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Original Article

High-dose mizoribine combined with calcineurin inhibitor (cyclosporine or tacrolimus), basiliximab and corticosteroids for renal transplantation: A Japanese multicenter study

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Abbreviations & Acronyms
AE = adverse event
CMV = cytomegalovirus
CNI = calcineurin inhibitor
CsA = cyclosporine
HLA-AB = human leukocyte antigen-AB
HLA-DR = human leukocyte antigen-DR
MMF = mycophenolate mofetil
MZR = mizoribine
TAC = tacrolimus

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Objective: To evaluate the utility and safety of high-dose mizoribine combination therapy using cyclosporine and tacrolimus as calcineurin inhibitors in patients undergoing kidney transplant.

Methods: The present study enrolled 156 patients who received kidney transplants in 18 institutions between 2009 and 2013. ABO-incompatible and/or pre-sensitized recipients were excluded. Immunosuppression used cyclosporine (88) or tacrolimus (68) as a calcineurin inhibitor, and the dosage was adjusted based on blood concentrations. Mizoribine was started at 6 mg/kg/day, and the target trough level was 1–2 ng/mL. Primary efficacy end-points of this study were 2-year patient survival, 2-year graft survival and the acute rejection rate within 2 years after transplantation.

Results: The 2-year patient and graft survival rates in the cyclosporine group were 98.9% and 94.3%, respectively, whereas those in the tacrolimus group were 100% and 98.5%, respectively, with no significant difference between groups. Rates of onset of rejection during the observation period were also equivalent, at 22.7% in the cyclosporine group and 17.6% in the tacrolimus group. Furthermore, groups showed no significant differences in transplanted renal function. No notable differences in adverse events were observed between groups.

Conclusions: A regimen of high-dose mizoribine in combination with calcineurin inhibitors basiliximab, and corticosteroids can provide effective immunosuppression while lowering the rate of cytomegalovirus infection in kidney transplant patients.

Key words: calcineurin inhibitors, combination therapy, cytomegalovirus, mizoribine, renal transplantation.

Introduction

Recent advances in immunosuppressive agents have been marked, and research and development into new drugs continue to be active. As a result, many drugs are available, and proper use in combination with immunosuppression is key to graft survival of renal transplantation. The current concept of immunosuppression is to adeptly combine various kinds of drugs so that the characteristics are taken advantage of to control rejection reactions, while avoiding side-effects.

MZR, a novel nucleoside analog, has been developed as an immunosuppressant. The initially approved daily dosage of MZR for renal transplant recipients was 1–3 mg/kg/day, but this dose was found to be too low to suppress rejection reactions. A 2005 multicenter collaborative study showed that a MZR dose ≥5 mg/kg/day was appropriate when combined with TAC.[1] In 2016, a multicenter collaborative study was carried out on the utility and safety of high-dose MZR (6 mg/kg/day) combined with CsA, basiliximab and corticosteroids. In that

study, 2-year graft survival rates in the MZR and MMF groups were 97.8% and 97.5%, and rejection rates within 2 years after transplantation were 21.1% (MZR) and 16.0% (MMF); this difference was not significant. The incidence of CMV infection was significantly higher in the MMF group. They concluded that combination therapy is effective for kidney transplantation recipients.[2]

We report herein a multicenter collaborative study carried out after the aforementioned study. The purpose of the present study was to evaluate the utility and safety of high-dose MZR combination therapy using CsA and TAC as CNIs.

Methods

Patients and immunosuppression therapy

The present study enrolled 156 patients who received kidney transplants in 18 institutions between 2009 and 2013. A total of 148 kidney transplant recipients were from living donors, and eight kidney transplant recipients were from cadaveric donors. ABO-incompatible and/or pre-sensitized recipients were excluded. Immunosuppression therapy was initiated with MZR, corticosteroids, basiliximab and CsA or TAC as a CNI. In the present study, the allocation to the TAC and CsA groups was not random. The dosage of drugs was adjusted based on blood concentrations (Fig. 1). CsA (n = 88) was administered to keep the trough level at 200 ng/mL during the first week, and at 150–200 ng/mL 1 week after kidney transplantation. TAC (n = 68) was administered to keep the trough level at 10–15 ng/mL during the first 3 weeks and at 5–10 ng/mL until 3 month after kidney transplantation. MZR was started at 6 mg/kg/day, and the target trough level was 1–2 ng/mL. Basiliximab at 20 mg was given intravenously on day 0 and at the same dose on day 4. The maintenance dose of corticosteroids was 5–10 mg/day after initial dose reduction.

Ethical approval

All study protocols were approved by the ethics committees at each study center, and all patients provided written informed consent. The study was carried out in accordance with the guidelines of the Declaration of Helsinki and the ethical guidelines outlined by the Transplantation Society. The study information was disclosed in the University Hospital Medical Information Center (registration no. 8751).

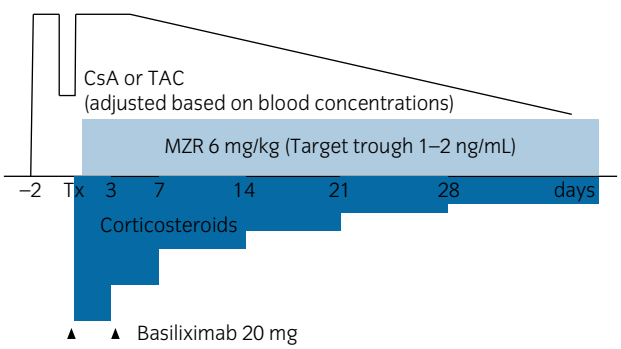

Fig. 1 Immunosuppression used CsA or TAC as a CNI, and the dosage was adjusted based on blood concentrations. MZR was started at 6 mg/kg/day, and the target trough level was 1–2 ng/mL. Basiliximab at 20 mg was administered intravenously on days 0 and 4. The maintenance dose of corticosteroids was 5–10 mg/day after initial dose reduction.

End-points

Primary efficacy end-points of the present study were 2-year patient survival, 2-year graft survival and the acute rejection rate within 2 years after transplantation. When rejection was suspected, diagnosis of rejection was established from graft biopsy. Rejection was diagnosed according to the Banff classification (1997) after carrying out a graft biopsy. Adverse events occurring within 2 years were also recorded as an end-point.

Statistical analysis

Student's t-test, the χ^2-test and the log–rank test were used for statistical analysis.

Results

Demographic characteristics

Baseline characteristics of patients and pre-transplant complications among recipients and donors are shown in Table 1. CsA and TAC groups showed similar demographic and baseline characteristics.

Immunosuppression

CNI trough levels are shown in Figure 2. CsA trough levels were 261 ± 100 ng/mL at 1 week, 215 ± 72 ng/mL at 1 month, 139 ± 69 ng/mL at 1 year and 123 ± 85 ng/mL at 2 years. TAC trough level was 10.7 ± 4.3 ng/mL at 1 week, 8.8 ± 2.9 ng/mL at 1 month, 5.6 ± 2.1 ng/mL at 1 year and 5.6 ± 2.4 ng/mL at 2 years. Administered doses of MZR were 316 ± 96 mg/day at 1 month, 273 ± 86 mg/day at 1 year and 258 ± 76 mg/day at 2 years in the CsA group, and 328 ± 89 mg/day at 1 month, 276 ± 82 mg/day at 1 year and 256 ± 73 mg/day at 2 years in the TAC group. There was no significant difference in dose of MZR administered between CsA and TAC groups.

Patient and graft survivals

The 2-year patient survival rates using Kaplan–Meier analysis were 98.9% in the CsA group and 100% in the TAC group. One patient in the CsA group died of acute cardiac failure on postoperative day 47. The 2-year graft survival rates were 94.3% in the CsA group and 98.5% in the TAC group.

Acute rejection rate and graft function

Acute rejection rates within 2 years after transplantation were 22.7% in the CsA group and 17.6% in the TAC group. The Banff classifications of AR are shown Table 2. The difference in rejection rate between CsA and TAC groups was not significant. Serum creatinine levels in both groups are shown

Table 1 Baseline characteristics of patients among recipients and donors

	CsA (n = 88)	TAC (n = 68)	P
Recipient			
Sex (male/female)	66/22	42/26	0.0757
Age (years)	44.4 ± 14.2	45.4 ± 14.8	0.6885
Bodyweight (kg)	61.9 ± 13.5	57.1 ± 11.7	0.0218
Cause of uremia			0.7795
Diabetic nephropathy	18	9	
Chronic glomerulonephritis	33	32	
Focal glomerulosclerosis	6	4	
Loops nephritis	1	1	
Other	18	15	
Unknown	12	7	
Duration of hemodialysis (months)	45.7 ± 66.4	73.9 ± 48.0	0.0705
Pre-emptive transplantation	24	20	
Donor			
Donor source (living/cadaveric)	86/2	62/6	0.0795
Sex (male/female)	28/60	30/38	0.1761
Age (years)	56.5 ± 10.0	54.1 ± 12.0	0.0804
Donor type			
Father	15	11	
Mother	31	13	
Siblings	10	13	
Spouse	28	19	
Other	2	6	
ABO blood type			
Identical	65	48	0.6498
Compatible	23	20	
HLA-AB mismatches	2.1 ± 1.0	2.0 ± 1.3	0.6735
HLA-DR mismatches	1.0 ± 0.6	0.9 ± 0.7	0.3448

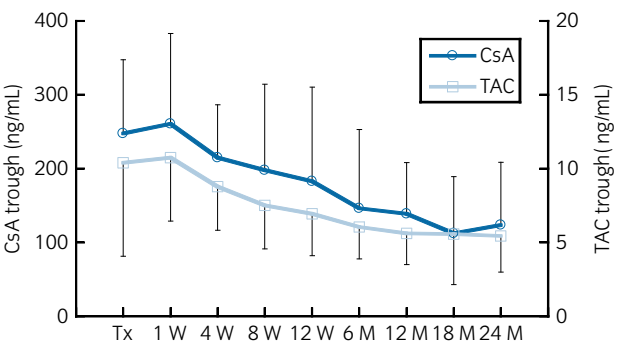

Fig. 2 CsA trough levels were 261 ± 100 ng/mL at 1 week, 215 ± 72 ng/mL at 1 month, 139 ± 69 ng/mL at 1 year and 123 ± 85 ng/mL at 2 years. TAC trough levels were 10.7 ± 4.3 ng/mL at 1 week, 8.8 ± 2.9 ng/mL at 1 month, 5.6 ± 2.1 ng/mL at 1 year and 5.6 ± 2.4 ng/mL at 2 years.

in Figure 3. No significant difference in graft function was evident between the CsA and TAC groups.

Adverse events

Incidences of adverse events during the 2-year period after transplantation in the CsA and TAC groups are shown in Table 3. Rates of adverse events were 64.8% in the CsA

Table 2 Incidences of acute rejection in the CsA and TAC groups

	CsA		TAC	
No. patients	20/88 (22.7%)		12/68 (17.6%)	
Frequency of rejection	27		13	
	Patients	Frequency	Patients	Frequency
Banff classification				
Acute antibody mediated rejection	0	0	4	4
Border line changes	2	2	1	1
Acute rejection type 1A	6	8	3	4
Acute rejection type 1B	6	9	1	1
Acute rejection type 2A	0	0	1	1
Acute rejection type 2B	0	0	0	0
Acute rejection type 3	0	0	0	0
Clinical rejection (without biopsy)	6	8	2	2

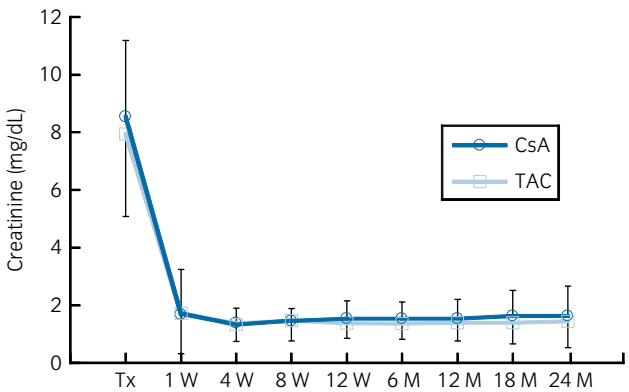

Fig. 3 Serum creatinine levels in both groups are shown. No significant difference in graft function was evident between the CsA and TAC groups.

Table 3 Incidences of adverse events in the CsA and TAC groups

	CsA (n = 88)	TAC (n = 68)
No. patients	57 (64.8%)	41 (60.3%)
No. AEs	81	66
Blood disorder		
Leukocytopenia	2	2
Thrombocytopenia	2	3
Anemia	0	2
Infection		
CMV disease	0	1
CMV antigenemia-positive	26	19
Herpes zoster	4	2
Urinary tract infection	1	0
Genital herpes	1	0
Liver dysfunction	1	4
Hyperglycemia	1	2
Hyperuricemia	18	13
Diarrhea	1	0
Other	24	18

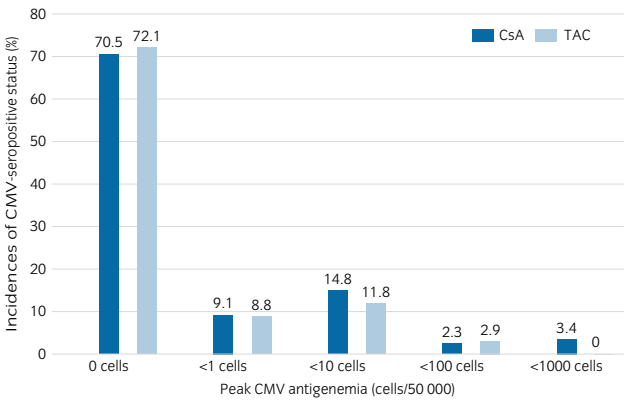

Fig. 4 Incidences of CMV-seropositive status in both groups are shown. No significant difference in peak CMV antigenemia was evident between the CsA and TAC groups.

group and 60.3% in the TAC group. Profiles of adverse events were similar in both groups.

Incidences of CMV-seropositive status were 29.5% in the CsA group and 27.9% in the TAC group (30.0% and 25.8% after excluding the risk status of donor-positive/recipient-negative). Incidences of CMV-seropositive status in both groups are shown in Figure 4. One patient in the TAC group experienced CMV disease (pneumonia treated with ganciclovir).

Rates of uricemia onset were 20.5% in the CsA group and 19.1% in the TAC group. Incidences of CMV-seropositive status in both groups are shown in Figure 4. All patients with hyperuricemia received treatment. Hyperuricemia was easily controlled with drugs in both groups. Mean uric acid levels were 6.41 mg/dL at 6 month, 6.07 mg/dL at 1 year and 5.94 mg/dL at 2 years in the CsA group and 6.37 mg/dL at 6 month, 6.27 mg/dL at 1 year and 6.35 mg/dL at 2 years in the TAC group. There was no significant difference in uric acid levels between the CsA and TAC groups.

Discussion

MZR is a novel imidazole nucleoside, isolated from the culture filtrate of *Eupenicillin brefeldianum* M-2166. MZR is an immunosuppressive agent that inhibits the proliferation of lymphocytes, and entered the Japanese market in 1984.[3,4] MZR has been registered in Japan not only for the prevention of rejection in kidney transplantation recipients, but also for the treatment of lupus nephritis, rheumatoid arthritis and nephrotic syndrome.

The initially approved daily dosage of MZR for renal transplant recipients was 1–3 mg/kg/day, determined on the basis of animal experiments and the *in vitro* mixed lymphocyte reaction.[5,6] However, this was found to be inadequate to suppress rejection reactions. Tanabe reported that MZR at 1–3 mg/kg/day did not bring the blood concentration to an effective range in some cases, and a higher dosage of 4–5 mg/kg/day did bring the blood concentration to an effective range without major adverse events.[7] In 2005, a multicenter collaborative study carried out by Akiyama *et al.* investigated the utility of TAC, MZR and corticosteroids combination therapy.[1] They noted that combination therapy was safe and efficacious, and that "a loading dose of 5 mg/kg/day is beneficial to increase the rejection free rate."

In 2013, Yoshimura *et al.* tried combination therapy using MZR at 6 mg/kg/day together with CsA, basiliximab and corticosteroids, and showed the efficacy of this regimen.[8,9] A nationwide multicenter collaborative study using a similar regimen was undertaken in 2016.[2] Combination therapy using high-dose MZR with CsA, basiliximab and corticosteroids has been reported as an effective regimen for inhibiting rejection, and has led to renal transplant survival. The results also suggested that the risk of CMV infection was reduced compared with MMF, and that benefits were also seen in regard to events such as gastrointestinal disorders, hyperlipidemia and blood disorders. In 2013, Oshiro *et al.* concluded that four-drug combination therapy using high-dose MZR (8 mg/kg/day), CyA, basiliximab and corticosteroids in renal transplant recipients was effective and safe.[10] They also reported MZR trough levels >2.5 mg/mL were effective to prevent acute rejection episodes.

In contrast, in 2005, Akiyama *et al.* reported high-dose MZR at ≥5 mg/kg/day used in combination with TAC is beneficial to increase the rejection-free rate.[1] In 2013, Takahara *et al.* reported that 12 mg/kg/day MZR and 2 g/day MMF with TAC were considered almost equivalent in terms of efficacy and safety.[11] In 2016, Ishida *et al.* reported the results of a regimen in which an increased MZR dosage of 12 mg/kg/day was used in combination with TAC, basiliximab and corticosteroids.[12] They concluded that "high-dose MZR at 12 mg/kg/day was a safe and efficacious immunosuppressive alternative to MMF in living donor kidney transplant recipients."

It goes without saying that TAC, along with CsA, is a main drug of post-transplantation immunosuppressant therapy. The mechanism of immunosuppression by TAC resembles that of CsA, binding to and inhibiting the activity of calcineurin. However, the profile of side-effects differs from that of CsA, and caution is required for use in combination with other drugs.

The present domestic multicenter study carried out immunosuppressive therapy with four drugs, including high-dose MZR, following ABO blood type-compatible kidney transplantation. The results showed that the 2-year patient and graft survival rates in the CsA group were 98.9% and 94.3%, respectively, whereas those in the TAC group were 100% and 98.5%, respectively, with no significant difference between groups. Rates of onset of rejection during the observation period were also equivalent, at 22.7% in the CsA group and 17.6% in the TAC group. However, these AR rates show rather high incidence, as they include borderline change and the clinical diagnosis. Furthermore, groups showed no significant differences in transplanted renal function. MZR dosages during the study period also did not differ between groups. Both TAC and CsA were administered at standard blood concentrations. That is, like CsA, TAC was shown to be an effective immunosuppressive agent in combination with high-dose MZR, basiliximab and corticosteroids.

No notable differences in adverse events were observed between groups. CMV antigenemia-positive rates were

29.5% in the CsA group and 27.9% in the TAC group, and were 30% and 25.8% after excluding negative recipient cases from CMV-positive donors. Only one case of CMV disease was seen in the TAC group. In contrast, when using MMF ($n = 81$), the earlier nationwide multicenter study found that the CMV antigenemia-positive rate was 46.9% and the frequency of CMV disease was 12.3%.[2] Shiraki *et al.* suggested that CsA and corticosteroids enhanced the replication of human cytomegalovirus *in vitro*, and MZR and azathioprine suppressed replication at the concentrations used *in vivo*.[13] They also reported that the combination of CsA, corticosteroids and MZR at immunosuppressive doses suppressed replication of human cytomegalovirus *in vitro*, with suppression dependent on the concentration of MZR. Kuramoto *et al.* reported MZR as an immunosuppressive and anti-CMV drug in the clinical regimen was suggested to suppress replication of CMV *in vivo* and control CMV infection in transplant recipients in combination with ganciclovir.[14] The present results suggest that MZR exerts antiviral activity, and can be considered an advantageous agent for use in combination therapy for CMV infection in renal transplant recipients.

In contrast, caution is required in regard to hyperuricemia, which is a characteristic side-effect of MZR.[15,16] The mechanism should be associated with hypoxanthine-guanine phosphoribosyl transferase, which catalyzes the conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate.[17] It plays a central role in the generation of purine nucleotides through the purine salvage pathway. However, there is no report that MZR has the ability to enhance hypoxanthine-guanine phosphoribosyl transferase activity, which suggests why serum uric acid cannot be kept at stable normal levels under MZR treatment. Ding *et al.* reported that recipients who receive MZR should be monitored frequently for serum uric acid during the first 5 months, followed by standard monitoring after 18 months.[18] MZR-induced hyperuricemia is known to correlate strongly with blood concentrations of MZR. As MZR is excreted by the kidney, blood concentrations correlate with the function of the transplanted kidney. Therefore, if changes in renal function occur during the course of combination therapy, MZR blood levels should be determined and the dosage adjusted accordingly. In addition, MZR-induced hyperuricemia is easily controlled with drugs, and blood concentrations of uric acid were able to be properly controlled in the present study.

The limitations of this study are that the allocation to the TAC and CsA groups was not random, there might be a bias and the observation period was just 2 years. Another point is that we were not able to verify the extent to which adjustment of the MZR dosage was carried out.

Continuation of MMF can be difficult as a result of hematological toxicity, gastrointestinal problems and so on. The high-dose MZR can be expected to have a rejection reaction suppressing effect comparable with that of the usual dosage of MMF. It is also advantageous for CMV infection. In conclusion, the regimen of high-dose MZR in combination with CNIs (CsA as well as TAC), basiliximab and corticosteroids can establish not only satisfactory immunosuppression, but also a low rate of CMV infection *in vivo*.

Acknowledgments

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Conflict of interest

None declared.

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