0001$), and 6 h (C6, $r^2=0.6219$, $P<0.0001$). Three-point estimates at C0, C0.5, and C2 resulted in an acceptable correlation between predicted AUC and AUC from the full profile when we used the formula AUC = 10.01391 + 3.94791×C0 + 3.24253×C0.5 + 1.0108×C2, Pearson's $r=0.8996$, 95% confidence interval 0.8277–0.9424 (Fig. 2). However, even better results could be obtained when we used AUC = 8.217 + 3.163×C0 + 0.994×C1 + 1.334×C2 + 4.183×C4, Pearson's $r=0.9456$, 95% confidence interval 0.9051–0.9691 (Fig. 3). Bland and Altman analysis revealed good agreement between predicted AUC based on C0, C0.5, and C2 and AUC from the full profile (Fig. 4), but was inferior to the four-point approach when the mountain plot analysis (Fig. 5) was used.

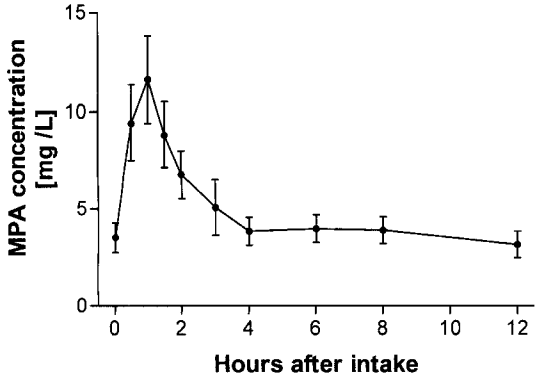

Fig. 1 MPA concentration in 49 pharmacokinetic profiles in steady state from 29 pediatric renal transplant patients on combination therapy with mycophenolate mofetil and tacrolimus

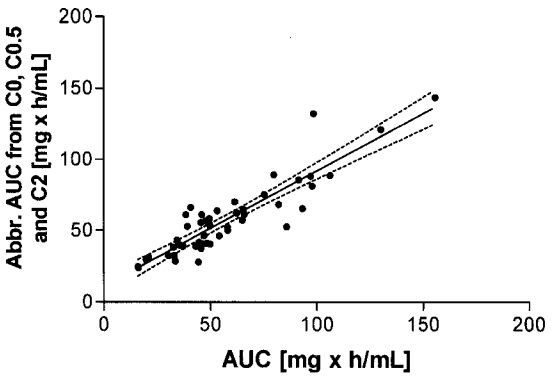

Fig. 2 Relationship between the abbreviated pharmacokinetic profile derived from C0, C0.5, and C2 and the corresponding regression line with the 95% confidence interval. The following formula derived from multiple stepwise linear regression analysis was used: AUC = 10.01391 + 3.94791×C0 + 3.24253×C0.5 + 1.0108×C2, Pearson's $r=0.8996$, 95% confidence interval 0.8277–0.9424

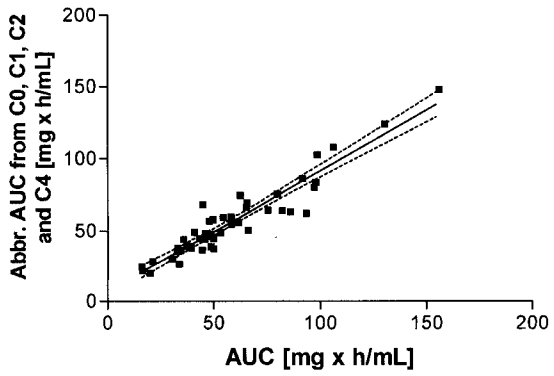

Fig. 3 Relationship between the abbreviated pharmacokinetic profile derived from C0, C1, C2, and C4 and the corresponding regression line with the 95% confidence interval. The following formula derived from multiple stepwise linear regression analysis was used: AUC = 8.217 + 3.163×C0 + 0.994×C1 + 1.334×C2 + 4.183×C4, Pearson's $r=0.9456$, 95% confidence interval 0.9051–0.9691

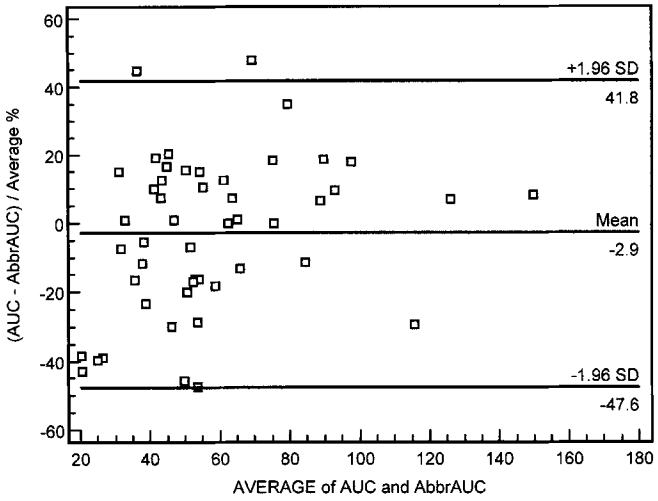

Fig. 4 Bland and Altman analysis testing agreement between the abbreviated AUC derived from C0, C0.5, and C2 and the full ten-point AUC. There was an average error of 2.9%, and while most values were within an error margin of 20%, the outliers of up to 40% might not be clinically acceptable

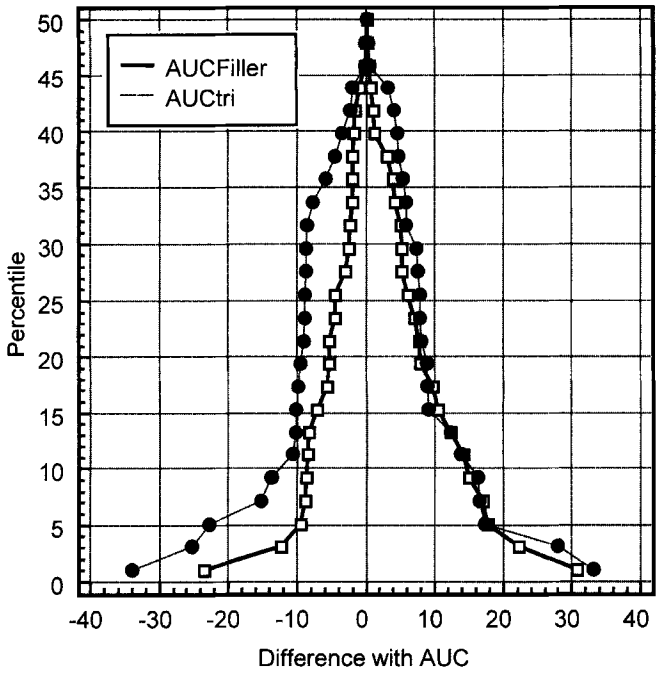

Fig. 5 Mountain plot analysis testing agreement between the abbreviated AUC derived from C0, C0.5, and C2 (*AUCtri*, modified according to Pawinski [18], *black circles*) on the one hand, and the abbreviated AUC derived from C0, C1, C2, and C4 (*AUCFiller* [14], *open squares*) on the other hand, with the full ten-point AUC. The four-point approach was better than the three-point approach; however, the three-point method yields acceptable results

We also tested whether the formula derived by Pawinski et al. [18] for adults with combination therapy would be applicable for children. If it were assumed that

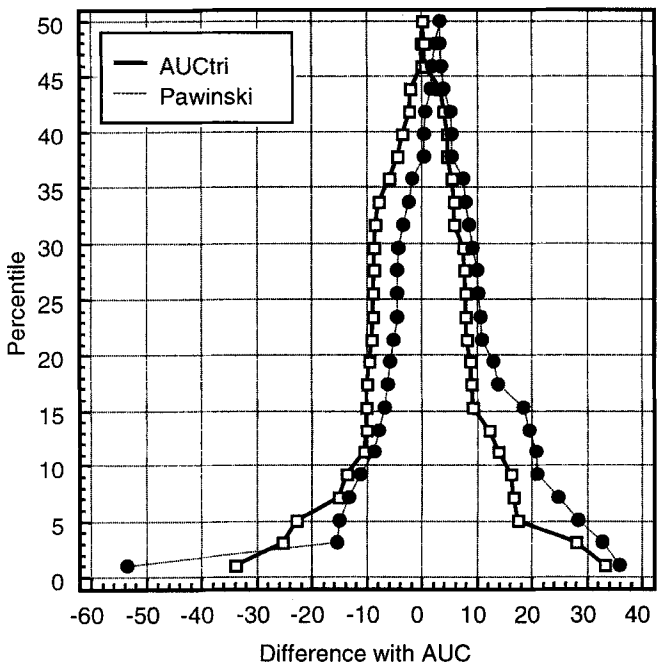

Fig. 6 Mountain plot analysis testing agreement between the abbreviated AUC derived from C0, C0.5, and C2 (*AUCtri, open squares*) here, on the one hand, and the abbreviated AUC derived from C0, C0.5, and C2 using the adult formula (*Pawinski* [18], *black circles*) on the other hand, with the full ten-point AUC. There was a shift between the two curves with an average mistake of 3% and a much wider base when the formula derived by Pawinski et al. [18] was used. One has to conclude that the adult formula cannot be applied in children

the overall slopes were identical, there was a 95% chance of data points with slopes this different being randomly chosen ($P=0.9486$). Thus, the differences between the slopes were not significant. Since the slopes were not significantly different, it is possible for us to calculate one slope for all the data. The pooled slope equaled 0.812183. When asking whether the elevations were different, we found that there was a 17% chance of randomly choosing data points with elevations this different. The differences between the elevations were thus also not significant ($P=0.1744$). However, in the mountain plot analysis there was considerable bias when the formula AUC = $7.75+6.49 \times C0+0.76 \times C0.5+2.43 \times C2$ [18] was used. The comparison of the two, via Bland and Altman analysis, is shown in Fig. 6. One has to conclude that the formula derived for adults cannot be used in children.

Discussion

The objective of this study was to compare the usability of the abbreviated MPA PK profile formula derived in Pawinski et al. [18] for children, with a separate formula derived from pediatric data and other formulae pub-

lished in the literature [14]. Obviously, the retrospective nature of this study imposes limitations. Also, not all the patients were on this therapy primarily. However, all PK profiles were obtained in steady state with at least 1 week on a steady dosage. Thus, only the pharmacodynamics after transplantation cannot be analyzed, while the dataset allows the derivation of formulae across the entire pediatric age range. Only infants under 1 year of age were not included in the study. Children cannot simply be compared to adults. Their clearance can often be higher than that of adults. In fact, when we analyzed MPA clearance versus age, there was a weak, but significant, negative correlation between age and MPA clearance ($r^2 = 0.2282$, $P = 0.0007$, data not shown). The mountain plot analyzing the difference in the Bland and Altman analysis when comparing the limited sampling strategy from C0, C0.5, and C2 derived in this manuscript and the Pawinski formula [18] clearly showed a systematic bias for the Pawinski formula. These data clearly point out that a separate limited sampling strategy has to be used for children.

So far, TDM has not yet been fully established and does not reflect the immunosuppressive action on the key enzyme IMPDH [19]. However, measurement of IMPDH is not generally available. A 50% inhibition of IMPDH, proposed to be sufficient for immunosuppression, was found at an average AUC of 59 μg×h/ml and MPA trough concentrations between 2–5 μg/ml [19], and, therefore, most clinicians use these ranges as target ranges. Interestingly, our mean MPA AUC was 57.6 ± 28.8 mg×h/l (or μg×h/ml), not significantly different from the 59 proposed by Langman et al. [19]. Suffice to say, target MPA AUCs have not yet been defined, particularly not in a pediatric patient cohort. The correlation between AUC and trough level is poor, and only a limited sampling strategy involving three time points yields satisfactory information on the actual AUC [13]. If the limited sampling procedure proposed from C1, C2, and C6 is used, the costs are less than 1/3 that of a full profile, and there is good agreement with the AUC calculated from the full PK profile [17]. The limited sampling approach chosen here showed a similarly acceptable Pearson's correlation coefficient. However, while the Bland and Altman analysis did not show a bias with this pediatric formula, it did show considerable lack of agreement with the full AUC, and up to 40% error might not be clinically acceptable. The four-point approach previously described for all of the three most commonly used immunosuppressive drugs in pediatric renal transplantation [14] appears to be a preferable approach. These academic discussions about limited sampling strategies notwithstanding, all such efforts remain meaningless if appropriate target AUCs are not established. We recommend the establishment of target AUCs, which has still not been done sufficiently in children, with abbreviated AUCs measured at given time points after renal transplantation, and the correlation of these findings to glomerular filtration rate and histology.

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