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Influence of Serum Uric Acid Levels in Response to the Conversion From Mycophenolate Mofetil to Mizoribine in Kidney Transplant Recipients

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ABSTRACT

Background. Currently, hyperuricemia is reported to be one of the most common side effects of mizoribine (MZR). However, previous clinical trials have not specially focused on MZR-related hyperuricemia.

Methods. Thirty-nine kidney recipients underwent a switch from mycophenolate mofetil (MMF) to MZR due to adverse clinical events. The control group included 39 kidney recipients continuing MMF without obvious adverse clinical events. They all received cyclosporine (CsA), prednisone, and MMF before enrollment. Every control was matched with a study group patient based on each pair undergoing kidney transplantations during the same week. Over the 24-month follow-up, every recipient underwent a physical examination including blood pressure, and laboratory screening of serum uric acid (sUA), creatinine, albumin, and CsA trough concentrations. We also calculated the estimated glomerular filtration rate (eGFR).

Results. The sUA level of the study group significantly and consistently increased during the first 5 months, and then decreased. By 18 months, sUA concentrations were not significantly different between the 2 groups ($P = .068$). There were more new hyperuricemic cases in the study group at 24 months. There were no significant differences in blood pressure, eGFR, and CsA levels between the 2 groups.

Conclusion. Administration of MZR was associated with a significant, transient increase in sUA. The increased uric acid suggested that MZR interferes with purine metabolism. The decrease should be associated with hypoxanthine-guanine phosphoribosyl transferase (HGPRT) enzyme activity, which improved under long-term MZR treatment. Therefore, recipients who receive MZR should be monitored more frequently for sUA during the first 5 months, followed by standard monitoring after 18 months.

THE CURRENT antimetabolite immunosuppressive agents prescribed to kidney transplant recipients include azathioprine (AZA), mycophenolate mofetil (MMF), and mizoribine (MZR). The use of AZA is gradually decreasing due to its serious adverse effects.[1] MMF has strong immunosuppressive properties to reduce the incidence of acute rejection episodes and improve long-term results following renal transplantation.[2–4] Accordingly, MMF is the most commonly prescribed immunosuppressive drug for kidney transplant recipients. However, when a patient treated with MMF experiences serious adverse events, such as a pulmonary infection or severe leukocytopenia, the dose should be adjusted or the drug discontinued. In the latter case, the prescription can be changed to

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MZR, a nucleoside of the imidazole class that has been shown to inhibit both humoral and cellular immunity by selectively inhibiting lymphocyte proliferation via inhibition of de novo purine biosynthesis.[5–7] One of the most common side effects associated with MZR is hyperuricemia.[8] However, no previous clinical trials have specially focused on MZR-related hyperuricemia. Nevertheless, our previous clinical experience, showed serum uric acid (sUA) levels of many patients to decrease after long-term versus de novo MZR treatment. Therefore, this case-controlled study was designed to evaluate sUA levels in kidney transplant recipients who underwent conversion from MMF to MZR.

MATERIALS AND METHODS

Study Design

Between April 2006 and January 2011, 244 patients received immunosuppressive therapy with cyclosporine (CsA; 2–5 $mg.kg^{-1}.d^{-1}$), prednisone (Pred; 10–15 $mg.d^{-1}$), and MMF (1000–1500 $mg.d^{-1}$) to achieve stable normal graft function for at least 3 months following transplantation. However, a study group of 39 patients required the initial MMF prescription to be discontinued due to the development of respiratory infections, ie, repeated upper respiratory infection or vital pulmonary infection (n = 19; 48.7%); leukocytopenia (n = 13; 33.3%); abnormal liver function (n = 5; 12.8%); or serious diarrhea (n = 2; 2.5%). To replace MMF, MZR prescribed at a dose of 100 $mg.d^{-1}$ was administered as two 50 mg tablets. A second set of kidney transplant recipients (n = 39) were chosen from the remaining 205 cases based on an ability to maintain continuous prescription of MMF independent of adverse clinical effects. For each study group patient, a matched control group patient was selected based on cadaveric kidney transplantation during the same week.

The patients enrolled in this study were Chinese. All study protocols were reviewed and approved by \ill\ ethics committee; written informed consent was obtained from each patient prior to initiation of the study. No prisoners were used and no organs from prisoners grafted in the study.

The 2 groups showed no significant differences in gender, age, or body mass index (BMI). In addition, there were no significant differences in sUA levels, creatinine, estimated glomerular filtration rate (eGFR), or CsA trough levels at the start of the study (Table 1).

Prior to the study and at each follow-up visit (ie, 1, 2, 3, 4, 5, 6, 8, 10, 12, 18, and 24 months after enrollment) patients underwent a physical examination and laboratory screening of sUA, creatinine, albumin, and whole blood CsA trough concentrations. The eGFR was calculated using a version of the Modification of Diet in Renal Disease (MDRD) formula as follows: GFR (mL/min/1.73 m^2) = 170 × (serum creatinine)$^{-0.999}$ × (age)$^{-0.176}$ × (albumin)$^{0.318}$ × 0.762 (if female).[9]

Exclusion criteria included the following: (1) an acute rejection episode or a >50% increase in serum creatinine level; (2) gout, pregnancy, or diabetes mellitus; (3) serum creatinine level >2 mg/mL, BMI >25 kg/m^2, fasting plasma glucose (FPG) level >7.9 mmol/L; (4) prescription of antihyperuricemic drugs, diuretics, angiotensin-converting enzyme inhibitors (ACEIs), angiotensin II type 1 receptor blockers (ARBs); (5) alcohol or consumption of internal organs from animals 3 days prior to each follow-up examination; (6) unsuccessfully treated infection, or diarrhea 2 weeks before each follow-up examination; or (7) significant abnormal function of the heart, lung, or liver.

Statistical Analysis

Statistical analyses were performed using SPSS 11.5 software with the characteristics of the 2 groups analyzed using a t-test. Comparisons between other data were evaluated using an analysis of variance of repeated data. $P < .05$ was considered to be statistically significant.

RESULTS

Among the study group, 1 young woman experienced rejection due to discontinuation of Pred without the doctor's approval; another middle-aged man developed diabetes mellitus; these 2 cases were removed from the study. In the control group, a female patient was removed from the study due to pregnancy. Based on the design principle that a patient in the study group was matched with 1 in the control group, we also removed 2 control and 1 study patient from the research. Clinical data for these 6 patients were not included in the analyses.

The study group showed a significant increase in sUA through the 5 months after conversion. Compared with the baseline, increasing amplitudes from 85.3 to 123.4 μmol/L indicated clinical significance. Compared with each preceeding month, the amplitude of the increases were 85.3, 3.3, 3.2, 23.9, and 7.7 μmol/L, respectively. It was obvious that month 1 showed the greatest increase in sUA. After 5

Table 1. Baseline Characteristics of the Patients Enrolled in This Study

Variable	MZR-Treated Patients	Control Patients	Statistical Analysis	*P*
Male/female	13/23	12/24	$\chi^2 = 0.061$	.804
Age, y	40.1 ± 7.1	39.4 ± 8.9	$t = 0.382$	.704
BMI (kg/m^2)	20.7 ± 26	21.3 ± 2.4	$t = -0.94$	.346
sUA (μmol/L)	317.6 ± 46.5	322.4 ± 58.4	$t = -0.388$	.699
Serum creatine (μmol/L)	86.0 ± 16.9	81.9 ± 14.1	$t = -1.107$	.272
eGFR (mL/min/1.73m^2)	80.2 ± 10.0	80.0 ± 10.8	$t = -0.215$	.830
CsA trough level (ng/mL)	248.6 ± 100.5	241.4 ± 68.9	$t = 0.353$	.725
Blood pressure (mm Hg)				
SBP	129.5 ± 11.2	125.8 ± 6.5	$t = -0.024$	.981
DBP	71.8 ± 9.1	74.7 ± 11.0	$t = 1.240$	.219

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.
Note: This excludes cases dropped during the study.

months, the sUA values began to decrease; by 18 months, the mean level was still higher than that of the control group, albeit the difference was not significant ($P = .068$). Moreover, a relatively "normal" sUA level fluctuation at every time was detected in the control group (compared with baseline, 5.1–37.5 μmol/L; compared with each preceeding month, −8.5–20.7 μmol/L). There seemed to be no noticeable increase or decrease throughout 24 months.

Analysis of variance of repeated data was used to compare sUA, eGFR, blood pressure (systolic blood pressure [SBP] and diastolic blood pressure [DBP]), and CsA levels between the 2 groups (Table 2).

The statistical tendency of sUA in the study group differed significantly from that of the control group ($P_{time*group} < .0001$). Moreover, the P value of time was <.0001, indicating that total sUA levels of the 2 groups changed significantly after enrollment. Excluding the time factor, there was a significant difference between the 2 groups ($P_{group} = .0024$).

In addition, more new cases of hyperuricemia (compared with month 0) occurred among the MZR than the MMF group. In particular, 7 (19.4%) and 6 (16.7%) new cases of hyperuricemia in the MZR group were reported at months 3 and 4, respectively.

eGFR values were similar between the 2 groups (Table 2). For the CsA concentrations, we observed a constant decrease with no significance between the 2 groups (Table 2).

Blood pressure, including SBP and DBP, showed P values for group and time*group >.05, suggesting no significant difference (Table 2). However, the P value of time with SBP and DBP were all <.0001, a significant difference between the 2 groups in SBP and DBP levels at various times, but we believe it to be a natural fluctuation and doubt it to be responsible for the differences in sUA levels between the 2 groups.

The main side effects were also monitored (Table 3): the MZR group had more man-time (times of the cases) of hyperuricemia. However, there was no clinical gout in either group. Fewer respiratory tract infections and leukopenia occurred among the MZR group. There was no difference in liver damage between the 2 groups.

Table 2. Analysis of Variance for Repeated Data

	Group		Time		Group*Time	
	F	P	F	P	F	P
sUA	9.89	.0024	18.23	<.0001	7.15	<.0001
eGFR	0.25	.6162	0.55	.8719	0.21	.9970
CsA	0.73	.3943	65.73	<.0001	0.82	.6227
SBP	0.05	.8248	10.58	<.0001	1.50	.1424
DBP	3.71	.0583	35.3	<.0001	0.70	.7127

Note: According to the principle of analysis of variance for repeated data, we are aware that there are 3 P values: Group, Time, and Group*Time. With $P <$ .0001 of Group*Time, the 24-month sUA tendencies of the 2 groups are significantly different. Because the P value of Time contains 2 groups' total sUA, it indicates that the total sUA of the 2 groups were fluctuating throughout the 24 months, consistant with our clinical experience. F, statistic value of Analysis of Variance for repeated data.

Table 3. Main Side Effects Through the Study

	MZR Group	MMF Group	Statistic	P
Leukopenia	5	16	$\chi^2 = 5.905$	.015
Abnormal GPT	2	7	$\chi^2 = 2.807$	.094
URTI	26	73	$\chi^2 = 10.163$	.001
Pulmonary infection	1	7	$\chi^2 = 4.073$	.044
ARF	1*	0		
Gout	0	0		
Hyperuricemia†	21	8	$\chi^2 = 6.030$	.014

Abbreviations: URTI, upper respiratory tract infection; ARF, acute renal failure; GPT, glutamate pyruvate transaminase.

Note: All of the units are man-time. Main side effects related to MZR and MMF were monitored. The frequency of leukopenia, URTI, and pulmonary infection were lower in the MZR group. No gout happened in both groups. Hyperuricemia was more common in the MZR group. With abnormal GPT, there was no statistical difference.

*The case of ARF in the MZR group was attributed to the discontinuation of Pred without doctor approval.

†Hyperuricemia: male >420 μmol/L, female >357 μmol/L.

DISCUSSION

Purines play a crucial role in DNA and RNA. Abnormal purine production and breakdown can severely alter a cell's DNA. In addition, purine metabolic abnormalities can influence sUA levels. Purine synthesis occurs via 2 separate pathways: de novo and salvage. In the de novo pathway, the ribose phosphate portion of purine nucleotides is derived from 5-phosphoribosyl 1-pyrophosphate (PRPP), which is synthesized from adenosine triphosphate (ATP) and ribose 5-phosphate. Lymphocytes are primarily dependent on the de novo pathway.[10–12] The salvage pathway essentially recycled purine bases, sugars, and other products.

In contrast to the great majority of other somatic cells, including polymorphonuclear leukocytes and neurons, B and T lymphocytes do not extensively utilize the salvage pathway of purine biosynthesis. The immunosuppressive mechanisms of MZR and MMF action in the de novo pathway show inhibition of the key enzyme IMPDH. This inhibits lymphocyte proliferation promoting graft survival.

sUA metabolism is abnormal by IMPDH (inosine monophosphate dehydrogenase) inhibition. MMF seldom causes hyperuricemia, but a large proportion of patients suffer hyperuricemia with MZR. The mechanism should be associated with hypoxanthine-guanine phosphoribosyl transferase (HGPRT), a transferase that catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate. It plays a central role in the generation of purine nucleotides through the purine salvage pathway. Deficiencies of HGPRT can result in hyperuricemia.[13] In turn, HGPRT is a crucial enzyme to maintain normal levels of sUA According to Weigel et al,[14] MMF enhances HGPRT activity significantly. So we believe it is of great concern for MMF to maintain a stable normal sUA level. But there is no report that MZR has the ability to enhance HGPRT activity, which suggests why the sUA can not be kept at stable normal levels under MZR treatment. However, Terai et al[15] reported that MZR reduces HGPRT-deficient somatic cells. The population of

HGPRT normal cells increases gradually under long-term MZR treatment, with increased HGPRT enzyme activity, which explains why sUA in patients returns.

In addition, Koyama et al[16] reported that the levels of adenine phosphoribosyl (APRT) activity in MZR-resistant mouse mammary tumor cell lines F28-7 and FC-1 were lower than those of the wild-type cells. We believe that during long-term MZR treatment recipient somatic cells are resistant in varying degrees to MZR. Therefore, APRT enzyme activity change might also contribute to sUA level fluctuation.

A combination of the theoretical mechanisms and our clinical observations suggest that doctors and recipients should not be worried about MZR-induced hyperuricemia after 5 to 18 months of treatment. However, Tanaka et al[17] reported a 64-year-old woman with rheumatoid arthritis/Sjogren's syndrome, who developed acute renal failure due to hyperuricemia associated with MZR. In this case, the authors considered that a delay in MZR discharge by transient renal dysfunction may have caused the hyperuricemia, followed by aggravation of renal dysfunction. Guo[18] also reported that a kidney transplant recipient experienced acute renal failure (ARF) associated with marked hyperuricemia during MZR administration. Renal function recovered after active hemodialysis treatments.

Through our study, we were vigilant to MZR-related adverse effects, such as serious hyperuricemia and even ARF, leukopenia, bone marrow suppression, infection, as well as liver damage. We observed more serious hyperuricemia than in the control group, but leukopenia, infection, and liver function damage were less. We did not observe a case of ARF and severe bone marrow suppression in the 2 groups (data not shown).

In conclusion, our study provided insight into MZR treatment of kidney transplant recipients. MZR patients must be monitored more frequently for the first 5 months, undergoing standard monitoring after 18 months when we observed little MZR hyperuricemia. These patients are more sensitive to sUA metabolism than those under MMF treatment due to deficient HGPRT and APRT activity.

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