Beneficial Effects of High-Dose Mizoribine on ABO-Incompatible Living-Related Kidney Transplantation: Two-Year Results by a Japanese Multicenter Study

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ABSTRACT

Background. Mizoribine (MZ) has been developed as an immunosuppressive agent in Japan, but it has a less-potent immunosuppressive effect up to 3 mg/kg/d. In the previous study, a Japanese multicenter study, we reported that high-dose MZ, at 6 mg/kg/d, with a calcineurin inhibitor was effective and safe in reducing the frequency of cytomegalovirus (CMV)-related events in ABO-incompatible (ABO-i) living-related kidney transplantation (LKT). In the present study, therefore, we investigated the effects of high-dose MZ with a CNI in ABO-i LKT recipients in a Japanese multicenter study.

Methods. A total of 37 patients were treated with high-dose MZ (6 mg/kg), a CNI (cyclosporine [CsA] or tacrolimus [Tac]), basiliximab (Bas), rituximab (Rit), and corticosteroids. CsA was started at a dose of 7 mg/kg to maintain blood levels [200 ng/mL (C0), 6000 ng-h/mL (AUC 0–9)]. Tac was started at a dose of 0.2 mg/kg to maintain blood levels [8–10 ng/mL (C0), 100 ng-h/mL (AUC 0–9)]. Bas (20 mg/body) was administrated on day 0 and day 4 after transplantation. Rit (100–200 mg/body) was administrated on day −14 and day −7 before transplantation. MZ was adjusted to maintain target C0 levels of 1.5 to 2.0 μg/mL.

Results. Patient and graft survival rates for 2 years were 100% in the CsA group (n = 22) and 93.3% in the Tac group (n = 15) (not significant, NS). Overall incidence of acute rejection for 2 years was 22.7% in the CsA group and 26.7% in the Tac group. Mean serum creatinine levels at 2 years were 1.29 ± 0.2 mg/dL in the CsA group and 1.21 ± 0.34 mg/dL in the Tac group (NS). The incidence of CMV disease was 0% in both groups, and positive rates of CMV antigenemia were 50.0% and 26.7% in the CsA and Tac groups, respectively (NS). Mean serum uric acid levels were 5.5 ± 1.3 mg/dL and 6.4 ± 1.2 mg/dL at 2 years (NS) in the CsA and Tac groups, respectively.

Conclusions. A high-dose MZ regimen including calcineurin inhibitor (CsA or Tac), Bas, Rit, and steroids was effective and safe in reducing the frequency of CMV-related events in ABO-i LKT.

ABO-INCOMPATIBLE (ABO-i) living-donor kidney transplantation (LKT) has been performed in Japan since 1989 and has currently developed as a standard and high–success rate treatment. Mycophenolate mofetil (MMF) is generally used as an antimetabolite in post-transplant regimens for ABO-i LKT, but the high rate of cytomegalovirus (CMV) infection is a serious problem. Mizoribine (MZ), a kind of immunosuppressive agent, is a nucleoside of the imidazole class [1]. MZ was found to

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inhibit both humoral and cellular immunity by specifically inhibiting the proliferation of lymphocytes. From 1978 to 1982, the clinical usefulness and safety of MZ in kidney transplantation was studied in various Japanese institutions, and the Ministry of Health and Welfare in Japan approved the use of MZ in 1984. MZ exhibits a low incidence of severe adverse reactions, does not promote oncogenicity, and acts on the same target molecule as does MMF [2]. The original dosage of MZ in the 1990s was 1 to 3 mg/kg/d as a substitute for azathioprine (AZP), combined with steroids and cyclosporine (CsA) [3]. Some studies reported that a low dose of MZ (up to 3 mg/kg/d) has a less-potent immunosuppressive effect but fewer adverse events [4,5]. In the previous study (a 3-year study in a Japanese single center), we investigated and reported that high-dose MZ (at 6 mg/kg/d) was useful and safe for ABO-i LKT in conjunction with CNI (CsA or tacrolimus [Tac]), corticosteroids, and anti-CD20 and anti-CD25 antibodies without splenectomy [6]. The present study reports the results of the effects of high-dose MZ with a CNI (CsA or Tac) in ABO-i LKT recipients in a Japanese multicenter study.

METHODS

Study Subjects and Immunosuppression

A total of 37 ABO-i LKT were performed at Kyoto Prefectural University of Medicine, National Hospital Organization Chiba-East-Hospital, Ohmihachiman Community Medical Center, and Osaka City University between January 2011 and July 2015. Analysis of the background characteristics of 22 patients in the CsA group and the 15 patients in the Tac group showed no statistical differences, as shown in Table 1. The immunosuppressive regimens of high-dose MZ were developed at Kyoto Prefectural University of Medicine (Fig 1). Plasmapheresis (double-filtration plasmapheresis or plasma exchange) was performed before ABO-i LKT, based on the anti–blood type antibody titer, to decrease the titer <32-fold.

Table 1. Demographic Characteristics

Characteristic	CsA Group (n = 22)	Tac Group (n = 15)	*P* Value
Recipient			
Sex (male/female)	10/12	11/4	.0974
Age (years)	47.5 ± 12.3	52.1 ± 10.5	.2370
Weight (kg)	59.7 ± 10.9	62.3 ± 12.2	.4976
Incompatible blood type			
A:B:AB	7:8:7	8:7:0	
Duration of dialysis (mo)	36.1 ± 45.9	42.9 ± 47.3	.7209
Preemptive	4	6	
Donor			
Sex (male/female)	8/14	4/11	.7235
Age (y)	58.4 ± 7.7	55.3 ± 9.0	.2794
Relationship			
Father	2	2	.0240
Mother	7	0	
Sibling	0	2	
Spouse	13	11	
HLA-AB mismatch	2.3 ± 1.1	2.2 ± 1.0	.8384
HLA-DR mismatch	1.2 ± 0.7	1.3 ± 0.8	.7356

Abbreviation: HLA, human leukocyte antigen.

Two weeks before surgery, the pre-transplant regimen with MMF (1000 mg/d) and prednisolone (PSL) (10 mg/d; po, bid) was initiated. The oral administration of CsA or Tac was initiated from 1 week before LKT. CsA was started at a dose of 7 mg/kg/d to maintain blood levels [200 ng/mL (C0), 6000 ng-h/mL (AUC 0–9)]. Tac was started at a dose of 0.2 mg/kg/d to maintain blood levels [8–10 ng/mL (C0), 100 ng-h/mL (AUC 0–9)]. Rituximab (anti-CD20 monoclonal antibody) was administered 7 and 14 days before LKT at a dose of 100 to 200 mg. Basiliximab (anti-CD25 monoclonal antibody) was administrated on the operation day and 4 days after LKT at a dose of 20 mg. Post-transplant immunosuppressive regimen for ABO-i LKT consisting of CsA or Tac + high-dose MZ + PSL with anticoagulation therapy was used [7]. Because the administration of MZ in the end-stage renal disease period is difficult because of renal excretion, MMF was administrated in the desensitization period (before transplantation) and then was changed to MZ from the day after transplantation. MZ was adjusted to maintain target C0 level of 1.5 to 2.0 μg/mL.

Protocol biopsies were performed 1 month and 1 year after LKT. All patients gave informed consent, and the study was approved by the institutional review board and complied with the Declaration of Helsinki and the ethical guidelines outlined by The Transplantation Society.

Statistical Analysis

The Kaplan-Meier method was used to determine patient and graft survival rates, and statistical significance was tested by use of the log-rank test and the Student *t* test. The study was approved by the Ethics Committee of Kyoto Prefectural University of Medicine.

RESULTS

Demographic Characteristics

The demographic and baseline characteristics were similar between patients and donors assigned to the CsA and Tac groups (Table 1).

Patient and Graft Survival Rates

The 2-year patient survival rates for the CsA and Tac groups were 100% and 93.3%, respectively, and graft survival rates for the CsA and Tac groups were 100% and 93.3%, respectively. There was no significant difference between the 2 groups in terms of graft or patient survival rates.

Graft Function and Rejection

Serum creatinine levels of the CsA (n = 22) and Tac (n = 15) groups were 1.38 ± 0.41 mg/dL and 1.26 ± 0.35 mg/dL at 6 months, 1.32 ± 0.44 mg/dL and 1.33 ± 0.38 mg/dL at 1 year, and 1.29 ± 0.70 mg/dL and 1.21 ± 0.34 mg/dL at 2 years, respectively. There was no significant difference in serum creatinine levels between the 2 groups. Graft rejection was diagnosed according to Banff classification. Overall incidence of acute rejection for 2 years was 22.7% in the CsA group and 26.7% in the Tac group. One and 2 AMR, 1 and 2 ACR, and 3 and 1 borderline rejections were observed in the CsA and Tac groups, respectively (Table 2).

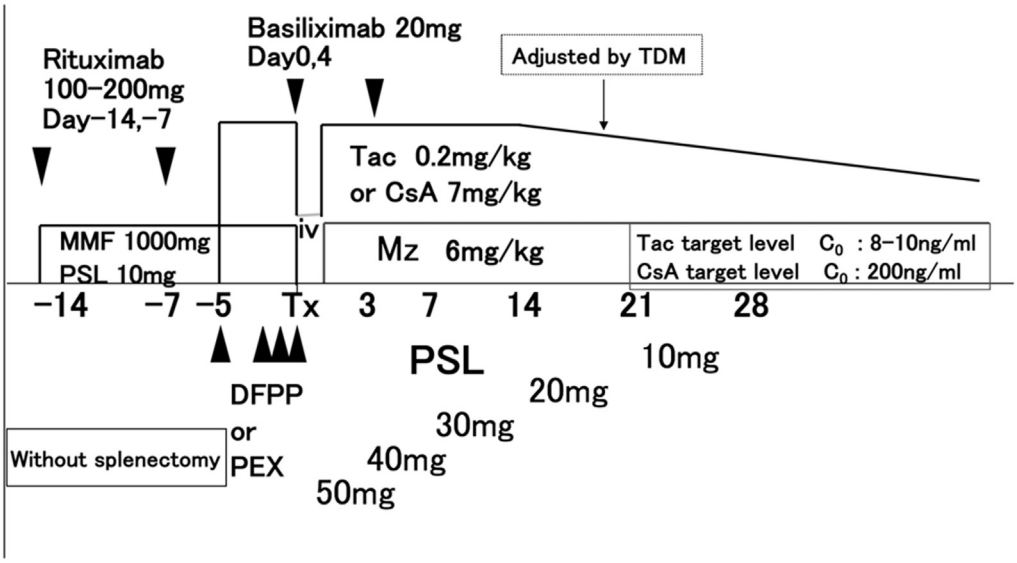

Fig 1. Immunosuppressive protocol. Immunosuppressive regimen in ABO-i kidney transplantation. The pre-transplant regimen was initiated 2 weeks before surgery, with MMF (1000 mg/d) and prednisolone (PSL) (10 mg/d; po, bid). The initial dose of CsA (7 mg/kg/d; po, bid) or Tac (0.2 mg/kg/d; po, bid) is administered orally for 1 week before LKT. Rituximab (anti-CD20 monoclonal antibody) was administered 7 and 14 days before LKT at a dose of 100 to 200 mg. Basiliximab (anti-CD25 monoclonal antibody) was administered on the operation day and 4 days after LKT at a dose of 20 mg. Abbreviations: DFPP, double-filtration plasmapheresis; PEX, plasma exchange.

Adverse Events

The incidences of adverse events in 2 years after renal transplantation were compared between the 2 groups (Table 3). The incidence of CMV disease was 0% in both groups, and positive rates of CMV antigenemia were 11 of 22 (50.0%) and 4 of 15 (26.7%) in the CsA and Tac groups, respectively (not significant, NS). Nine and 2 patients had elevation of uremic acid, and mean serum uremic acid levels were 5.5 ± 1.3 mg/dL and 6.4 ± 1.2 mg/dL at 2 years (NS) in the CsA and Tac groups, respectively.

DISCUSSION

In 1989, Tanabe et al [8] reported that MZ had an immunosuppressive effect comparable to AZP and fewer side effects such as myelosuppression and liver dysfunction in a randomized trial to compare CsA + AZP and CsA + MZ. Recently, MZ has been used commonly as an immunosuppressant in combination with CsA or FK506 and corticosteroids in kidney transplantation [9]. The original dosage of MZ, 1 to 3 mg/kg/d, was based on both animal experiments and the in vitro mixed lymphocyte reaction [10]. However, some Japanese papers reported that MZ at 1 to 3 mg/kg/d did not bring enough blood concentration to an effective range in several cases and that a higher dosage of MZ was as effective and safe as MMF without major side effects.

Table 2. Graft Function and Rejection

	CsA Group (n = 22)	Tac Group (n = 15)	
Serum creatinine (mg/dL)			
6 Months	1.38 ± 0.41	1.26 ± 0.35	NS
1 Year	1.32 ± 0.44	1.33 ± 0.38	NS
2 Years	1.29 ± 0.70	1.21 ± 0.34	NS
Rejection	5/22 (22.7%)	4/15 (26.7%)	
Banff classification			
AMR	1	2	
ACR	1	2	
Borderline	0	0	
Clinical rejection	3	1	

AMR, antibody-mediated rejection; ACR, acute cellular rejection.

Table 3. Adverse Event Profile Within 2 Years

	CsA Group (n = 22)	Tac Group (n = 15)	*P* Value
Anemia	1	0	NS
Pneumonia	0	1	NS
Sepsis	0	1	NS
VZV	2	0	NS
CMV	11	4	NS
Renal dysfunction	0	2	NS
Proteinuria	1	0	NS
Liver dysfunction	0	1	NS
Elevation of ALT	1	0	NS
Elevation of γ-GTP	3	0	NS
Hyperglycemia	0	1	NS
Elevation of uremic acid	9	2	NS
Tinnitus	1	0	NS
Others	3*	2†	NS

VZV, varicella zoster virus; ALT, alanine aminotransferase; GTP, guanosine triphosphate.
*Congestive heart failure, hyponatremia.
†Brain infarction, thrombotic micro-angiopathy.

Nakamura et al [11] reported that the graft survival rates of the MMF and the high-dose MZ (6 mg/kg/d) groups were not significantly different. Takahara et al [12] reported that patient and graft survival rate at 1 year after transplantation was 100% in the high-dose MZ (12 mg/kg/d) group and the MMF group, with no significant difference in rejection rate apparent between groups. In the previous study, we reported that a combination of high-dose MZ (6 mg/kg/d) with CsA yielded results similar to the MMF (25 mg/kg/d) and CsA treatment groups in terms of patient and graft survival and renal function [7].

Recently, the rates of overall patient and graft survival for ABO-i LKT in Japan are comparable to those for ABO-compatible LKT. Okumi et al [13] reported that ABO-i LKT and ABO-compatible LKT recipients yielded almost equivalent outcomes with respect to the 9-year graft survival rates, which were 86.9% and 92.0%, respectively. In this study, ABO-i LKT was performed with the use of anti-CD20 and anti-CD25 antibodies, without splenectomy. Golay et al [14] reported that the infusion of rituximab lyses CD20-positive cells and complement-dependent cell-killing processes, although it does not affect antibody-producing plasma cells. Mitsuhata et al [15] reported that PE decreased the therapeutic effect of rituximab when rituximab was administrated to treat humoral rejection. Rituximab should be administrated early before ABO-i LKT, in consideration of the cell cycle of plasma cells. Therefore, we administrated rituximab twice, 7 days and 14 days before ABO-i LKT.

In the present study, CsA or Tac was used as the CNI. There were no significant differences in patient and graft survival, serum creatinine, rejection, and adverse events. This result suggests that an immunosuppressive protocol, including high-dose MZ, is effective and safe, whether CsA or Tac is selected as the CNI.

With regard to adverse events in this study, the rate of CMV antigenemia-positive was 50% (11/22) and 26.7% (4/15) in the CsA and Tac groups, respectively. However, no patient developed CMV infection. CMV infection is one of the serious complications in LKT. Our previous study showed that the rate of CMV antigenemia-positive was significantly lower in the MZ group than in the MMF group [7]. Moreover, the numbers of patients who developed CMV infection and were treated with ganciclovir were not seen in the MZ group. Kuramoto et al [16] reported that MZ suppresses the replication of CMV in vivo and control CMV infection. Immunosuppressive protocol including high-dose MZ might contribute to a decrease in CMV infection in LKT.

There were no significant differences regarding patient and graft survival, rejection, and adverse events between the CsA and Tac groups in this study. Therefore, a high-dose MZ protocol on ABO-i LKT will lead to satisfactory results with the use of either CsA or Tac as the CNI. This result would give us a wider variety of choices of an effective and safe immunosuppressive protocol for ABO-i LKT. We conclude that a high-dose MZ regimen, including a CNI (CsA or Tac), basiliximab, rituximab, and steroids, was effective and safe in reducing the frequency of CMV-related events in ABO-i LKT.

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