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The Efficacy and Safety of High-Dose Mizoribine in ABO-Incompatible Kidney Transplantation Using Anti-CD20 and Anti-CD25 Antibody Without Splenectomy Treatment

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ABSTRACT

Background. Mizoribine (MZR) has been developed as an immunosuppressive agent in Japan, but it shows less potent immunosuppressive effects at doses up to 3 mg/kg/d. In this study, we investigated whether high-dose MZR (6 mg/kg/d) was effective for ABO-incompatible (ABO-i) living donor kidney transplantation (LKT) using treatment with anti-CD25 and anti-CD20 monoclonal antibodies without splenectomy.

Methods. Since 2007, we encountered 24 cases of ABO-i LKT using anti-CD20 and anti-CD25 monoclonal antibody without splenectomy. The pretransplant immunosuppressive regimen consisted of two doses of anti-CD20 antibody, mycophenolate mofetil (MMF), prednisolone, a calcineurin inhibitor (cyclosporine [7 mg/kg] or tacrolimus [0.2 mg/kg] and two doses of anti-CD25 antibody. Antibody removal by plasmapheresis was performed before LKT up to several times according to the antibody titer. The posttransplant regimen consisted of high-dose mizoribine (6 mg/kg/d) instead of MMF (MZR group, $n = 12$).

Results. The 1-year graft survival rates for the MZR and MMF groups were both 100%. The rejection rate in the MZR group (eight %) was not significantly higher than that in the MMF group (seventeen %) Serum creatinine level was not significantly different between the two groups. In the MZR group 6 (50%) patients developed CMV antigenemia-positivity versus 11 (92%) in the MMF group ($P < .05$). The number of patients who developed CMV disease was 0 in the MZR group and 1 (8%) in the MMF group. The number of patients treated with ganciclovir was 0% and 8%, respectively (not significant).

Conclusions. We obtain good clinical results with high-dose MZR in ABO-i LKT using anti-CD20 and anti-CD25 antibody treatment without splenectomy.

DUE TO THE SHORTAGE of deceased donors, ABO-incompatible (ABO-i) living donor kidney transplantation (LKT) has been performed in Japan since 1989. Currently ABO-i LKT affords a high success level. Mycophenolate mofetil (MMF) is generally used as an antimetabolite in posttransplant regimens for ABO-i LKT. In such cases, we have experienced high rate of cytomegalovirus (CMV) infection and diarrhea. Mizoribine (MZR), a novel nucleoside analog developed as an immunosuppressive agent,[1] was placed on the market in Japan in 1984 where it has now been widely used for 23 years. MZR exhibits a low incidence of severe adverse reactions and does not enhance oncogenicity.[2] In the 1990s the standard dosage was 1 to 3 mg/kg/d as a substitute for azathioprine (AZP) combined with steroids and cyclosporine (CsA).[3] However, some reports have indicated that even 3 mg/kg/d MZR achieves less potent immunosuppressive effects albeit with fewer

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adverse events.[4,5] In a previous study, therefore, we reported that high-dose MZR (6 mg/kg/d) was effective and safe for ABO-compatible LKT in conjunction with CsA, corticosteroid, and anti-CD-25 antibody. Therefore, in the present study, we employed high-dose MZR instead of MMF in ABO-i LKT patients using anti-CD-20 and anti-CD25 without splenectomy.

PATIENTS AND METHODS

Study Subjects and Immunosuppression

Between October 2007 and April 2010, we performed 24 ABO-i LKT. The background characteristics of 12 patients in the MZR and 12 patients in the MMF group showed no significant differences (Table 1). The immunosuppressive regimen (Fig 1) included plasmapheresis [double filtration plasmapheresis or plasma exchange (PE)] to remove anti-blood group antibody, before ABO-i LKT based on the titer. The ideal goal was to decrease the anti-blood group antibody titer to less than 1:32.[6] The pretransplant regimen was initiated 2 weeks prior to surgery with MMF (1000 mg/d) and prednisolone (PSL; 10 mg/d orally twice daily). The initial dose of CsA (7 mg/kg/d orally twice a day) or tacrolimus (0.2 mg/kg/d, orally twice a day) was administered orally for 1 week before LKT. Rituximab (anti-CD20monoclonal antibody; 200 mg) was administered at 7 and 14 days before LKT. Basiliximab (anti-CD25 monoclonal antibody; 20 mg) was administered on the operation day and 4 days after LKT.[7] Renal grafts were located in the right hemipelvis without splenectomy under general anesthesia. The posttransplant regimen was the same as that used in ABD-compatible cases, consisting of CsA or tacrolimus + high-dose MZR + PSL including anticoagulation therapy.[7] Protocol biopsies were performed at 1 month and 1 year after LKT.

Endpoints

The primary efficacy endpoints of this study were 1-year patient survival, 1-year graft survival, and acute rejection-free rates at 3 months after transplantation. Major side effects and complications within 6 months were also used as endpoints. The diagnosis of rejection was established by biopsy. An acute rejection episode was defined as histopathologic findings over grade I according to the Banff classification.

All patients gave informed consent and the study was approved by our Ethics Committee institutional review board to comply with the Declaration of Helsinki.

Table 1. Demographic Characteristics

	Mz Group	MMF Group
Number of recipients	12	12
Examination time	2009–2010	2007–2009
Recipient age (y)	50 ± 10 (35–61)	50 ± 13 (20–60)
Gender (male/female)	7:5	5:7
Incompatible blood type		
A:B:AB	4:7:1	8:4:0
Antibdoy titer before treatment		
IgG	262 ± 614 (2–2048)	276 ± 590 (2–2048)
IgM	60 ± 77 (2–256)	43 ± 45 (8–128)
Antibody titer after treatment		
IgG	20 ± 38 (2–128)	29 ± 42 (2–128)
IgM	7 ± 8 (2–32)	3 ± 2 (1–8)
DSA positive case	1 (8.3%)	1 (8.3%)
CNI (Tac/CsA)	7:5	6:6
Donor type		
parents: siblings: consorts	3:2:7	4:0:8

MZ, mizoribine; MMF, mycophenolate mofetil; CNI, calcineurin inhibitor; Tac, tacrolimus; CsA, cyclosporine; Ig, immunoglobulin; DSA.

Statistical Analysis

The Kaplan-Meier method was used to determine patient and graft survivals with statistical significance tested using the log-rank test.

RESULTS

Demographic Characteristics

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

Rejection and Graft Function

The 1-year patient survival rates for the MZR and MMF groups were 100% and 100%, respectively (Table 2). There was no significant difference in graft or patient survival.

Acute Rejection-Free Rate Within 3 Months After Transplantation

Table 2 shows the ejection rate at 3 months after transplantation for each group: it was not significantly higher in the MZR group (25%) than in the MMF group (16%).

Serum Creatinine Level

The serum creatinine levels among the MZR versus MMF groups were 1.24 ± 0.4 mg/dL versus 1.20 ± 0.5 mg/dL at 1; 1.35 ± 0.4 mg/dL versus 1.28 ± 0.4 mg/dL at 3 and 1.32 ± 0.55 mg/dL versus 1.44 ± 0.5 mg/dL versus and 1.31 ± 0.4 mg/dL at 12 months after transplantation, respectively (not significant; Table 2).

CMV Infection

The incidence of CMV infection was significantly higher in the MMF group. (Table 3) The number of CMV antigenemia-positive patients was 6 (50%) in the MZR versus and 11 (92%) in the MMF group ($P < .05$). The number who developed CMV disease was 0 in the MZR as versus 1 (8%) in the MMF group (not significant). Moreover, the number of patients treated with gancyclovir was in the MZR versus 1 (8%) in the MMF group (not significant). The average peak level of CMV antigenemia was 1 ± 0 in the MZR group and 11 ± 25 in the MMF group ($P = .007$).

Adverse Effects

The incidences of adverse effects and infections during the first year after renal transplantation were compared between the two groups (Table 3). The MZR group demon-

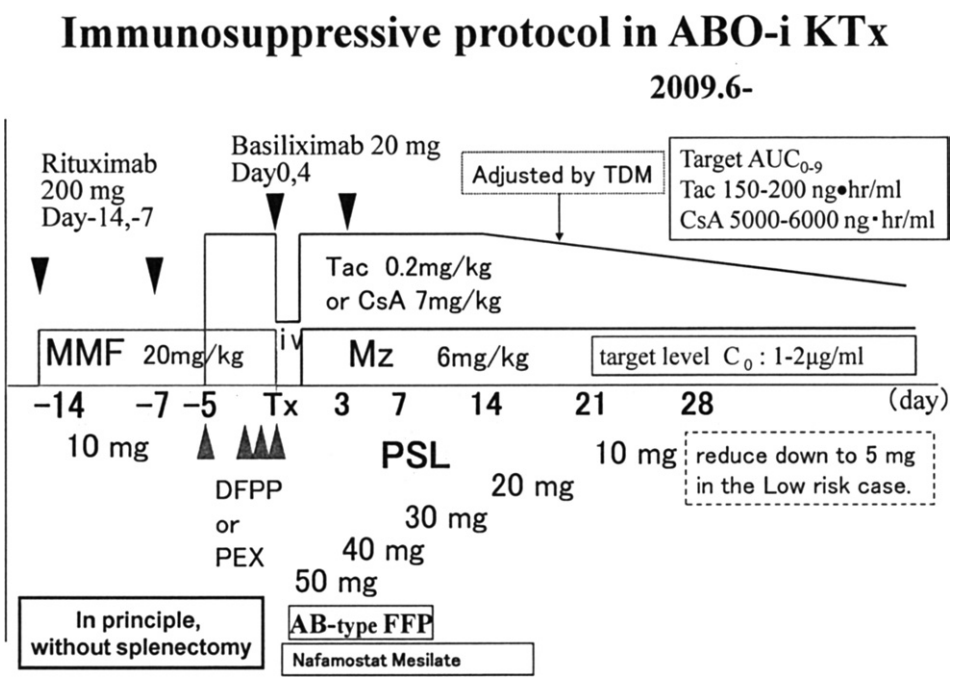

Fig 1. Immunosuppressive regimen in ABO-incompatible (ABO-i) kidney transplantation. The pretransplant regimen was initiated, 2 weeks prior to surgery, with mycophendlate mofetil (MMF; 1,000 mg/d) and prednisolone (PSL; 10 mg/d orally twice a day). The initial dose of cyclosporine (7 mg/kg/d orally twice a day) or tacrolimus (Tac; 0.2 mg/kg/d orally twice a day) is administered orally for 1 week before living donor kidney transplantation (LKT). Rituximab (anti-CD20 monoclonal antibody) was administered 7 and 14 days before LKT at a dose of 200 mg. Basiliximab (anti-CD25 monoclonal antibody) was administered on the operation day and 4 days after LKT at a dose of 20 mg. TDM, AuC, area under the curve; CsA, cyclosporine; Mz, mizoribone; FFP.

strated a significantly higher rate of elevated serum uric acid values. The number of patients treated with allopurinol was 4/12 (33.0%) in the MZR and 3/12 (25%) in the MMF group (not significant). There was no significant difference between the two groups in terms of bone marrow suppression or liver dysfunction. There was one occurrences of neutropenia in the MZR and there were three in the MMF group.

Table 2. Rejection and graft function

	Mz Group (n = 12)		MMF Group (n = 12)
Acute rejection (clinical rejection)	1 (8%)	NS	2 (17%)
Banff grade	2A (D2)		1A (D82), 2A (D8)
Treatment	MP + PE		MP, MP + PE
Protocol biopsy (1 mo)	12 (100%)	NS	9 (75%)
Banff grade	NR 12 (100%)		NR 8 (88.9%) 2A 1 (11.1%)
C4d0-positive	11/12 (91.6%)		5/6 (83.3%)
Serum creatinine (mg/dL)			
1 mo	1.28 ± 0.4	NS	1.20 ± 0.5
3 mo	1.35 ± 0.4	NS	1.28 ± 0.4
1 y	1.44 ± 0.5	NS	1.31 ± 0.4

MZ, mizoribine; MMF, mycophenolate mofetil; NS, not significant; MP, PE, plasma exchange.

DISCUSSION

A recent Japanese study demonstrated that the rates of overall patient and graft survival for ABO-i LKT are similar to those for ABO-compatible LKT. Takahashi and Saito[8] reported that the overall patient survival rates at 1, 3, 5, and 10 years following transplantation were 94%, 91%,

Table 3. Adverse Event Profile

	Mz Group (n = 12)		MMF Group (n = 12)
CMV infection	0 (0%)	NS	1 (8%)
CMV-Ag	6 (50%)	$P < .05$	11 (92%)
CMV-Ag (>5/500,000)	0 (0%)	$P < .05$	6 (50%)
GCV treatment	0 (0%)	NS	1 (8%)
Ag-peak (/50,000)	1 ± 0	NS	11 ± 25
Mean occurrence time after transplantation (d)	25 ± 15	NS	27 ± 13
Other virus infections	Herpes zoster 1		Herpes zoster 1 HSV 1
Other adverse events	Neutropenia 1	NS	Neutropenia 3 Hemorrhagic ulcer 1
Hyperuricemia (treatment)	4 (33%)	NS	3 (25%)

Mz, mizoribine; MMF, mycophenolate mofetil; NS, not significant; CMV, cytomegalovirus; Ag: GCV, HSV.

88%,and 81%, with uncensored graft survival rates of 86%, 82%, 74%, and 53%, respectively. In this study, ABO-i LKT were performed using anti-CD20 and anti-CD25 antibody without splenectomy. Although rituximab (anti-CD 20 antibody) infusion does not affect antibody-producing plasma cells, it lyses CD20-positive cells and complement-dependent cytulytic processes.[9] Mitsuhata et al have reported that PE decreased the therapeutic effect of rituximab when the antibody was administered to treat humoral rejection.[10] When the cell cycle of plasma cells is considered, rituximab needs to be administered early before LKT. Therefore, in our regimen, rituximab was administered twice, at 7 and 14 days before LKT.

MZR, a nucleoside of the imidazole class, was isolated from culture media 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 inhibiting the proliferation of lymphocytes. It has been developed as an immunosuppressive agent in Japan. From 1978 to 1982 the clinical efficacy of MZR in renal transplantation was documented in various Japanese institutions and approved by the Ministry of Health and Welfare, Japan, in 1984. Recently, it has been used most commonly in combination with other immunosuppressants, such as CsA or tacrolimus, and corticosteroids in transplantation. In 1989, a randomized trial comparing CsA + AZP versus CsA + MZR revealed MZR to show equal immunosuppressive effects and fewer side effects such as myelosuppression and liver dysfunction then AZP.[11] The original 1 to 3 mg/kg/d dosage of MZR was based on 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.[4] Mixed lymphocyte reactions revealed that 1 μg/mL suppressed lymphocyte proliferation by 50% (IC_{50}).[12]

In 2004, a multicenter trial in Japan[13] reported that high-dose MZR (5 mg/kg/d) concomitant 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%) or the 3 to 5 mg/kg/d group (65.1%). In the past study, we showed that a combination of high-dose MZR (6 mg/kg/d) with CsA yielded similar results to a MMF plus CsA treatment group in terms of patient and graft survivals as well as renal function. Regarding adverse events, many Japanese studies have reported MZR to cause little myelosuppression or hepatic disorder in comparison with AZP. Furthermore MZR produces fewer gastrointestinal disturbance and viral infections than MMF.[13] Our study showed similar results. The most major side effect of MZR is hyperuricemia, which is easily controlled by allopurinol administration in most cases.

Although several previous studies have failed to not an increased incidence of CMV disease among kidney transplant recipients treated with MMF, our everyday clinical experience suggests that the number of patients with CMV disease has increased after MMF introduction. Sarmiento et al.[14] also reported MMF to be associated with more clinically severe forms of CMV disease than AZA. Patients who received MMF were more likely to show CMV disease with organ involvement: 7/12 (58%) in the MMF versus 3/17 (18%) in the AZA group ($P = .03$). Furthermore, the median number of organs involved was greater in the MMF than the AZA group: 2.0 versus 1.0 ($P = .015$).

Our study weaked the positive rate of CMV antigenemia to be significantly lower in the MZR than the MMF group. No patient developed CMV disease or was treated with gancyclovir among the MZR group.

Since MZR is eliminated via the urinary system, it is difficult to administered it during end-stage renal disease. Therefore, we used MMF for desensitization before transplantation changing to MZR on the day after transplantation.

Our findings showed that the present combination and dosage of immunosuppressants established not only satisfactory immunosuppression but also suppression of CMV infection in vivo. We obtain good clinical results with high-dose MZR in ABO-i LKT using anti-CD20 and anti-CD25 antibody without splenectomy.

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