Efficacy and Safety of High-Dose Mizoribine Combined With Cyclosporine, Basiliximab, and Corticosteroids in Renal Transplantation: A Japanese Multicenter Study

H. Ushigome[a,*], K. Uchida[b], K. Nishimura[c], K. Akioka[d], Y. Fukuda[e], K. Yuzawa[f], M. Fujisawa[g], A. Sugitani[h], S.-I. Ito[i], T. Nakatani[j], T. Horimi[k], and N. Yoshimura[a]

[a]Department of Transplantation and Regenerative Surgery, Kyoto Prefectural University of Medicine, Kyoto City, Japan; [b]Department of Organ Transplant Surgery, Aichi Medical University, Nagakute, Aichi, Japan; [c]Department of Urology, Hyogo Prefectural Nishinomiya Hospital, Nishinomiya, Hyogo, Japan; [d]Department of Surgery, Omihachiman Community Medical Center, Omihachiman, Shiga, Japan; [e]Department of Surgery, Koseiren Hiroshima General Hospital, Hatsukaichi, Hiroshima, Japan; [f]Department of Surgery, Mito Medical Center, Ibaragi-cho, Ibaragi, Japan; [g]Department of Urology, Kobe University, Kobe, Hyogo, Japan; [h]Department of Surgery, Yonago Medical Center, Yonago, Tottori, Japan; [i]Department of Urology, Gifu University, Gifu, Japan; [j]Department of Urology, Osaka City University, Abeno-ku, Osaka, Japan; and [k]Department of Transplant Surgery, Kochi Health Sciences Center, Kochi, Japan

ABSTRACT

Mizoribine (MZR) is an immunosuppressive agent that exhibits a less potent immunosuppressive effect at doses up to 3 mg/kg/d. We investigated whether high-dose MZR is effective and safe for renal transplant patients in conjunction with cyclosporine (CsA), basiliximab, and corticosteroids. Ninety Japanese renal transplant patients were administered MZR (6 mg/kg/d), CsA (7 mg/kg/d), prednisolone (maintenance dose, 10 mg/d), and basiliximab (20 mg/body). They were compared with a control group of 81 renal transplant patients who received mycophenolate mofetil (MMF; 1500 mg/d), CsA, prednisolone, and basiliximab. The 2-year patient and graft survival rates were 98.9% and 97.8% in the MZR group and 98.8% and 97.5% in the MMF group, respectively. The rejection rate within 2 years after transplantation was 21.1% in the MZR group and 16.0% in the MMF group; the difference was nonsignificant. None of the MZR group developed cytomegalovirus (CMV) disease, whereas 12.3% of the MMF group contracted CMV ($P < .0001$). CMV viremia developed in 28.9% of the MZR group vs 46.9% of the MMF group ($P < .0001$); their peak antigen levels were 20.4 ± 44.1 and 252.8 ± 527.0 ($P < .01$). Furthermore, the incidence of gastrointestinal disorder, hyperlipidemia, and blood disorder was significantly lower in the MZR group than in the MMF group. The combination of high-dose MZR with CsA, basiliximab, and corticosteroids not only provides satisfactory immunosuppression but is also associated with a low incidence of CMV infection and gastrointestinal and blood disorders.

MIZORIBINE (MZR) IS A NUCLEOSIDE compound with an imidazole skeleton isolated from soil on Hachijo Island, Tokyo, Japan in 1971 [1]. MZR was found to specifically inhibit the proliferation of lymphocytes. MZR is phosphorylated by adenosine kinase within cells, and it selectively inhibits inosine monophosphate dehydrogenase, which is a rate-limiting enzyme for de novo purine synthesis, suppressing the proliferation of lymphocytes and exerting immunosuppressive effects. Although the inhibition patterns of MZR and mycophenolate mofetil (MMF) differ, MZR acts on the same target molecule as MMF [2]. Although the original dose of MZR administered was 1 to 3 mg/kg/d, larger doses may be required. In fact, the results of a multicenter trial in Japan in 2005 [3] suggest that the rejection-free rate within 3 months after transplantation in the group administered ≥5 mg/kg/d of MZR together with

This study was supported by the Japan Kidney Foundation.

*Address correspondence to Hidetaka Ushigome, MD, PhD, Department of Transplantation and Regenerative Surgery, Kyoto Prefectural University of Medicine, 465 Kajii-cho, Kamigyo-ku, Kyoto city 602-8566, Japan. E-mail: ushi@koto.kpu-m.ac.jp

0041-1345/16
http://dx.doi.org/10.1016/j.transproceed.2015.12.117

tacrolimus (85.0%) was significantly higher than that in groups given <3 mg/kg/d (64.9%) or 3 to 5 mg/kg/d (65.1%).

In the present study, we therefore investigated the efficacy of high-dose MZR (6 mg/kg/d) administered in conjunction with cyclosporine (CsA), basiliximab, and corticosteroids. The preliminary results of this study at 1 year were presented at the XIIth Congress of the Asian Society of Transplantation, in Seoul, Republic of Korea, on September 25 to 28, 2011, and have been published in *Transplantation Proceedings* [4]. The present report details the results of efficacy and safety studies at the 2-year point, and these are summarized compared with historical data for the MMF group obtained from studies performed at the participating medical institutions.

PATIENTS AND METHODS

Study Subjects and Immunosuppression

The study subjects comprised 90 patients who underwent renal transplantation (86 transplants from living donors, 3 from cadavers, and 1 from a brain-dead donor) at 1 of 11 institutions in Japan between February 2006 and June 2008, excluding patients with ABO blood type incompatibility. The patients were administered MZR (6 mg/kg/d, given orally twice a day), CsA (7 mg/kg/d; trough level, 200–250 ng/mL during the first month after transplantation), prednisolone (maintenance dose, 5–10 mg/d, after the initial dose reduction), and basiliximab (20 mg/body at days 0 and 4) (Fig 1). The control group comprised 81 patients who underwent renal transplantation (78 transplants from living donors and 3 from cadavers) at participating institutions between May 2002 and September 2007. Patients in the control group were administered MMF (30 mg/kg/d), CsA, prednisolone, and basiliximab, and the 2 groups were compared. CsA, basiliximab, and steroids were administered according to the same protocol as that for the MZR group at the same institutions. There were no intergroup differences in patient background characteristics such as the underlying disease in the recipient, sex, age, body weight, dialysis period, and number of HLA mismatches (Table 1). All patients were aged between 13 and 66 years; patients with an ABO-incompatible relationship or who were crossmatch-positive were excluded.

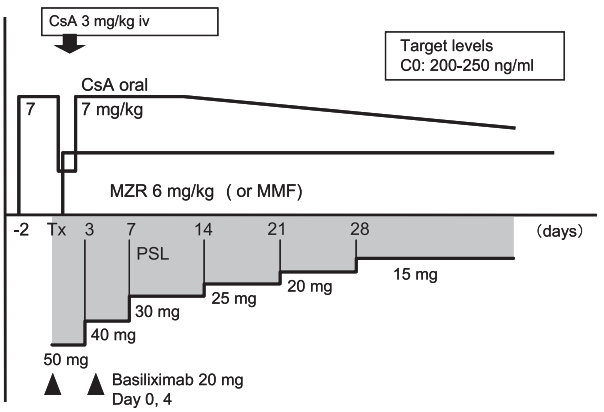

Fig 1. Immunosuppressive regimens: cyclosporine (CsA) + mizoribine (MZR) (or mycophenolate mofetil [MMF]) + prednisolone (PSL) + basiliximab. Abbreviation: Tx, transplantation.

Table 1. Demographic and Baseline Characteristics

Characteristic	MZR (n = 90)	MMF (n = 81)	*P*
Cause of uremia			
Diabetic nephropathy	14 (16%)	12 (15%)	.3057
Chronic glomerulonephritis	41 (46%)	31 (38%)	
Focal glomerulosclerosis	5 (6%)	1 (1%)	
Other	17 (19%)	24 (30%)	
Unknown	13 (14%)	13 (16%)	
Recipient sex			
Male/female	56/34	50/31	.9470
Recipient age, y*	42.5 ± 13.5	39.2 ± 13.1	.1040
Recipient weight, kg*	56.0 ± 11.1	57.8 ± 14.2	.3797
Duration of hemodialysis, mo†	27 (0–364)	9.5 (0–348)	.1575
Donation source			
Living donors	86 (96%)	78 (96%)	.6316
Cardiac death donors	3 (3%)	3 (4%)	
Brain death donor	1 (1%)	0	
Donor type			
Father	18 (20%)	19 (23%)	.5943
Mother	26 (29%)	24 (39%)	
Siblings	19 (21%)	9 (11%)	
Children	3 (3%)	2 (2%)	
Grandparents	0	1 (1%)	
Uncle or aunt	0	1 (1%)	
Spouse	20 (22%)	22 (27%)	
Death donor	4 (4%)	3 (4%)	
HLA-AB mismatches*	1.85 ± 0.98	1.96 ± 0.99	.5611
HLA-DR mismatches*	0.94 ± 0.63	0.96 ± 0.64	.8177
ABO blood type			
Identical	63 (70%)	51 (63%)	.3297
Compatible	27 (30%)	30 (37%)	

Abbreviations: MMF, mycophenolate mofetil; MZR, mizoribine.
*Mean ± standard deviation.
†Median (range).

The study was approved by the ethics committees of the participating facilities. All patients gave informed consent. The study was approved by an institutional review board and complied with the Declaration of Helsinki and the ethical guidelines outlined by The Transplantation Society.

Endpoints

The primary study endpoints used to reflect efficacy were 2-year patient survival, 2-year graft survival, and the acute rejection-free rate within 2 years of transplantation. Major side effects and complications occurring within 2 years were also recorded as an endpoint. The diagnosis of rejection was established by using biopsy results. Any patients who required antirejection therapy were considered to have acute rejection of their grafts.

Statistical Analysis

The Student *t* test, χ^2 test, and log-rank test were used for statistical analyses.

RESULTS

Demographic Characteristics

The patients and donors assigned to the MZR and MMF groups had similar demographic and baseline characteristics (Table 1).

Immunosuppressive Therapy

Transit of the administered dose of CsA in the MZR and MMF groups is shown in Fig 2A. The administered doses of CsA and steroid did not differ significantly between groups. Transit of the administered doses of MZR and MMF is shown in Fig 2B. The dose of MZR administered was 316 ± 73 mg at 1 week, 312 ± 65 mg at 1 month, 289 ± 70 mg at 6 months, 291 ± 82 mg at 12 months, and 276 ± 77 mg at 24 months. The dose of MMF administered was 1805 ± 845 mg at 1 week, 1481 ± 434 mg at 1 month, 1367 ± 512 mg at 6 months, 1307 ± 509 mg at 12 months, and 1247 ± 473 mg at 24 months.

Patient and Graft Survival

The 2-year patient survival rates in the MZR and MMF groups were 98.9% and 98.8%, respectively. One patient in the MZR group died of sepsis caused by methicillin-resistant *Staphylococcus aureus* infection 1 month after transplantation, and 1 patient in the MMF group died of type B fulminant hepatitis 5 months after transplantation; renal function was normal in both patients. The 2-year graft survival rates in the MZR group and MMF group were 97.8% and 97.5% (renal graft loss was observed from 1 year after transplantation in 1 case each from both groups) (Table 2).

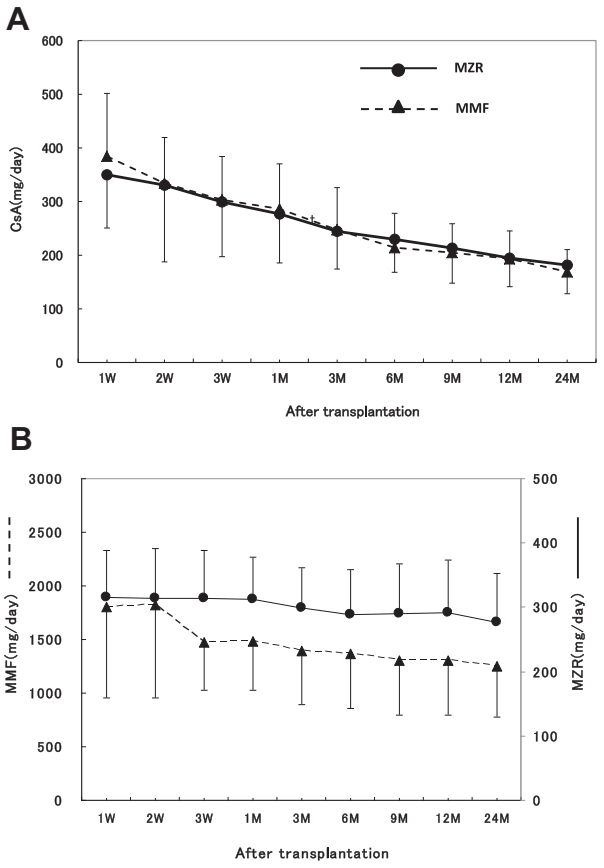

Fig 2. (**A**) Administration dose of cyclosporine (CsA). (**B**) Administration dose of mizoribine (MZR) and mycophenolate mofetil (MMF). Each value indicates mean ± standard deviation. Abbreviations: M, month; W, week.

Table 2. Graft Survival Rate, Rejection Episodes, and Outcome of Renal Function

Variable	MZR (n = 90)	MMF (n = 81)	*P*
Cumulative graft survival rate			
0 mo after transplantation	1.000	1.000	1.0
6 mo after transplantation	0.989	0.988	.9972
12 mo after transplantation	0.989	0.975	.8992
18 mo after transplantation	0.977	0.975	.9793
24 mo after transplantation	0.977	0.975	.9793
Rejection episodes	19 (21.1%)	13 (16.0%)	.3968
Serum creatinine level, mg/dL*			
1 mo after transplantation	1.37 ± 0.67	1.36 ± 0.75	.9382
3 mo after transplantation	1.46 ± 0.51	1.46 ± 0.57	.9951
6 mo after transplantation	1.51 ± 0.53	1.49 ± 0.61	.8770
12 mo after transplantation	1.52 ± 0.61	1.54 ± 0.72	.8843
24 mo after transplantation	1.51 ± 0.59	1.44 ± 0.46	.4170

Abbreviations: MMF, mycophenolate mofetil; MZR, mizoribine.
*Mean ± standard deviation.

Acute Rejection–Free Rate Within 2 Years of Transplantation and Renal Function

The graft rejection rate at 2 years after transplantation in each group is shown in Table 2. The rejection rate observed in the MZR group (21.1%) was not significantly higher than that in the MMF group (16.0%). Serum creatinine levels in the MZR and MMF groups at 1, 3, 6, 12, and 24 months after transplantation are shown in Table 3. There were no significant differences between the 2 groups at any time point.

Adverse Effects

The incidence of adverse effects and infections, both drug related and overall, during the 2-year period after renal transplantation was compared between the 2 groups (Table 3). The rate of hyperuricemia onset was significantly higher in the MZR group (52 of 90 [57.8%]) than in the MMF group (23 of 81 [28.4%]) ($P = .0001$). Gastrointestinal disorder occurred in 1 patient from the MZR group and 7 patients from the MMF group ($P = .0199$). Diarrhea occurred in 5 patients in the MMF group; the severity of the diarrhea in 3 patients required a reduction in MMF dose that resulted in symptom abatement. Abdominal distension and nausea also occurred with high frequency in the MMF group. In addition, leukocytopenia or anemia was observed in 2 patients from the MZR group and 8 patients from the MMF group ($P = .0315$). There were no reports of hyperlipidemia in the MZR group; 8 patients from the MMF group reported hyperlipidemia ($P = .0021$). However, no significant intergroup difference was detected in the incidence of impaired glucose tolerance or hepatitis.

Cytomegalovirus Infection

The incidence of cytomegalovirus (CMV) infection was significantly higher in the MMF group (Table 3). None of

Table 3. Adverse Events Profile

Adverse Event	MZR (n = 90)	MMF (n = 81)	*P*
Infection			
CMV disease	0	10 (12.3%)	<.0001
CMV antigenemia-positive	26 (28.9%)	38 (46.9%)	<.0001
Treatment with GCV	5 (19.2%)	21 (55.3%)	.0039
Antigen peak level (/50,000)	20.4 ± 44.1*	252.8 ± 527.0*	.0082
BKV	2 (2.2%)	1 (1.2%)	NS
HSV	2 (2.2%)	2 (2.5%)	NS
Herpes zoster	2 (2.2%)	5 (6.2%)	NS
Sepsis (MRSA)	1 (1.1%)	0	NS
PTLD	1 (1.1%)	0	NS
Pneumocystis carinii	0	1 (1.2%)	NS
Pneumonia	0	1 (1.2%)	NS
Peritonitis	0	1 (1.2%)	NS
Urinary tract infection	2 (2.2%)	2 (2.5%)	NS
Hyperuricemia	55 (61.1%)	30 (37.0%)	.0017
Gastrointestinal disorder (inappetence, nausea, vomiting, diarrhea)	1 (1.1%)	7 (8.6%)	.0199
Blood disorder (leukocytopenia, anemia)	2 (2.2%)	8 (9.9%)	.0315
Hyperlipidemia	0	8 (9.9%)	.0021
Impaired glucose tolerance	2 (2.2%)	2 (2.5%)	NS
Boredom, fever, trembling	3 (3.3%)	6 (7.4%)	NS
Hepatitis	0	2 (2.5%)	NS

Abbreviations: CMV, cytomegalovirus; GCV, ganciclovir; BKV, BK virus; HSV, herpes simplex virus; MMF, mycophenolate mofetil; MRSA, methicillin-resistant *Staphylococcus aureus*; MZR, mizoribine; NS, not significant; PTLD, post-transplant lymphoproliferative disease.
*Mean ± standard deviation.

the patients in the MZR group developed CMV disease; 10 (12.3%) patients in the MMF group ($P < .0001$) developed CMV disease. Twenty-six patients (28.9%) in the MZR group were CMV antigenemia-positive, compared with 38 (46.9%) in the MMF group ($P < .0001$), and the average peak level of CMV antigenemia was 20.4 ± 44.1 in the MZR group and 252.8 ± 527.0 in the MMF group ($P = .0082$), respectively. Ganciclovir (GCV) was administered to 5 (19.2%) patients in the MZR group and 21 (55.3%) in the MMF group ($P = .0039$).

DISCUSSION

The present prospective study compared renal transplant patients with no ABO blood group incompatibility treated at 11 transplant institutions in Japan with patients administered MMF at the same institutions during almost the same period. The results indicate that patients in the MZR group had outcomes equivalent to those of the MMF group in terms of 2-year patient survival, 2-year graft survival, and renal function. The acute rejection rate was 18.9% in the MZR group and 16.0% in the MMF group, the difference being nonsignificant. With regard to adverse events, it is known that MZR is associated with a lower risk of gastrointestinal disorders or viral infection than MMF [3]. The most troublesome side effect of MZR is hyperuricemia, and this scenario was also the case in the present series. However, previous reports have suggested that even if MZR is used in conjunction with allopurinol (a drug used to treat hyperuricemia), its metabolism is not affected, as is the case with azathioprine (AZA) [5]. For this reason, in the present study, MZR-induced hyperuricemia was easily controlled in most cases by administration of allopurinol or benzbromarone.

Some papers [6,7] have suggested that the number of patients with CMV disease has increased since the introduction of MMF. Basic-Jukic et al [6] suggested that use of MMF was associated with an increased incidence of CMV disease after cadaveric renal transplantation compared with AZA. In their study, the group treated with AZA included 280 patients who were treated for 17,672 months with AZA/CsA/steroids or AZA/steroids; the MMF group included 219 patients who were treated for 5079 months with MMF/CsA/steroids or MMF/steroids. The AZA group had 51 CMV disease episodes (1 episode per 346.5 treatment months), and the MMF group had 43 episodes (1 per 118.1 months) ($P < .01$). Mean onset of CMV disease occurred 32.65 ± 47.69 months and 3.72 ± 4.43 months after transplantation in the AZA and MMF groups, respectively.

Shiraki et al [8] reported the effect of MZR on suppressing CMV replication in vitro. They showed that the combination of CsA and prednisolone enhances CMV replication, whereas addition of MZR suppresses it. The anti-CMV activity of MZR is not as potent as that of GCV but may help suppress the early stages of CMV infection and reduce the frequency and severity of CMV infection in vivo. The synergistic suppressive effects of MZR and GCV on CMV replication may exert a more effective therapeutic effect when GCV therapy is required. The immunosuppressive effects of MZR on in vitro human CMV replication in cells treated with CsA and prednisolone is dose dependent, with MZR concentrations of 1 μg/mL, 2 μg/mL, and 10 μg/mL inducing ~60%, 80%, and 90% suppression. In our study, the peak (C4) MZR blood concentration during the first 14 and 28 days after transplantation was 2.9 to 3.3 μg/mL, and the trough concentration was ~1.0 μg/mL (data not shown). These blood levels may have been appropriate for inducing anti-CMV activity.

MMF is also known to increase the frequency of gastrointestinal disorders such as diarrhea, which complicate patient management after transplantation. In the present study, diarrhea was reported in 5 of 81 patients in the MMF group, and this side effect was managed by reducing or discontinuing MMF. Diarrhea was not reported as a side effect in the MZR group. Although Gastrointestinal Symptom Rating Scale scores [9] were not used in this study, there were clearly fewer adverse events involving gastrointestinal disorders in the MZR group than in the MMF group.

CONCLUSIONS

Our findings suggest that a combination of high-dose MZR with CsA, basiliximab, and corticosteroids at the indicated

dosages is associated not only with satisfactory immunosuppression but also a low incidence of CMV infection, gastrointestinal disorder, hyperlipidemia, and blood disorder in renal transplant patients.

ACKNOWLEDGMENTS

The authors thank Dr. Takashi Ando from the Japan Clinical Research Support Unit, Tokyo, Japan, for conducting the statistical analysis. Part of this study was presented at the American Transplantation Congress (May 18–22, 2013) in Seattle, Washington.

REFERENCES

[1] Mizuno K, Tsujino M, Takada M, Hayashi M, Atsumi K. Studies on bredinin. I. Isolation, characterization and biological properties. J Antibiot 1974;27:775–82.

[2] Ishikawa H. Mizoribine and mycophenolate mofetil. Curr Med Chem 1999;6:575–97.

[3] Akiyama T, Okazaki H, Takahashi K, Hasegawa A, Tanabe K, Uchida K, et al. Mizoribine in combination therapy with tacrolimus for living donor renal transplantation. Transplant Proc 2005;37:843–5.

[4] Nishimura K, Uchida K, Yuzawa K, Fukuda Y, Ichikawa Y, Akioka K, et al. Excellent results with high-dose mizoribine combined with cyclosporine, corticosteroid, and basiliximab in renal transplant recipients: multicenter study in Japan. Transplant Proc 2012;44:147–9.

[5] Jain AK, Lee HA. No myelosuppression when mizoribine is combined with allopurinol for the treatment of hyperuricemia in renal transplant patients. Clin Exp Nephrol 2001;5:173–6.

[6] Basic-Jukic N, Kes P, Bubic-Filipi LJ, Puretic Z, Brunetta B, Pasini J. Does mycophenolate mofetil increase the incidence of cytomegalovirus disease compared with azathioprine after cadaveric kidney transplantation? Transplant Proc 2005;37:850–1.

[7] Sarmiento JM, Dockrell DH, Schwab TR, Munn SR, Paya CV. Mycophenolate mofetil increases cytomegalovirus invasive organ disease in renal transplant patients. Clin Transplant 2000;14:136–8.

[8] Shiraki K, Ishibashi M, Okuno T, Kokado Y, Takahara S, Yamanishi K, et al. Effects of cyclosporine, azathioprine, mizoribine, and prednisolone on replication of human cytomegalovirus. Transplant Proc 1990;22:1682–5.

[9] Darji P, Vijayaraghavan R, Thiagarajan CM, Sharma RK, Subbarao B, Pishardy R, et al. Conversion from mycophenolate mofetil to enteric-coated mycophenolate sodium in renal transplant recipients with gastrointestinal tract disorders. Transplant Proc 2008;40:2262–7.