Effect of clinical condition and mycophenolate mofetil on plasma retinol, α-tocopherol and β-carotene in renal transplant recipients

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Abstract

Introduction: Plasma antioxidant vitamins (retinol, α-tocopherol, β-carotene) were measured to establish the influence of clinical condition and mycophenolate mofetil (MMF) treatment on the nutritional status of renal transplant recipients.
Material and methods: In 106 adult patients plasma vitamins were measured and 24-h diet history questionnaires were conducted. The MMF influence on plasma vitamins was verified in 61 patients.
Results: The current dietary intakes of vitamins in daily food rations were lower than recommended. Plasma retinol was lower in patients suffering from gastrointestinal disorders (1.25 ±0.48 mg/l vs. 1.55 ±0.70 mg/l) and inversely associated with aminotransferases activity (p = 0.019) and creatinine clearance (p = 0.021). Retinol concentrations were positively associated with plasma creatinine (p = 0.027) and pharmacokinetic parameters of MMF phenyl glucuronide. β-Carotene concentrations were higher in women (0.39 ±0.46 mg/l vs. 0.28 ±0.23 mg/l; p = 0.041) and when MMF was co-administered with cyclosporine vs. tacrolimus (0.45 ±0.62 mg/l vs. 0.25 ±0.19 mg/l). Plasma α-tocopherol correlated negatively with the mycophenolic acid pre-dose concentration (p = 0.027) and was significantly lower in patients treated with calcineurin inhibitors (8.90 ±5.23 mg/l vs. 12.25 ±5.62 mg/l). A positive correlation was observed between α-tocopherol levels and aspartate aminotransferase (p = 0.006). In multivariate regression aspartate aminotransferase and MMF treatment significantly influenced retinol (p < 0.001).
Conclusions: The MMF treatment was associated with significantly lower retinol concentrations. The gastrointestinal disorders occurrence in MMF-treated patients may cause a decrease in retinol absorption. Diet adjustment and/or vitamin A supplementation should be considered.

Key words: antioxidant vitamins, clinical condition, mycophenolate mofetil, renal transplantation, nutritional status.

Introduction

Malnutrition in renal transplant recipients intensifies coexisting diseases, deteriorates prognosis, increases mortality and morbidity as well

as the cost of health care and, generally, it deteriorates the life quality of patients [1]. Malnutrition may result from gastrointestinal disorders, often leading to digestion and absorption disorders, as well as from concomitant diseases such as post-transplant diabetes, chronic liver disease and infections, which often occur in transplant recipients due to the various administered medications [2-6]. The majority of complications may be prevented or treated by early nutritional intervention and follow-up. Therefore, evaluation of the nutritional status as well as the analysis of risk factors concerning malnutrition have become more prevalent among physicians [1].

One of the factors influencing nutritional status in patients after solid organ transplantation may be the immunosuppressive agent administered. At present, mycophenolate mofetil (MMF) is the most widely used antiproliferative drug after solid organ transplantation [7]. The MMF was also found to be beneficial in children with idiopathic nephritic syndrome, primary glomerulonephritis and auto-antibody associated glomerulonephritis. Some authors recommend MMF to be a part of the standard treatment regimen for lupus nephropathy [8]. The MMF is often co-administered with calcineurin inhibitors (CNI) and corticosteroids [7, 9]. After oral administration, pharmacologically inactive MMF is rapidly and entirely hydrolyzed into an active metabolite, mycophenolic acid (MPA) [2, 10], which is further converted into an inactive phenyl glucuronide (MPAG) [11]. Although the appropriate diet may be contributory in reducing metabolic disorders [12], which often occur during immunosuppressive therapy, there have been, to our knowledge, few studies concerning MMF treatment and the nutritional status of renal transplant recipients.

Some of the disorders noted have been associated with oxidative stress. Therefore, an appropriate intake of antioxidant vitamins (tocopherols, retinol and carotenoids) may help prevent diabetes mellitus, cardiovascular and central neurodegenerative diseases as well as cancer [13-17]. However, in clinical practice, vitamin concentrations are rarely determined in patients during the post-transplant period, and there are few studies focusing on MMF [18].

The aim of the study was therefore to assess the influence of clinical condition, MMF treatment and the pharmacokinetics of its metabolites (MPA and MPAG) on vitamin nutritional status (retinol, α-tocopherol and β-carotene) in renal transplant recipients.

Material and methods

Patients

The study included 106 renal transplant recipients aged 20-72 years in the late post-transplant period. In all patients sex and clinical condition such as diabetes, gastrointestinal disorders, liver function (alanine aminotransferase [ALT] and aspartate aminotransferase activity [AST] above or below 36 U/l) as well as kidney function (plasma creatinine [C_{cr}] above or below 1.5 mg/dl; creatinine clearance [CL_{cr}] above or below 60 ml/min) in relation to plasma vitamin concentrations were examined.

The MMF influence on plasma vitamins was studied in 61 renal transplant recipients aged 23-69 years treated with MMF (MMF group) with cyclosporine (CsA) (n = 28), tacrolimus (Tac) (n = 24) or without CNI (n = 9). Almost all patients in this group were treated with corticosteroids (n = 54). Additionally, the results were compared with a group of 45 renal transplant recipients aged 20-72 years, treated with different immunosuppressive regimens excluding MMF and mycophenolate sodium (non-MMF group). Immunosuppressive regimens included corticosteroids (n = 42) in combination with CNI (n = 36), azathioprine (n = 16) or sirolimus (n = 12). Clinical characteristics of the patients are listed in Table I.

For vitamin analysis, blood samples from patients were collected into EDTA tubes after an overnight fast. The samples were centrifuged to obtain plasma and stored at –20°C until analyzed.

The vitamin concentrations were analyzed in order to establish whether MMF treatment or MPA and MPAG pharmacokinetics could have influenced vitamin concentrations.

The study was approved by the Bioethical Committee at Poznan University of Medical Sciences and is in accordance with the Helsinki Declaration of 1975. Informed consent was obtained from the patients prior to initiating the study.

Determination of vitamins

The determination of plasma α-tocopherol, retinol and β-carotene was based on standard methods [19-21] with some modifications. The reverse-phase HPLC method (Hewlett-Packard 1100) with a Supelcosil LC-18-DB column was applied for retinol and α-tocopherol analyses. The normal-phase HPLC method (WATERS 600) with a LiChrosorb Si 60 column was used for β-carotene analysis. The vitamin concentrations of standard solutions were prepared according to their absorbance values (1 cm/1%) (for retinol 1835 at 325 nm, for α-tocopherol 75.8 at 290 nm, for β-carotene 2590 at 450 nm).

Into 200 µl of plasma, 20 µl of internal standard (IS) (retinol acetate 25 mg/l in ethanol) was added. For β-carotene determination, 20 µl of hexane was added instead of IS. Subsequently, 500 µl of water, 500 µl of ethanol with 0.04% of BHT (2,6-di-*tert*-butyl-4-methylphenol) and 1000 µl of hexane with 0.04% BHT were added. The resulting solutions were shaken for 5 min, vortex-mixed (10 min at 1000 × g) and 700 µl of the organic layer was col-

Table I. Characteristics of patients included in the study (mean ± SD)

Parameter	MMF group (n = 61)	Non-MMF group (n = 45)	All patients (n = 106)
Age [years]	43 ±12*	49 ±13*	46 ±13
Post-transplant period [years]	4.7 ±2.9*	6.6 ±3.1*	5.5 ±3.1
Body weight [kg]	74.9 ±16.7	70.0 ±13.6	72.9 ±15.6
BMI [kg/m^2]	25.9 ±4.7	24.8 ±4.1	25.5 ±4.5
Creatinine concentration [mg/dl]	1.80 ±0.69	1.76 ±0.74	1.78 ±0.70
Creatinine clearance [ml/min]	58.4 ±24.4	51.5 ±19.7	55.5 ±22.7
Alanine aminotransferase [U/l]	31.5 ±21.5	31.3 ±17.9	31.4 ±20.0
Aspartate aminotransferase [U/l]	26.6 ±10.1	26.1 ±10.4	26.4 ±10.2
Number of patients			
Sex (male/female)	34/27*	19/26*	53/53
CsA/Tac	28/24	21/15	49/39
Corticosteroids treatment	54 (88.5%)	42 (93.3%)	96 (90.6%)
Gastrointestinal disorders	45 (73.8%)	22 (48.9%)	67 (63.2%)
Diabetes	18 (29.5%)	13 (28.9%)	31 (29.2%)

$p < 0.05$ (MMF group vs. non-MMF group); BMI – body mass index, CsA – cyclosporine, MMF – mycophenolate mofetil, Tac – tacrolimus

lected. The hexane was evaporated to dryness under nitrogen at 40°C. The residue was dissolved in 200 µl of mobile phase (methanol with 0.025% of BHT for retinol and α-tocopherol; hexane with dioxane in 97 : 3 volumetric ratio for β-carotene), transferred into vials and 20 µl was injected into the HPLC system. For retinol and α-tocopherol, the flow rate was 1.2 ml/min and the column was thermostatic at 20°C with simultaneous ultraviolet (325 nm for retinol) and fluorescence (excitation wavelength of 295 nm and emission wavelength of 325 nm for α-tocopherol) detections. For β-carotene, the flow rate was 1.5 ml/min with photodiode-array ultraviolet detection (450 nm). The sample preparation was processed under a stream of nitrogen and in dim light.

The inter-day precision, expressed as mean coefficient of variation, was 7.4% for retinol (within concentration range 0.05-2.00 mg/l), 8.8% for α-tocopherol (within concentration range 1.0-30.0 mg/l) and 7.8% for β-carotene (within concentration range 0.1-2.0 mg/l). The inter-day absolute accuracy was 3.5%, 5.7% and 4.1% for retinol, α-tocopherol and β-carotene, respectively.

Pharmacokinetic parameters of mycophenolate mofetil metabolites

For pharmacokinetic analyses plasma MPA and MPAG concentrations were determined using the HPLC method described previously [22, 23]. The following pharmacokinetic parameters for MPA and MPAG were calculated: pre-dose concentration (C_0), maximum concentration (C_{max}) and area under the plasma concentration – time curve from 0 to 4 h ($AUC_{0\text{-}4\ h}$) using the linear trapezoidal rule based on six time points determination (before the morning dose of MMF, 40 min, 1, 2, 3, and 4 h after dosing). Pharmacokinetic parameters were normalized to the MMF dose (per gram of MMF) (data not shown).

Dietary intake

Each patient was subjected to a diet history questionnaire to estimate 24-h food intake on the day preceding the collection of blood samples. The antioxidant vitamin intakes were analyzed on the basis of the patients' dietary history using Microsoft Access 2000 databases, which are the extended version of 'Tables of food composition and nutritive value' [24]. The reference norms of 10 mg/day, 1 mg/day and 5 mg/day for α-tocopherol, retinol and β-carotene, respectively, were assumed [25].

Statistical analysis

All statistical tests were performed using Statistica software version 8.0 (StatSoft) and a p value lower than 0.05 was considered significant. Normality was determined by the Shapiro-Wilk test. The differences between variables were estimated using the Mann-Whitney test and for normally distributed data Student's t test was applied. The results are presented as mean ± standard deviation (SD). The correlations of data were tested using Pearson or Spearman correlation analysis for normally and non-normally distributed data, respectively. Pearson's χ^2 test was used for the evalua-

tion of qualitative data. Multivariate regression analysis was applied to evaluate the interactions between several variables and their influence on the levels of plasma antioxidant vitamins.

Results

Plasma retinol concentrations ranged from 0.58 mg/l to 3.83 mg/l and were statistically lower in patients treated with MMF compared to the non-MMF group. Plasma retinol levels were also found to be significantly lower in patients suffering from gastrointestinal disorders. Retinol concentrations were statistically higher when CL_{cr} was lower than 60 ml/min and when C_{cr} was above 1.5 mg/dl. The ALT above 36 U/l was associated with lower retinol levels (Table II). Retinol concentrations were negatively associated with AST and positively with MPAG $AUC_{0\text{-}4\ h}$ and MPAG C_0 (Table III).

Plasma α-tocopherol concentrations ranged from values below the lower limit of quantification (LLOQ) to 25.63 mg/l. The concentrations of α-tocopherol were significantly lower in patients treated with CNI (Table II). Moreover, a positive correlation was observed between α-tocopherol levels and AST. Plasma α-tocopherol concentrations correlated negatively with MPA C_0 (Table III).

β-Carotene concentrations ranged from values below the LLOQ to 3.22 mg/l. Despite the lack of difference in dietary intake between men and women, β-carotene concentrations were higher in women and when MMF was co-administered with CsA compared to MMF co-administered with Tac. The concentrations of β-carotene were significantly lower in patients suffering from diabetes and from gastrointestinal disorders (Table II).

We also verified whether MMF treatment and pharmacokinetics of MMF metabolites (MPA and MPAG) correlate with patients' clinical condition. MMF treatment was associated with more frequent gastrointestinal disorders (Table I). Additionally, some MPA and MPAG pharmacokinetic parameters correlated with liver or kidney function. MPA C_0 was inversely dependent on ALT (p = 0.018), AST (p = 0.050) and CL_{cr} (p < 0.001), while all MPAG

Table II. Concentrations of retinol, α-tocopherol and β-carotene (mean ± SD) in relation to MMF, CNI co-administered and patients' clinical condition

Parameter		*n*	Retinol [mg/l]	Value of *p*	α-Tocopherol [mg/l]	Value of *p*	β-Carotene [mg/l]	Value of *p*
All patients		106	1.56 ±0.76		9.47 ±5.42		0.34 ±0.37	
MMF	Yes	61	1.22 ±0.45	< 0.001	9.39 ±4.85	0.812	0.32 ±0.43	0.200
	No	45	2.03 ±0.86		9.59 ±6.19		0.35 ±0.26	
MMF with	CsA	28	1.12 ±0.39	0.220	9.77 ±4.99	0.484	0.45 ±0.62	0.046
	Tac	24	1.28 ±0.51		9.16 ±5.17		0.25 ±0.19	
CNI	Yes	88	1.58 ±0.81	0.807	8.90 ±5.23	0.026	0.36 ±0.39	0.153
	No	18	1.47 ±0.46		12.25 ±5.62		0.24 ±0.16	
Gastrointestinal disorders	Yes	67	1.25 ±0.48	0.049	9.35 ±5.32	0.533	0.26 ±0.18	0.031
	No	39	1.55 ±0.70		10.34 ±5.56		0.46 ±0.56	
CL_{cr} [ml/min]	< 60	71	1.68 ±0.83	0.021	8.93 ±5.56	0.108	0.36 ±0.43	0.853
	≥ 60	35	1.32 ±0.54		10.58 ±5.04		0.29 ±0.20	
C_{cr} [mg/dl]	> 1.5	56	1.70 ±0.82	0.027	9.04 ±6.00	0.327	0.37 ±0.45	0.296
	≤ 1.5	50	1.40 ±0.66		9.96 ±4.71		0.30 ±0.25	
ALT [U/l]	> 36	28	1.35 ±0.74	0.019	10.03 ±4.40	0.322	0.28 ±0.22	0.311
	≤ 36	78	1.64 ±0.76		9.10 ±5.67		0.32 ±0.24	
AST [U/l]	> 36	13	1.36 ±0.51	0.491	11.05 ±3.28	0.190	0.23 ±0.16	0.170
	≤ 36	93	1.59 ±0.79		9.16 ±5.58		0.32 ±0.24	
Sex	Male	53	1.53 ±0.77	0.750	9.42 ±5.30	0.813	0.28 ±0.23	0.041
	Female	53	1.59 ±0.76		9.53 ±5.59		0.39 ±0.46	
Diabetes	Yes	31	1.64 ±0.83	0.418	9.17 ±5.59	0.929	0.25 ±0.23	0.001
	No	75	1.54 ±0.75		9.25 ±5.34		0.34 ±0.24	

MMF – mycophenolate mofetil, CNI – calcineurin inhibitors, CsA – cyclosporine, Tac – tacrolimus, CL_{cr} – creatinine clearance, C_{cr} – plasma creatinine, ALT – alanine aminotransferase, AST – aspartate aminotransferase

Table III. Univariate regression analysis for retinol, α-tocopherol and β-carotene in relation to MMF metabolites pharmacokinetic parameters and patients' clinical condition

Parameter	*n*	Retinol [mg/l]		α-Tocopherol [mg/l]		β-Carotene [mg/l]	
		r	*p*	*r*	*p*	*r*	*p*
ALT [U/l]	106	–0.145	0.140	0.051	0.603	–0.155	0.114
AST [U/l]	106	–0.248	0.011	0.265	0.006	–0.137	0.165
MPA $AUC_{0\text{-}4\ h}$ [mg×h/l]	61	0.082	0.534	–0.152	0.247	0.151	0.249
MPA C_0 [mg/l]	61	0.211	0.105	–0.286	0.027	0.021	0.875
MPAG $AUC_{0\text{-}4\ h}$ [mg×h/l]	61	0.312	0.026	0.025	0.861	0.158	0.267
MPAG C_0 [mg/l]	61	0.295	0.035	0.071	0.621	0.071	0.618

ALT – alanine aminotransferase, AST – aspartate aminotransferase, MPA – mycophenolic acid, $AUC_{0\text{-}4\ h}$ – area under the plasma concentration – time curve from 0 to 4 h, C_0 – predose concentration, MPAG – 7-O-mycophenolic acid glucuronide

pharmacokinetic parameters ($AUC_{0\text{-}4\ h}$, C_0 and C_{max}) were inversely dependent on CL_{cr} (CL_{cr} > 60 ml/ min and CL_{cr} < 60 ml/min) (p < 0.001 for each pharmacokinetic parameter).

The factors which significantly influenced the concentrations of vitamins as well as MMF metabolites in univariate analysis were analyzed in multivariate regression. It was found that the influences of some factors, e.g. diabetes and gastrointestinal disorders on β-carotene concentration, CL_{cr} on retinol concentration, and kind of CNI applied on α-tocopherol, were statistically insignificant in multivariate regression. However, in the same way as in the univariate analysis, the significant influence of AST and MMF treatment on retinol concentration (p < 0.001) as well as the β-carotene concentration dependence on sex (p = 0.037) were also observed in the multivariate regression models. In the multivariate regression analysis β-carotene was not dependent on type of CNI co-administered.

The current dietary intakes of studied vitamins in the daily food ration showed lower than recommended intakes in all studied patients. Moreover, similar intakes of analyzed vitamins were observed in patients treated and not treated with MMF as well as within the MMF group in relation to CNI applied (Table IV).

Discussion

The determination of plasma vitamins constitutes part of a study concerning the influence of MMF on the nutritional status of renal transplant recipients. Although the concentrations of retinol, β-carotene and α-tocopherol detected in our study are comparable to those previously described, the latter concerned different populations or groups of patients [18, 19, 21, 26, 27]. Our results showed that the β-carotene concentration was dependent on sex. This corroborates the findings of other authors [28-30]. Although some studies indicated a relationship between retinol [29] as well as α-tocopherol [30] and gender, we found no such correlation.

In our study only retinol concentrations were lower in patients treated with MMF. We did not observe any differences in α-tocopherol and β-carotene concentrations between the MMF and non-MMF group. However, β-carotene was dependent on the administered CNI since β-carotene concentrations were significantly lower in patients treated with MMF and with Tac compared to those

Table IV. Dietary intake of antioxidant vitamins in patients treated in relation to MMF and MMF co-administered with CNI

Vitamin		MMF*		MMF in combination with*		All patients (n = 106)
		Yes (n = 61)	No (n = 45)	CsA (n = 28)	Tac (n = 24)	
Retinol	[mg]	0.79 ±0.62	0.80 ±0.60	0.88 ±0.79	0.68 ±0.30	0.79 ±0.61
	%RDA	87.2 ±66.4	88.3 ±60.5	95.9 ±81.1	76.2 ±35.5	87.7 ±63.7
α-Tocopherol	[mg]	7.10 ±4.41	7.63 ±5.28	7.47 ±5.53	6.59 ±3.48	7.32 ±4.77
	%RDA	73.7 ±44.2	80.3 ±54.1	77.0 ±55.6	69.3 ±35.2	76.4 ±48.5
β-Carotene	[mg]	0.20 ±0.46	0.13 ±0.17	0.22 ±0.60	0.13 ±0.18	0.17 ±0.37
	%RDA	40.6 ±92.8	26.0 ±34.5	44.3 ±120.7	26.5 ±36.5	34.5 ±74.2

*MMF – mycophenolate mofetil, CsA – cyclosporine, Tac – tacrolimus, RDA – recommended daily allowance; *differences statistically insignificant*

who received the MMF and CsA regimen. To our knowledge, ours is the first such study in adult patients; however, in children [31] there were no differences observed in β-carotene concentrations in relation to CNI applied, probably due to the small number of patients in particular groups (14 and 9 patients for CsA and Tac, respectively).

Additionally, we observed lower retinol as well as β-carotene concentrations in patients suffering from gastrointestinal disorders. More frequent gastrointestinal disorders in patients treated with MMF appeared to have led to a decrease in retinol absorption.

Our results indicate that plasma retinol may be associated with kidney function. High retinol concentrations were related to increased C_{cr} and decreased CL_{cr}. Furthermore, we observed positive correlations between retinol concentration and MPAG pharmacokinetic parameters, which may have resulted from the MPAG and retinol concentration dependence on renal function. These correlations are consistent with those of other authors [26, 32, 33]. In fact, Botella-Carretero *et al.* [26] described a positive correlation between the retinol concentration and C_{cr} ($r = 0.464$; $p < 0.001$) in 80 obese patients (BMI ≥ 40 kg/m^2) suffering from non-alcoholic fatty liver disease. Gavrilov *et al.* [32] showed in multiple myeloma patients, and Abahusain *et al.* [33] in diabetics, a higher serum retinol concentration associated with chronic renal insufficiency. It may suggest a decreased retinol elimination in these patients.

In addition, our results indicate that plasma retinol may also be related to liver function, as low retinol concentrations were associated with higher ALT and AST activity. Botella-Carretero *et al.* [26] found a negative correlation between retinol and AST activity ($r = -0.236$; $p = 0.036$) and also between retinol and ALT activity ($r = -0.241$; $p = 0.032$). Consequently, the authors suggested a protective retinol effect on the liver.

Unlike other authors, we observed a positive correlation between α-tocopherol and AST, whereas we did not find any correlation with ALT. The relationship between ALT and α-tocopherol has been presented in several studies [34-36], while a similar relationship with AST has been described only in one article [34]; however, the research was conducted in rats. In some studies, the favorable impact of vitamin E supplementation on either ALT [34, 35] or AST decreases [34] was indicated. In addition, low α-tocopherol concentration was stated to be a reliable marker of hepatocyte damage, independently of the increase in aminotransferase activity [36].

The negative correlation between α-tocopherol and MPA C_0 may be caused by the dependence of α-tocopherol and MPA C_0 on AST. The present study seems to be the first to report an influence of MMF on α-tocopherol.

In the present study, the significantly lower β-carotene concentrations in diabetic patients corroborates published data [37-39]. It makes our results more relevant, as the protective role of β-carotene in diabetes prophylaxis has been suggested by many authors [37-41].

In conclusion, we found that MMF treatment significantly influences retinol concentrations. MMF treatment as well as the incidence of gastrointestinal disorders were associated with lower plasma retinol concentrations, which may be caused by decreased retinol absorption. Diet adjustment and/or including vitamin A supplementation should be considered in such patients.

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