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Excellent Results of High-Dose Mizoribine Combined With Cyclosporine, Basiliximab, and Corticosteroids in Renal Transplant Recipients—4-Year Results

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ABSTRACT

Background. Mizoribine (MZR) at 3 mg/kg/d shows less potent immunosuppressive effects, but high-dose MZR (6 mg/kg/d) was effective and safe in a 2-year study in conjunction with a regimen of cyclosporine (CsA), basiliximab, and corticosteroids.

Methods. We compared 40 living-related kidney recipients administered MZR (6 mg/kg/d), CsA (7 mg/kg/d), prednisolone (maintenance dose 10 mg/d), and basiliximab (20 mg/body) with control group (n = 38) treated with CsA, mycophenolate mofetil (MMF; 25 mg/kg/d), basiliximab, and corticosteroids.

Results. The 4-year graft survival rates for the MZR vs MMF groups were 92.5% vs 94.7%, respectively, with serum creatinine levels of 1.66 ± 1.0 mg/dL vs 1.41 ± 0.42 mg/dL at 3 years, and 1.72 ± 1.16 mg/dL vs 1.56 ± 1.26 mg/dL at 4 years. There was no significant difference in serum creatinine levels between the 2 groups. The MZR group demonstrated a significantly higher rate of elevated serum uric acid values (29.7%). The numbers of patients treated with allopurinol at 4 years were 11/37 (29.7%) for MZR vs 2/36 (5.6%) for the MMF subjects ($P < .05$). Mean serum uric acid levels of the MZR vs MMF group at 4 years were 7.1 ± 1.9 mg/dL vs 7.0 ± 1.6 mg/dL, respectively (NS). There was no significant difference between the 2 groups regarding bone marrow suppression or liver dysfunction. Severe cytomegalovirus infection was not observed at 3 and 4 years in either group. There were no severe gastrointestinal symptoms among the MZR or the MMF group at 3 or 4 years.

Conclusions. The combination of high-dose MZR with CsA, basiliximab, and corticosteroids displayed excellent results over a 4-year follow-up.

Mizoribine (MZR), a novel nucleoside analogue developed as an immunosuppressive agent[1] was placed on the market in Japan in 1984, where it has been widely used for 28 years. MZR has been shown to exhibit a low incidence of severe adverse drug reactions and does not enhance oncogenicity.[2,3] The standard dosage used in the 1990s was 1–3 mg/kg/d as a substitute for azathioprine (AZA) in combination with steroids and cyclosporine (CsA).[4] However, some reports have indicated that up to 3 mg/kg/d MZR has less potent immunosuppressive effects but few adverse events.[5,6] Therefore, we previously reported the results of a 2-years follow-up with high-dose MZR (6 mg/kg/d), documenting it to be effective and safe for kidney transplant patients in conjunction with CsA, basiliximab, and corticosteroids.[7] In the present study, we reported the results at 4 years.

PATIENTS AND METHODS

Study Subjects

Among 92 renal transplantations from October 2004 to July 2007 at our institution, the 78 living-donor cases constituted the focus of this study. CsA, basiliximab, and corticosteroids were concomitantly administered with either MZR or mycophenolate mofetil (MMF) treatment. Among the 78 cases, we obtained consent in 40

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0041-1345/13/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2013.01.055

cases for treatment with MZR. The patient characteristics of the MZR and MMF groups showed no significant differences between the 2 groups in the previous study, so we compared the safety and efficacy of MZR versus the MMF group in the present analysis.

Immunosuppression

Immunosuppressive therapy was initiated with MZR (daily dose 6 mg/kg, given orally twice a day), CsA (trough level 200 ng/mL during the first week and 150–200 ng/mL 1 week after transplantation), and prednisolone (maintenance dose 10 mg/d, after initial dose reduction). Basiliximab (20 mg/body) was administered on days 0 and 4 (Fig 1a). A control group treated with CsA, MMF, basiliximab, and corticosteroids was also employed in this study. MMF was administered at a dose of 25 mg/kg/d (Fig 1b). CsA, basiliximab, and steroids were administered using the same protocol as that for the MZR group. All patients were living related, aged 16–66 years. We excluded both ABO-incompatible and crossmatch-positive pairs. The number of patients who were switched from cyclosporine to tacrolimus within 4 years was 6 in MZR group and 4 in the MMF group (P = NS).

Each of the patients gave informed consent. The study was approved by the Institutional Review Board and complied with the Declaration of Helsinki.

Statistical Analysis

The Kaplan-Meier method was used to determine patient and graft survival rates, with statistical significance tested with the log-rank test. The study was approved by our Ethics Committee.

RESULTS

Patient and Graft Survival

The patient survival rates for the MZR versus MMF groups were, respectively, 100% versus 94.7% at 3 years and 97.5% versus 94.7%, at 4 years. The graft survival rates for the MZR vs MMF groups were, respectively, 100% versus 94.7% at 3 years and 92.5% versus 94.7% at 4 years. There was no significant difference between the groups regarding patient or graft survival rates.

Serum Creatinine Level (Table 1)

Serum creatinine levels for the MZR versus MMF groups were, respectively, 1.66 ± 1.07 mg/dL (n = 40) versus 1.41 ± 0.42 mg/dL (n = 36) at 3 years, and 1.72 ± 1.16 mg/dL (n = 37) versus 1.56 ± 1.26 mg/dL (n = 36) at 4 years. There was no significant difference in serum creatinine levels between the groups.

Adverse Effects

We compared the incidences of adverse effects and infections, both drug-related and overall, between 3 and 4 years after renal transplantation (Table 2). The MZR group demonstrated a significantly higher rate of elevated serum uric acid values (29.7%). The number treated with allopurinol was 11/38 (29.7%) MZR and 2/36 (5.6%) MMF subjects ($P < .05$). Mean serum urate levels of the MZR versus MMF group after transplantation were, respectively, 6.9 ± 1.5 mg/dL versus 7.1 ± 1.9 mg/dL at 3 years and 7.2 ± 1.8 mg/dL versus 7.0 ± 1.6 mg/dL at 4 years. There was no significant difference between the groups regarding bone marrow suppression or liver dysfunction.

Severe cytomegalovirus (CMV) infection was not observed at 3 or 4 years in either group. There were no gastrointestinal symptoms in the MZR or MMF group at 3 and 4 years. BK polyomavirus infection was not observed in either group.

DISCUSSION

MZR, a nucleoside of the imidazole class, was isolated from a culture medium of the mold *Eupenicillium brefeldianum* M-2166, which was found in the soil of Hachijo Island, Tokyo, Japan, in 1971.[1] The drug inhibits both humoral and cellular immunity by selectively blocking lymphocyte proliferation. MZR was approved as an immunosuppressant by the Ministry of Health and Welfare, Japan, in 1984. Recently, it has been used commonly in transplantation in

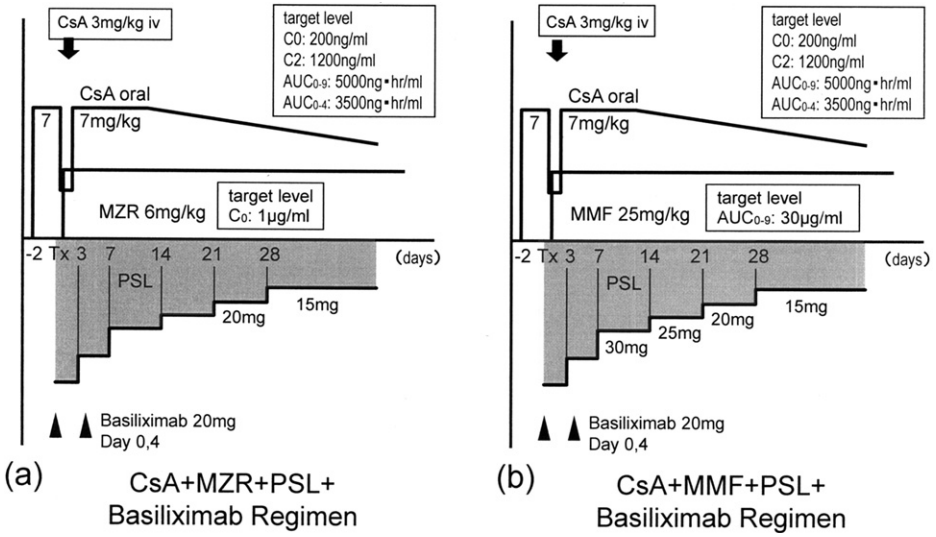

Fig 1. Immunosuppressive regimens: **(a)** MZR group; **(b)** MMF group. Abbreviations: CsA, cyclosporine; MZR, mizoribine; PSL, prednisolone; MMF, mycophenolate mofetil; Tx, transplant; AUC, area under receiver operating characteristic curve.

Table 1. Outcome of Kidney Function

	Mean Serum Creatinine Level (mg/dL)		
	MZR Group (n = 40)	MMF Group (n = 38)	*P* Value
1 y after transplantation	1.30 ± 0.56 (n = 40)	1.47 ± 0.50 (n = 37)	NS
2 y after transplantation	1.54 ± 0.69 (n = 40)	1.38 ± 0.36 (n = 36)	NS
3 y after transplantation	1.66 ± 1.07 (n = 40)	1.41 ± 0.42 (n = 36)	NS
4 y after transplantation	1.72 ± 1.16 (n = 37)	1.56 ± 1.26 (n = 36)	NS

Abbreviations: MZR, mizoribine; MMF, mycophenolate mofetil.

combination with other immunosuppressants, including CsA or tacrolimus, and corticosteroids.

In 1989, a randomized trial compared CsA/AZA with CsA/MZR revealing MZR to show equal immunosuppressive with fewer side effects of myelosuppression and liver dysfunction.[8]

The original dosage of MZR (1–3 mg/kg/d) was decided on the basis of animal experiments that showed good survival rates and no harmful side effects using 1.2 mg/kg in rats and 3 mg/kg in dogs.[5] The mixed lymphocyte reaction revealed that a blood concentration of 1 μg/mL suppressed lymphocyte proliferation by 50% (IC_{50}).[9] However, blood concentrations have not been measured in clinical cases. Several Japanese papers reported that 1–3 mg/kg/d MZR did not achieve effective blood concentrations in some cases and that a higher dosages of 4–5 mg/kg/d were as potent as MMF without major side effects.[10] Therefore, high-dose MZR (6 mg/kg/d) in conjunction with CsA, basiliximab, and corticosteroids was reported in a previous 2-year study.[7]

The 2-year graft survival rates for the MZR and MMF groups were 100% and 94.7%, respectively (Fig 2). The acute rejection rate in the MZR group (25%) was not significantly higher than in the MMF group (16%). Serum creatinine levels were not significantly different between the 2 groups. The number of patients who developed CMV infection was 0 in the MZR group and 7 (18.4%) in the MMF group ($P < .05$). The number of patients treated with gancyclovir was 3 (7.5%) and 11 (28.9%), respectively, ($P < .05$).

Our 2-year findings showed that a combination of high-dose MZR with CsA, basiliximab, and corticosteroids at indicated dosages yielded not only satisfactory immunosuppression but also suppression of CMV infection *in vivo*.

A multicenter trial in Japan[11] reported in 2004, that high doses of MZR (≥5 mg/kg/d) used concomitantly with tacrolimus achieved a significantly higher rejection-free rate within 3 months after transplantation (85.0%) compared with the <3 mg/kg/d group (64.9%) and with the 3-<5 mg/kg/d group (65.1%). In a long-term study, Tanabe et al[9] reported that a combination of MZR with CsA achieved 10-year patient and graft survival rates equivalent to that of a group receiving AZA and CsA. In the present study of 4-year follow-up, we showed that a combination of high-dose MZR with CsA yielded results similar to the MMF/CsA treatment group regarding patient and graft survivals and renal function. Regarding adverse events, many Japanese studies have reported MZR to cause little myelosuppression and hepatic dysfunction in compared with AZA and little gastrointestinal disturbance and viral infection compared with MMF.[11] Our results in this study were similar to those reported earlier. The most harmful side effect of MZR was hyperuricemia, as noted in the present study, however, it was easily controlled by allopurinol administration in most cases.

In our previous 2-year study, we reported the number of patients who developed CMV disease, gancyclovir treatment, and the average peak level of CMV antigenemia to be significantly lower among the MZR than the MMF group.

An inhibitory effect of MZR on CMV infection in vitro was reported by Shiraki et al.[12] The effect of combination

Table 2. Adverse Event Profile Between 3rd and 4th Years

	MZR Group (n = 37)	MMF Group (n = 36)	*P* Value
Leucocytopenia	0 (0%)	1 (2.8%)	NS
Grade 1	0 (0%)	1 (2.8%)	NS
Anemia	3 (8.1%)	1 (2.8%)	NS
Grade 1	3 (8.1%)	1 (2.8%)	NS
Hepatic function abnormal	1 (2.7%)	1 (2.8%)	NS
Grade 1	1 (2.7%)	1 (2.8%)	NS
Hyperuricemia	11 (29.7%)	2 (5.6%)	<0.05
Mean serum urate level (mg/dL)			
3 y after transplantation	6.9 ± 1.5	7.1 ± 1.9	NS
4 y after transplantation	7.2 ± 1.8	7.0 ± 1.6	NS

Abbreviations: MZR, mizoribine; MMF, mycophenolate mofetil.

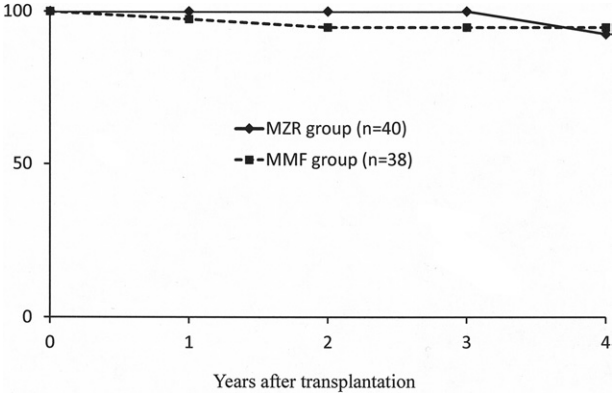

Fig 2. Graft survival rates in the mizoribine (MZR) and the mycophenolate mofetil (MMF) groups.

therapy (CsA, prednisolone, MZR) on CMV in vitro was evaluated with fixed concentrations of CsA and prednisolone. MMF has also been associated with gastrointestinal (GI) symptoms, particularly difficult to manage diarrhea. In our previous results, it was clear that there were few GI adverse events among the MZR versus MMF group.

In conclusion, the combination of high-dose MZR with CsA, basiliximab, and corticosteroids showed excellent results over 4 years.

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