Are in clinical practice measurements of concentrations and the calculation of mycophenolate mofetil pharmacokinetic parameters needed for optimizing therapy in patients with renal diseases or kidney transplantation?

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Abstract

Background. Modern pharmacotherapy requires an individual approach to patients, taking into account changes in pharmacokinetics in pathological states and between-subject variability. This procedure is of particular importance in immunosuppressive drug therapy. In recent years, the attention has been paid to the usefulness of calculating the kinetic parameters of the drug in the optimization of the immunosuppressive treatment.

Objectives. To assess the possibility of using mycophenolic acid (MPA) concentration measurements in order to optimize pharmacotherapy in patients with kidney diseases or after kidney transplantation.

Materials and methods. The study included 103 patients with renal diseases (group 1) and after kidney transplantation (group 2), treated at the Department of Nephrology and Transplantation Medicine at the University Clinical Hospital, Wrocław, Poland. The concentrations of MPA were measured using Enzyme Multiplied Immunoassay Technique (EMIT®) method. A total of 193 pharmacokinetic profiles were performed.

Results. The median of initial (C_0) concentration for all patients was 2.09 mg/L, in group 1 – 2.06 mg/L and in group 2 – 2.11 mg/L. The median concentration at 30 min after drug administration (C_{30}) was 11.72 mg/L in the whole study group, in group 1 – 11.52 mg/L and in group 2 – 12.72 mg/L. The median concentration at 120 min after the drug administration (C_{120}) was 4.73 mg/L, 4.45 mg/L and 5.57 mg/L, respectively. The median area under the curve from C_0 to C_{120} $(AUC)_{0–120}$ was 15.77 h × mg/L for the entire study group, in group 1 – 15.46 h × mg/L and in group 2 – 16.78 h × mg/L. Using the Spearman's rank correlation coefficient for both groups, statistically significant ($p < 0.05$) correlations were found between 1) C_0 and C_{30}, 2) C_0 and C_{120}, 3) C_0 and $AUC_{0–120}$, 4) C_0 and the daily dose, 5) C_{30} and $AUC_{0–120}$, and 6) C_{30} and the morning dose. There were also statistically significant correlations between C_{120} and $AUC_{0–120}$. Moreover, in group 1, a statistically significant correlation ($p < 0.05$) was found between 1) C_{120} and the daily dose, 2) C_{120} and albumin, 3) C_{120} and C_{30}, and 4) C_{120} and $AUC_{0–120}$. In the group 2, a statistically significant correlation was found between C_{120} and the morning dose.

Conclusions. Measurements of MPA concentrations are useful for optimizing immunosuppression in patients requiring an individualized treatment.

Key words: transplantation, renal disease, mycophenolate mofetil, therapeutic drug monitoring, pharmacokinetics

Background

Therapeutic drug monitoring (TDM), based on the measurement of the concentration of drugs in body fluids and adapting the dosing regimen to the therapeutic range, is currently a well-established procedure that significantly supports physicians in daily clinical practice. This procedure is of particular importance for drugs with high between-subject variability and low therapeutic index. The idea of TDM is based more on a close relationship between the concentration of a drug in the body (usually measured in the blood) and the clinical effect, than on the relationship between the dose and that effect. The therapeutic range of drugs is determined with the use of population-based retrospective investigations and pharmacokinetic modeling.[1] This method was introduced into clinical practice in the mid-1970s and is not only still widely used in clinical trials and in optimizing pharmacotherapy in hospitals, but it has also been improved with modern analytical techniques. In 1960, Buchthal et al. indicated a relationship between the plasma concentration of phenytoin in patients treated for epilepsy and the number of seizures in those patients.[2] It was one of the first publications pointing to the possibilities of optimizing the treatment using mathematical calculations. In the 1980s and 1990s, TDM was of interest for researchers, pharmacologists and medical practitioners. Currently, clinical medicine elucidates considerable benefits from these observations. However, it should be pointed out that, despite the development of numerous dosage regimens, there is still room for the improvement.

Meanwhile, the rapid development of transplantology and therapy of autoimmune diseases in recent years is strongly linked to the introduction of treatment regimens, based on the new generation of immunosuppressive drugs. The balance between the effectiveness of treatment and the frequency and severity of adverse reactions to drugs appears to be the issue in this area. In the late 20th century, expert communities consisting of physicians, pharmacologists and analytical chemists developed international pharmacotherapy guidelines aimed at patient care optimization.[3] The guidelines are periodically updated based on the results of meta-analyses, clinical observations and pharmacokinetic/pharmacodynamic (PK/PD) modeling. One of the groups of medicinal products for which various regimens were developed includes immunosuppressive drugs – calcineurin inhibitors (cyclosporine A (CSA), tacrolimus (TAC)), antiproliferative drugs (mycophenolate mofetil (MMF), mycophenolic acid sodium salt (EC-MPS), azathioprine (AZA)), mammalian target of rapamycin (mTOR) inhibitors (sirolimus (rapamycin), everolimus), and glucocortecosteroids (GCS). This is in line with evidence-based medicine (EBM) principles.

According to the report by the Scientific Registry of Transplant Recipients (SRTR), in 2018, 60% of transplant recipients followed a treatment regimen based on MMF+TAC+GCS, 30% of them followed a treatment regimen based on TAC+MMF, with the remaining transplant recipients following other treatment regimens. It can be said that MMF is one of the most commonly used immunosuppressive drugs in transplantation, but it is also an important element in the treatment of autoimmune diseases, which often lack registration for this drug despite its fairly widespread use.[4,5]

Compared to other medicinal products, MMF is related to lower rates of acute rejection of the transplanted organ, and reduced severity and a lower number of adverse reactions. Even though MMF was introduced over 20 years ago and initially, the doses recommended by the manufacturer were followed, clinical observations demonstrate the need for individualizing the dosage based on the concentration monitoring and PK/PD modeling, especially for organ transplant recipients.[6,7] The results of numerous studies indicate that the measurement of a single concentration (e.g., before the administration of the next steady-state drug dose) is not sufficient to predict properly the effectiveness of treatment.[5–8] Both previous observational works and the consensus recommendations point to the necessity of applying limited sampling strategies (LSSs) in order to calculate the area under the curve (AUC) in comparison to time curve, which increases predictive accuracy. Still, a uniform model is yet to be established.[8]

The generally accepted parameter that most closely reflects the pharmacokinetic processes of MMF in the body is AUC_{0-12h}, which is calculated based on steady-state concentrations.[9] However, it should be mentioned that the calculation of AUC should be based on many measurement timepoints, which is associated with high costs of the study. With this in mind, LSSs are more applicable to clinical practice.[9,10]

Objectives

The study aimed to assess the possibility of using mycophenolic acid (MPA) pharmacokinetic parameters calculated on the basis of concentration measurements in the abbreviated sampling profile, to optimize pharmacotherapy in a group of patients with various renal diseases or after kidney transplantation.

Materials and methods

Patients and study design

Initially, 164 patients were included in the study. The inclusion criteria were: the use of pharmacotherapy according to the guidelines for MMF at a fixed dose for at least 1 month, a stable status of the underlying disease and patient's informed consent to participate in the study. Ultimately, 103 people were included in the study from the Department of Nephrology and Transplantation Medicine

Table 1. Characteristics of patients

Parameter	Patients groups					
	whole study groups (n = 103)		glomerulonephritis patients (group 1) (n = 64)		kidney transplant recipients (group 2) (n = 39)	
Kinetic profiles	193		150		43	
Women	48		30		18	
Men	55		34		21	
Statistical parameters	mean (±SD)	CV [%]	mean (±SD)	CV [%]	mean (±SD)	CV [%]
Age [years]	42.83 ±18.4	34.24	42.3 ±13.94	32.98	44.88 ±16.89	37.85
BW [kg]	72.94 ±14.7	25.27	73.3 ±17.33	23.66	71.6 ±32.69	31.94
BMI [kg/m^2]	25.9 ±5.8	22.42	25.95 ±5.32	20.50	25.6 ±8.09	31.94
GFR [mL/min/1.73 m^2]	61.82 ±27.88	45.10	65.08 ±29.11	44.73	50.59 ±19.58	38.72
Duration of therapy [months]	36.7 ±101.4	275.9	20.00 ±15.94	79.67	95.13 ±204.00	214.45
Protein serum [g/L]	6.02 ±0.83	13.75	9.91 ±0.81	13.83	6.49 ±0.70	50.82
Albumin serum [g/L]	3.99 ±2.91	73.05	3.98 ±3.23	81.20	4.04 ±0.45	11.04
RBC [10^{12}/L]	4.54 ±0.89	19.76	4.55 ±0.91	19.82	4.45 ±0.88	19.81
HGB [g/dL]	13.12 ±2.40	18.31	13.18 ±2.45	18.59	12.88 ±2.24	17.40
WBC [10^9/L]	6.65 ±6.26	94.15	6.54 ±6.89	105.36	7.08 ±2.29	32.33
PLT [10^9/L]	247.36 ±86.85	35.11	268.29 ±84.85	31.63	206.21 ±79.97	38.78
CRP [mg/L]	6.61 ±14.43	218.22	7.63 ±13.04	170.88	7.13 ±18.85	264.54
MMF daily dose [mg]	1217.84 ±453.67	37.25	1222 ±423.14	34.60	1200 ±550.8	45.86
MMF morning dose [mg]	612.26 ±231.13	37.75	612.24 ±219.32	35.83	612.32 ±270.54	44.18

BW – body weight; BMI – body mass index; GFR – glomerular filtration rate; RBC – red blood cells; HGB – hemoglobin; WBC – white blood cells; PLT – platelets; CRP – C-reactive protein; CV – coefficient of variation; MMF – mycophenolate mofetil; SD – standard deviation.

at the University Clinical Hospital, Wrocław, Poland. A total of 193 separate pharmacokinetic profiles of the drug were determined. Patients were divided into 2 groups: group 1 consisted of individuals with chronic renal diseases requiring immunosuppressive treatment (n = 64), while group 2 included individuals after a renal transplant (n = 39). In group 1, the patients had PK profiles performed during the follow-up visit at the nephrology clinic (42 patients had 2–8 PK profiles performed at intervals of at least several weeks, most often after changing MMF dosage or for clinical monitoring; only 22 patients had PK parameters determined once), while in the group of people after kidney transplantation, only 4 patients had PK tests performed several times (2–3) and 35 patients had PK profiles performed once. Material for pharmacokinetic and biochemical analyses was collected between October 2013 and December 2017. The approval of the Bioethics Committee of the Medical University of Wroclaw (No. KB/174/2013 and KB-317/2015) was obtained and each patient signed informed consent to participate in the study. All procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

The characteristics of the study group are shown in Table 1.

In addition to the underlying disease, patients also suffered from other medical conditions. The following comorbidities were diagnosed in the patients included in the study: hypertension was observed in 57.7% of patients, diabetes mellitus – in 11.8%, thyroid dysfunction – in 91%, and frequent urinary tract infections – in 94%. In addition, the disorders of the gastrointestinal tract were observed.

Treatment

The patients in the study group were receiving immunosuppressive treatment for the underlying diseases, based on MMF and/or GCS and calcineurin inhibitors. To prevent the adverse effect of the interaction between MMF and CSA, consisting in reducing the bioavailability of MMF, patients treated with CSA were administered a higher dose of MMF, in accordance with current treatment protocols for transplant patients. Treatment regimens applied in the groups are presented in Table 2.

In addition, the patients were receiving medication specific to the treatment of comorbidities; on average, a single patient received 6 different preparations, including MMF.

The mean morning dose of MMF was 612.26 ±231.13 mg (median: 500 mg). The mean daily dose was 1217 ±453.67 mg/day (median: 1000 mg). There was no statistically significant difference in MMF dosage between groups 1 and 2 (p = 0.98; Mann–Whitney U test). The duration of MMF immunosuppressive therapy averaged 36.74 ±101.4 months (median:

Table 2. Immunosuppressive treatment in the entire study group and by subgroups

Immunosuppressive treatment regimens	Whole study group (n = 103) [%]	Percentage of patients in group 1 (n = 64) [%]	Percentage of patients in group 2 (n = 39) [%]
MMF+GCS	40.8	65.6	0
MMF in monotherapy	11.6	18.7	0
MMF+GCS+TAC	29.1	3.1	71.8
MMF+GCS+CSA	12.6	9.4	17.9
MMF+CSA	2.9	3.1	2.6
MMF+TAC	1.9	0	5.1
MMF+SIR	1	0	2.6

MMF – mykophenolate mofetil; GCS – glucocorticosteroids; TAC – tacrolimus; CSA – cyclosporine A; SIR – sirolimus.

20 months) in the entire study group, 20.01 ±15.94 months in the renal disease group (median: 18.7 months), and 95 ±204 months in the kidney transplant group (median: 60 months). The duration of therapy was statistically significantly longer in the kidney transplant group compared to the patients with renal disease ($p < 0.001$; Mann–Whitney U test).

Analytical methods

Concentrations of MPA, an active form of MMF, were measured in the Laboratory of Therapeutic Drug Monitoring of the Department of Clinical Pharmacology, Wroclaw Medical University, Poland, using the commercial immunoassay method – Enzyme Multiplied Immunoassay Technique (EMIT®) on a Siemens Viva-E Chemistry Analyzer (Siemens Healthcare Diagnostics Inc., Newark, USA). The Emit® 2000 Mycophenolic Acid Assay (Siemens Healthcare Diagnostics Inc.) was used. The analytical range of the test was 0.1–15 µg/mL. The material was plasma collected in accordance with a predetermined protocol. The samples for the study were collected just before the next morning dose of the drug (C_0), 30 min after the drug administration (C_{30}) and 2 h after the drug administration (C_{120}). The sampling profile was established based on the literature describing sampling methods in relation to MMF[11] and after previous own studies, consisting in controlling the selection of timepoints in 5 randomly selected patients who had more measurements at additional timepoints (C_{15}, C_{45} and C_{90}). In addition, 3 selected measuring points were used for the analysis of the practical, economic and ethical reasons. The patients were usually treated in a hospital outpatient clinic and the long waiting time for collecting the material for the study would be burdensome for them. Other biochemical tests and anthropometric measurements were performed during routine monitoring of therapy in the Transplantation and Nephrology Outpatient Clinic and in the Diagnostic Laboratory of the Mikulicz-Radecki University Clinical Hospital, Wrocław, Poland. Pharmacokinetic calculations were performed using Kinetica v. 5.0 (Thermo Fisher Scientific, Waltham, USA) and Phoenix computer software (Phoenix Software International, El Segundo, USA); statistical calculations were performed using STATISTICA v. 13.1 software (StatSoft Inc., Tulsa, USA).

Pharmacokinetics of MPA in the study subjects was characterized by determining the parameters of C_0, C_{30}, C_{120}, and area under the curve (AUC) to 120 min after the dose (AUC_{0-120}).

Statistical analyses

Statistical tests consisted of the calculation of basic statistics. The mean, median, minimum and maximum values, standard deviation (SD) and coefficient of variation (CV), 1st quartile (Q1), 3rd quartile (Q3), and interquartile range (IQR) were evaluated. In order to check the distribution of the examined variables and to test their compliance with the normal distribution, basic descriptive statistics were calculated, and Shapiro–Wilk distribution normality tests were performed. In patients who had more than one MPA concentration profile, the intervals between consecutive tests were at least 4 months; therefore, statistical calculations for unrelated samples were used for these patients. Correlations between quantitative variables were analyzed using the Spearman's rank correlation coefficient due to the nonparametric nature of the variables, with a value of $p < 0.05$ considered significant.

Results

The mean value of the initial (C_0) concentration for the entire study group was 2.79 mg/L (median: 2.09), in group 1 it was 2.77 mg/L (median: 2.06) and in group 2 it was 2.90 mg/L (median: 2.11). The concentration at 30 min after the administration of the drug (C_{30}) averaged 15.23 mg/L (median: 11.72), in group 1 it was 14.89 mg/L (median: 11.52) and in group 2 it was 16.41 mg/L (median: 12.72). The mean C_{120} was 5.66 mg/L (median: 4.73) in the whole group, while in group 1 and group 2 it was 5.19 mg/L (median: 4.45) and 7.38 mg/L (median: 5.57), respectively. The mean AUC_{0-120} was 18.62 h × mg/L (median: 15.77) for the entire study group, 18.34 h × mg/L

Table 3. Detailed values of pharmacokinetic parameters and statistical analyses in the studied group of patients, divided into groups of patients with renal diseases and kidney transplant recipients

Parameter	Patients/profiles											
	whole study group (n = 103/193)				glomerulonephritis patients (group 1) (n = 64/150)				kidney transplant recipients (group 2) (n = 39/43)			
	median	Q1	Q3	IQR	median	Q1	Q3	IQR	median	Q1	Q3	IQR
C_0 [mg/L]	2.09	1.3	3.5	2.5	2.06	1.3	3.7	2.4	2.11	1.4	3.8	2.4
C_{30} [mg/L]	11.72	6.7	20.5	13.6	11.52	7.0	21.0	13.9	12.72	4.5	20.3	15.8
C_{120} [mg/L]	4.73	2.8	7.5	4.3	4.45	2.7	6.7	4.0	5.57	4.2	9.2	5.0
$AUC_{0–120}$ [mg × h/L]	15.77	10.1	26.1	15.4	15.46	10.1	25.4	15.0	16.78	10.2	29.4	19.2

Q1 – 1st quartile; Q3 – 3rd quartile; IQR – interquartile range; AUC – area under the curve.

(median: 15.46) and 19.63 h × mg/L (median: 16.78) for group 1 and group 2, respectively.

The comparison between pharmacokinetic parameters calculated in the groups of patients treated with combined therapy of MMF with CSA and those treated with MMF without CSA showed no significant difference between these groups (Mann–Whitney U test; $p > 0.05$). Detailed values of pharmacokinetic parameters and statistical analyses are presented in Table 3.

The MPA concentrations measured at designated timepoints observed in groups 1 and 2 are shown in Fig. 1.

The statistical analysis of correlations between individual variables of pharmacokinetic parameters and in relation to variables characterizing a patient's clinical condition was then performed.

All of the correlations below were tested using the Spearman's rank correlation coefficient.

Patients with renal disease – group 1

In group 1, there was no statistically significant correlation ($p > 0.05$) between morning dose and C_0 ($r = 0.129$; $p < 0.2$), and between morning dose and C_{120} ($r = 0.08$; $p < 0.4$). There was also no statistically significant correlation between daily dose and C_{30} ($r = 0.14$; $p < 0.09$). However, a weak positive correlation was found between morning dose values and C_{30} ($r = 0$; $p < 0.013$) and $AUC_{0–120}$ ($r = 0.2204$; $p < 0.008$), and between daily dose values and C_0 ($r = 0.1853$; $p < 0.026$) and C_{120} ($r = 0.1951$; $p < 0.02$). There were statistically significant positive correlations of moderate strength between C_0 and C_{30} ($r = 0.3692$; $p < 0.001$); C_0 and C_{120} ($r = 0.5308$; $p < 0.001$); C_{30} and C_{120} ($r = 0.3066$; $p < 0.001$), and strong positive correlations between concentrations at each measurement point and $AUC_{0–120}$ ($r = 0.4985$; $p < 0.001$; $r = 0.8997$; $p < 0.001$; and $r = 0.5709$; $p < 0.001$, respectively). There was a weak but significant correlation between albumin levels and C_{30} ($r = 0.2195$; $p < 0.011$), as well as between albumin and $AUC_{0–120}$ ($r = 0.1937$; $p < 0.024$). In contrast, no correlation was found between the aforementioned parameters and total protein levels ($p > 0.05$). Weak but statistically significant negative correlations of glomerular filtration rate (GFR) with C_0 ($r = –0.3851$; $p < 0.001$) and C_{120} ($r = –0.4256$; $p < 0.001$) were found.

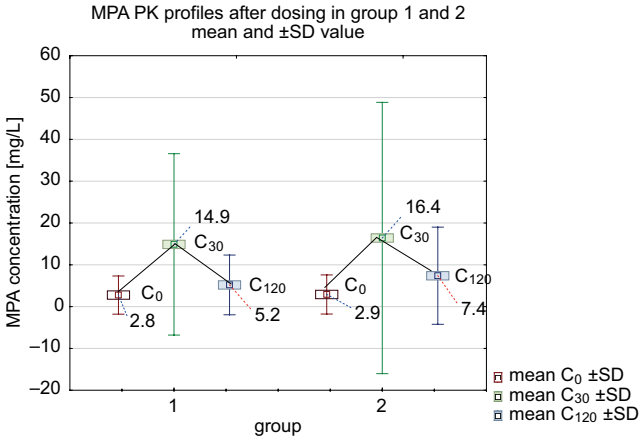

Fig. 1. Plasma concentrations of mycophenolic acid (MPA, mg/L) measured at designated timepoints observed in the entire study population (mean ± standard deviation (SD))

PK – pharmacokinetic.

Kidney transplant recipients – group 2

Similarly, group 2 showed no statistically significant correlation ($p > 0.05$; Spearman's rank correlation coefficient) between the morning dose and C_0 ($r = 0.2$; $p < 0.054$). In contrast, it was found that there is a statistically significant moderately strong positive correlation of morning dose values with C_{30} ($r = 0.5044$; $p < 0.001$), C_{120} ($r = 0.4318$; $p < 0.005$) and $AUC_{0–120}$ ($r = 0.6065$; $p < 0.001$), and of the daily dose values with C_0 ($r = 0.25$; $p < 0.02$), C_{30} ($r = 0.5128$; $p < 0.001$), C_{120} ($r = 0.4721$; $p < 0.002$), and $AUC_{0–120}$ ($r = 0.6418$; $p < 0.001$). There were statistically significant positive correlations of moderate strength between C_0 and C_{30} ($r = 0.3879$; $p < 0.012$), as well as between C_0 and C_{120} ($r = 0.4516$; $p < 0.003$), and C_0 and albumin level ($r = 0.3686$; $p < 0.038$).). There was a strong positive correlation of C_0, C_{30} and C_{120} with $AUC_{0–120}$ ($r = 0.5319$; $p < 0.001$, $r = 0.9017$; $p < 0.001$, and $r = 0.4938$; $p < 0.001$, respectively). There was no statistically significant correlation between albumin and total protein levels or GFR and any of the calculated pharmacokinetic parameters ($p > 0.05$; Spearman's rank correlation coefficient).

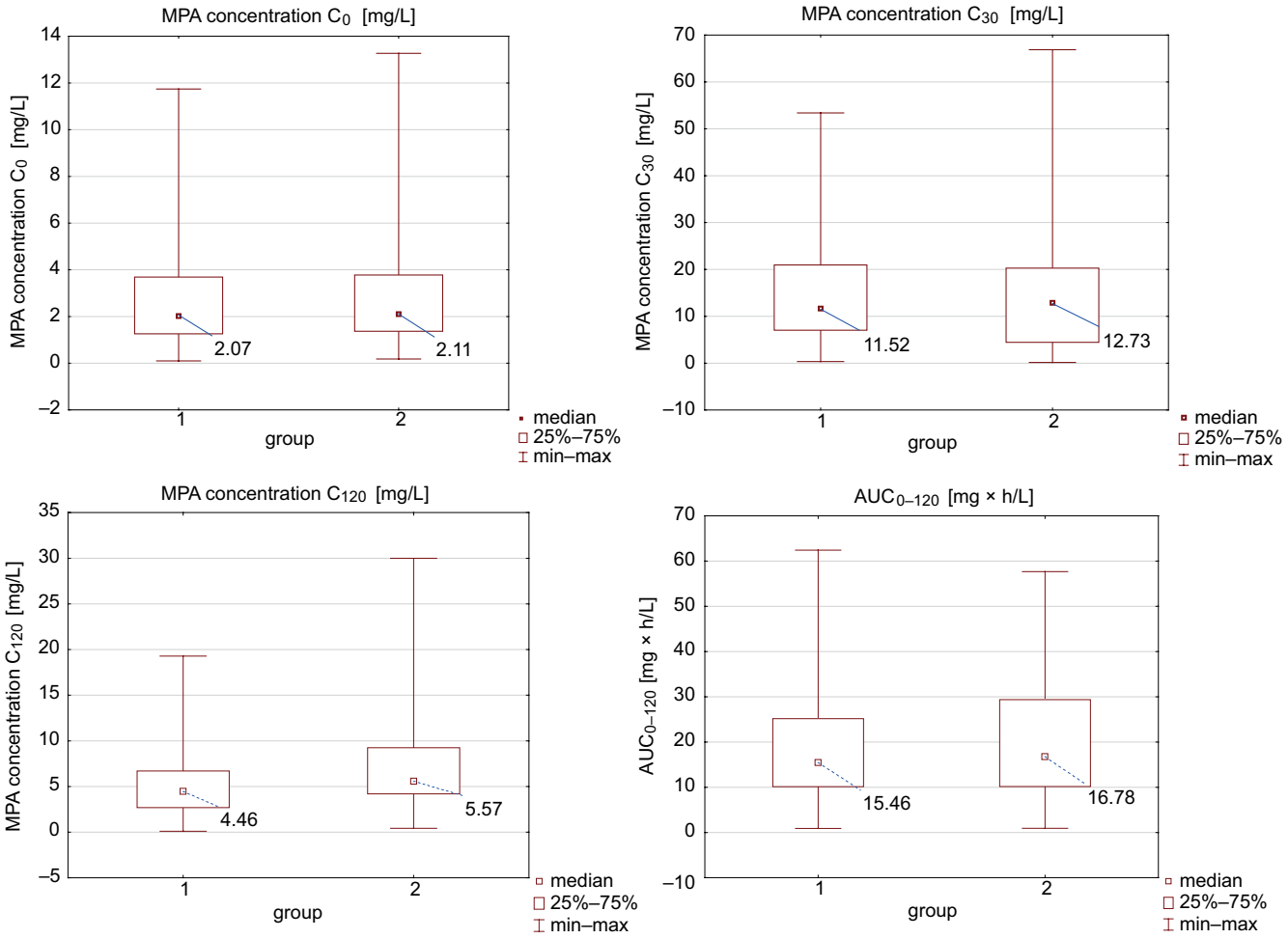

Fig. 2. Selected pharmacokinetic parameters of mycophenolic acid (MPA) in groups 1 and 2; box-whisker plots

AUC – area under the curve.

Efficacy and tolerability

The efficacy and safety of MMF pharmacotherapy were assessed based on the biochemical parameters and clinical symptoms. In the group of kidney transplant patients, the criterion of effectiveness is the lack of graft rejection. In this group (group 2), none of the patients had acute or chronic rejection. In the group of people with various renal diseases (group 1), the criteria of effectiveness are disease remission or no clinical deterioration, the assessment based mainly on estimated GFR (eGFR), serum albumin level and the presence or absence of proteinuria.

In group 2, no signs of rejection were observed, regardless of MPA concentration and AUC_{0-120}. In group 1, no significant signs of clinical deterioration were found, although there was a relationship between AUC_{0-120} and the level of albumin and total protein, which may indicate a significant influence of the drug on the course of the disease.

The pharmacological toxicity of MMF includes mainly the risk of opportunistic infections (bacterial, fungal, viral), neoplasm (lymphoma), bone marrow aplasia and digestive disorders, that are the clinical criteria of the safety of immunotherapy.

The selected pharmacokinetic parameters of MPA in groups 1 and 2 are shown in Fig. 2.

Discussion

The calculation of pharmacokinetic parameters of many drug substances is an important part of modern pharmacotherapy. A recent interest in pharmacometrics has contributed to an improved efficacy and safety of treatment. There are heated discussions on the possibility of developing an ideal pharmacokinetic and/or pharmacodynamic model for the initial phase of chronic treatment that would increase a patient's chances of optimal therapy. The influence of clinical factors other than those directly related to the drug, such as a patient's age, biomarker values, dietary habits, and genetic background, are also noteworthy.[8,12] The use of immunosuppressive drugs is a good example of how PK/PD modeling can be used to define the treatment profile. Unfortunately, the high

Table 4. Results of the Shapiro–Wilk test for the assessment of the normality of the distribution of selected variables

Parameter	Patients groups					
	whole study group		glomerulonephritis patients (group 1)		kidney transplant recipients (group 2)	
	W-value	p-value	W-value	p-value	W-value	p-value
C_0 [mg/L]	0.8350	<0.0001	0.8445	<0.0001	0.8013	<0.0001
C_{30} [mg/L]	0.8547	<0.0001	0.8999	<0.0001	0.8123	<0.0001
C_{120} [mg/L]	0.8468	<0.0001	0.9043	<0.0001	0.8455	<0.0001
$AUC_{0–120}$ [mg × h/L]	0.9232	<0.0001	0.8947	<0.0001	0.9389	<0.001
GRF	0.9454	<0.0001	0.9455	<0.0001	0.9653	0.021
Morning dose	0.8335	<0.0001	0.7551	<0.0001	0.8758	<0.0001
Daily dose	0.9171	<0.0001	0.9009	<0.0001	0.8947	<0.0001
Duration of therapy	0.9168	<0.0001	0.9168	<0.0001	0.3811	<0.0001

AUC – area under the curve; GFR – glomerular filtration rate.

interindividual variability and the dynamic changes in the health status of patients mean that there is still no clear consensus on the management of immunotherapy, especially in patients after vascular organ transplantation. The PK/PD modeling after kidney transplantation and in immune-mediated kidney diseases is a challenge for modern pharmacokinetics. The TDM is currently the gold standard in immunosuppression, but it does have some limitations due to the poor availability of analytical methods, the need for multiple sampling of the patient, the cost of the assay, and the insufficient knowledge of medical staff on interpreting the obtained results.

The present study measured MPA concentrations at 3 timepoints after MMF dosing, and calculated selected pharmacokinetic parameters of this drug in the group of subjects with renal disease and after kidney transplantation. The reported data showed that there was no statistically significant relationship between baseline concentration (C_0) and the morning dose, but there were statistically significant correlations between this concentration and the daily dose of MMF. These observations indicate that there are many individual factors that affect the absorption and distribution of the drug in the body, and that the use of the TDM method is one of the options for optimizing treatment.

In April 2021, the latest consensus report on MMF therapy from the International Association of Therapeutic Drug Monitoring and Clinical Toxicology was published.[7] According to this document, it is advisable to monitor MMF therapy due to the nonlinearity of the pharmacokinetics of the drug, as the drug is highly bound to plasma proteins, mainly albumin (97–99%).[13] The mean elimination half-life of MPA is 8–16 h; it is excreted through active tubular excretion in the form of the metabolite MPA glucuronide (MPAG) in the urine. The binding of MPA to albumin is reduced in diseases with significantly impaired renal function.[14]

The present observation showed a weak but significant correlation between albumin level and C_{30}, and also between albumin level and $AUC_{0–120}$ in the renal disease group, but no such correlation was found in kidney transplant patients. Weak but statistically significant negative correlations of GFR with C_0 and C_{120} were found. However, both albumin levels and GFRs in both study groups showed no signs of severe renal failure, and there was a considerable interindividual variability in the obtained results.

In both observed groups, a statistically significant correlation of $AUC_{0–120}$ with the morning and daily doses was demonstrated, which indicates the usefulness of calculations of this parameter for optimal immunotherapy, especially in patients with multiple comorbidities, even despite the shortened sampling time.

According to various observations with MPA concentration measurements, the concentration value at single timepoints is not a reliable predictor. It is recommended to calculate the AUC and adjust dosing regimens based on this parameter.[8] It seems that the main reason for the necessity of the individualized therapy is the incidence of adverse effects. On the other hand, dose mismatch may cause symptoms of chronic graft rejection.[15,16]

The recommended range of AUC values, most commonly determined between 0 h and 12 h after drug administration ($AUC_{0–12h}$) is 30–45 mg × h/L in patients with autoimmune diseases and 43.98–50.5 mg × h/L in kidney transplant patients.[17–19]

There is a rapid absorption phase for MMF and, after 0.5–1 h, its maximum concentration is observed in the blood. Several publications have evaluated the usefulness of the LSS method for estimating AUC or establishing a dosing regimen. It was shown that it is possible to estimate drug concentrations based on multiple linear regression; however, the accuracy of this method depends on the number of timepoints. For MMF, the LSS based on 2 or 3 timepoints was sufficient for an acceptable predictive value; however, for EC-MPS, a higher number of measurements (4–8 timepoints) was required, and also the last measurement, performed 4–9 h after drug administration, was necessary. This may be explained by a higher interindividual variability, depending on hepatic circulation and a time-varying stage of absorption.[20–22]

In the present study, all patients received MMF, which ensured the homogeneity of assay results and total drug exposure calculations. In most publications, 3 timepoints for concentration measurements were usually used, namely 20 min, 1 h and 3 h after the drug administration. In one of the cited studies, the last timepoint was 2 h after the drug administration.[23,24]

This confirms the advisability of using in this study a material collection limited to 2 h. According to the consensus, it would be more accurate to use Bayesian estimation, although this may be difficult in clinical practice, due to the need for appropriate computational tools and knowledge in this area.

In the present study, MPA concentration and PK parameters were measured at 3 timepoints limited to 2 h. The results of the calculations presented in this study show significant correlations of AUC_{0-120} with C_{30} and C_{120}, which is consistent with the calculations of other authors.[25–28] Moreover, statistically significant relationships between the concentration values measured at individual timepoints (C_0, C_{120}) and AUC_{0-120} were also demonstrated in both groups of patients and of C_0, C_{30} and C_{120} with AUC_{0-120} in group 1, which indicates the possibility of using these parameters for pharmacokinetic and drug dosing calculations. In line with the recommendations of the previously cited consensus, it is possible to calculate basic PK parameters for the correct prediction of drug exposure in different groups of patients, but this requires validation and determination of other parameters not included in this study, such as biomarkers. The role of pharmacogenetic studies of MPA metabolism in assessing the efficacy and safety of MPA immunosuppression is also emphasized.

Based on the measurements of MPA concentrations and the authors' observations, it can be concluded that the use of the TDM procedure is indicated to optimize the immunosuppressive treatment of MMF and reduce the severity of complications. Determining the best sampling profile and PK calculations requires further studies on a larger group of patients, taking into account genetic conditions or the use of a mathematical pharmacokinetic model, which would reduce the need for multiple samples for testing and the cost of measurements. Currently, based on the presented results and the literature, it can be concluded that the best predictive parameter is multiple blood sampling at short intervals and the calculation of AUC. The AUC_{0-12h} is often proposed in the literature, but it is difficult to carry out in an outpatient setting, mainly due to the long time of sample collection and the high cost. A biochemical parameter that is important in the interpretation of pharmacokinetic results is the albumin level, which should be analyzed in subsequent studies with respect to the clinical observation of drug effects, e.g., by analyzing a recognized quality of life scale, immunosuppressive effects and incidence of adverse effects.

Limitations

One limitation of the present study was the inability to compare the pharmacokinetic parameters used in this project with the pharmacokinetic profiles for 12-hour follow-up in a larger group of patients, in order to confirm the obtained results.

Conclusions

The measurements of MPA concentrations and the calculation of its pharmacokinetic parameters are useful in optimizing immunosuppression in groups of patients, requiring an individualized treatment due to individual differences and multidrug therapy, which is often the cause of drug interactions.

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