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ORIGINAL ARTICLE

Potential value of high-dose mizoribine as rescue therapy for ongoing acute humoral rejection

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Summary

Mizoribine (MZ) inhibits the proliferation of lymphocytes selectively via inhibition of inosine monophosphate dehydrogenase, like mycophenolate mofetil (MMF). The clinical dosage of MZ (2–5 mg/kg) is much lower than that of MMF (20–60 mg/kg). The purpose of this study was to examine whether high-dose MZ would be effective for treatment of acute humoral rejection. Renal transplantation was performed in a different pig strain combination. Group 1 ($n = 2$) received no treatment. Group 2 ($n = 4$) received cyclosporine microemulsion (6 mg/kg) and prednisolone (0.1 mg/kg) as baseline immunosuppression. Groups 3 ($n = 4$), 4 ($n = 4$) and 5 ($n = 4$) were additionally treated with MZ for rescue therapy, 30, 10 and 3 mg/kg, respectively, immediately after rejection was observed. All pigs developed acute vascular rejection between days 4 and 8. Complete reversal of acute rejection including reduction of elevated serum creatinine, suppression of anti-donor antibody production and pathological finding, was obtained in 3/4 (group 3), 1/4 (group 4) and 0/4 (group 5). Rescue with high-dose MZ (30 mg/kg) reversed ongoing acute humoral rejection. Such a high dose of MZ was tolerable for pigs. However, leukocytopenia was observed when MZ trough level was maintained over 10 μg/ml. Treatment with high-dose MZ would be applicable to a clinical trial, if blood level is carefully monitored.

Introduction

The incidence of acute rejection after transplantation has been reduced by the progress of immunosuppressive therapy, resulting in the prolongation of patient survival [1]. Even if acute cellular rejection would occur, most can be reversed. However, humoral rejection is still problematic because of the high possibility of graft failure [2,3].

Recently, the shortage of organs from deceased donors is increasing. To solve this problem, efforts have been made to extend the pool of available donors. Transplantation from living donors has already been realized as an alternative and viable option. Although the number of living transplant recipients is now increasing, some may inevitably develop the immunological complications such as ABO incompatibility or positive crossmatch because of the limited selection of living donors. The pre-existing or elicited anti-donor antibody is certainly involved in hyperacute or acute humoral rejection, which leads to poor transplant survival [2,3]. The establishment of prevention and treatment for acute humoral rejection is important to improve graft survival.

Mycophenolate mofetil (MMF), an anti-metabolite drug, has been shown to be effective not only as a prophylactic treatment, but also a rescue therapy for acute

rejection [4–7]. MMF at the dosage of 2–3 g (20–60 mg/kg), is now broadly used world-wide after transplantation. Mizoribine (MZ) selectively inhibits the proliferation of lymphocytes selectively via inhibition of inosine monophosphate dehydrogenase (IMPDH), similar to MMF. MZ at the dose of 2–3 mg/kg has been registered for prevention of rejection after renal transplantation in Japan. It has been recently reported that a relatively high dose (6 mg/kg) of MZ was effective for prevention of antibody-mediated rejection in ABO-incompatible renal transplantation [8]. The purpose of this study was to examine whether a much higher dose of MZ would also be effective in reversing ongoing acute humoral rejection like MMF, in an experimental transplantation model using pigs.

Materials and methods

Animals

Pitman-Moore/Taiwan-Small Ear (NIBS miniature) pigs, weighing 12–26 kg, were used as donors. Landrace/Yorkshire pigs, weighing 14–22 kg, were used as recipients. All animals used in this study received humane care in compliance with the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health. Experimental protocols were approved by the Committee on Research Animal Care, Institute for Laboratory Animal Research, Nagoya University School of Medicine.

Immunosuppressive protocol and group classification

Cyclosporine microemulsion (NEO), prednisolone (PSL) and MZ were administered through a gastrostomy tube, which was brought out onto the shoulder under a subcutaneous skin tunnel. The intravenous catheter and gastrostomy tube were put into a pocket of a jacket to prevent accidental removal. All recipient pigs were divided into the following five groups. Group 1 ($n = 2$) had no treatment. Group 2 ($n = 4$) received NEO (6 mg/kg/day) and PSL (0.1 mg/kg/day) as baseline immunosuppression. Groups 3 ($n = 4$), 4 ($n = 4$) and 5 ($n = 4$) were additionally treated with MZ as rescue therapy, at a dose of 30, 10 and 3 mg/kg/day, respectively, immediately after rejection was observed.

Pig kidney allotransplantation

Heterotopic renal transplantation was performed in a different pig strain combination. Pitman-Moore/Taiwan-Small Ear (NIBS miniature) pigs and Landrace/Yorkshire pigs were used as donors and recipients, respectively.

Recipient pigs were anesthetized using ketamine and maintained on inhaled halothane and oxygen. A left or right kidney which was typically excised from a donor pig was transplanted into a recipient pig. Through a midline laparotomy, the infrarenal aorta and inferior vena cava (IVC) were exposed. The donor renal artery and vein were anastomosed end-to-side to the recipient aorta and IVC. The ureter was implanted into the bladder. The ureteral stent catheter was inserted and brought out onto the lower limb through a bladder and a subcutaneous tunnel that allowed monitoring of urine output. Both native kidneys were removed. The recipient's jugular vein was cannulated. The catheter was brought out onto the back under a jacket to allow easy access to blood withdrawal and intravenous infusion of drugs.

Biopsies of transplanted kidneys were conducted prereperfusion and 1 h postreperfusion, and when serum creatinine level was elevated to greater than 4 mg/dl. Immediately after a biopsy was performed, MZ rescue therapy was initiated. Graft biopsies were also conducted at the end of the experiment. Biopsies of pig kidneys were formalin-fixed and stained with hematoxylin–eosin (HE). Follow up period was determined to be 21 days after transplantation.

Blood test

Blood cell counts were measured once a week. Serum biochemical and electrolyte values were measured every day. Serum concentration of MZ (trough level) was analyzed by high-performance liquid chromatography (HPLC) [9] after all experiments were completed.

Flow cytometry crossmatch

The production of anti-donor antibodies (IgG, IgM) was analyzed by flow cytometry crossmatch using donor lymphocytes. Donor pig lymphocytes were isolated from the excised spleen using Ficoll Hist-paque (Sigma-Aldrich, St Louis, MO, USA). T cells were separated from B cells using a nylon wool column. The effluent cells, mainly T cells were used for direct crossmatch assay. Recipient pig serum was incubated with 2×10^5 lymphocytes in phosphate-buffered saline (PBS) at 4 °C for 30 min. Afterward, cells were washed twice with PBS(−) containing 2% fetal bovine serum and reacted with fluorescein isothiocyanate (FITC)-labeled F(ab)′2 fragment goat anti-swine IgG (Jackson Immuno Research, West Grove, PA, USA) and goat anti-swine IgM (Bethyl Laboratories, Montgomery, TX, USA). The stained cells were subjected to flow cytometric analysis using EPIX XL (Beckman Coulter, Inc., Miami, FL, USA).

The mean fluorescence intensity (MFI) was used to quantify the change of anti-donor antibody level. The anti-donor IgG and IgM antibody levels were expressed relative to the MFI value at pretransplantation.

Results

The survival days were 6, 6 in Group 1 (control: no immunosuppression), and 5, 8, 8 and 8 in Group 2 which was treated with basic immunosuppression alone including 6 mg/kg of NEO and 0.1 mg/kg of PSL (Table 1).

Table 1. Immunosuppressive protocol and graft survival.

Group	Immunosuppression	Survival (days)
1 ($n = 2$)	None (control)	6, 6
2 ($n = 4$)	CsA (6) + PSL (0.1)	5, 8, 8, 8
Acute rejection		
3 ($n = 4$)	⟶ MZ (30) rescue	20, >21 × 3
4 ($n = 4$)	⟶ MZ (10) rescue	8, 10, 11, >21
5 ($n = 4$)	⟶ MZ (3) rescue	9, >21 × 3

CsA, cyclosporine; PSL, prednisolone; MZ, mizoribine.
Dosages (mg/kg) are given in parentheses.

Rescue with 30 mg/kg of MZ (group 3) was effective in reducing serum creatinine level and extended graft survival to over 21 days, except in one case for 20 days. Recipient pigs survived for 8, 10, 11 and >21 days in group 4 (rescue with 10 mg/kg of MZ), and 9 and >21 days for three pigs in group 5 (rescue with 3 mg/kg of MZ). All pigs developed acute rejection between days 4 and 8.

Figure 1a shows the change of serum creatinine level in group 2 (the time point of graft biopsy is indicated by an arrow). All recipient pigs in group 2 died of renal failure caused by acute rejection between days 5 and 8. HE staining of the biopsy specimens in group 2 is shown in Fig. 2a. Biopsy specimens taken before and 1 h after transplantation showed almost normal findings (data not shown). The resected graft on day 8 revealed severe acute rejection with hemorrhagic necrosis.

Figure 1b shows the change of serum creatinine level in group 3 with 30 mg/kg of MZ rescue therapy. Immediately after biopsy, MZ treatment was initiated. All biopsy specimens showed Banff grade II–III acute rejection. MZ treatment was effective in all cases and reduced serum creatinine level. Histopathology of the graft biopsy on day 7 shows typical acute humoral rejection, which is characterized by vascular endothelial hyperplasia, intimal

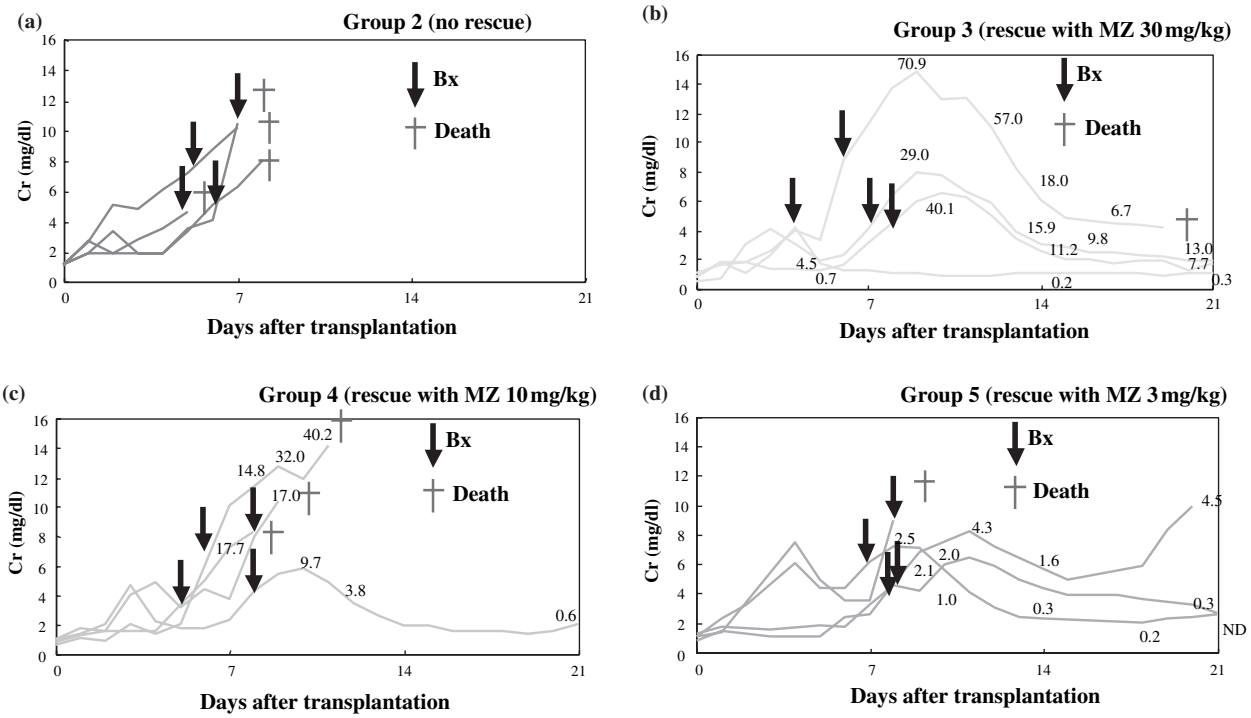

Figure 1 Changes in serum creatinine level and MZ trough level. (a) Group 2, (b) group 3, (c) group 4, (d) group 5. (a), (b), (c) and (d) show the change of serum creatinine level in groups 2, 3, 4 and 5. The time point of graft biopsy is indicated by an arrow. All recipient pigs developed acute rejection between days 4 and 8. Immediately after the graft biopsy was conducted, MZ rescue therapy was initiated. Serum MZ trough levels (μg/ml) were also described in (b)–(d).

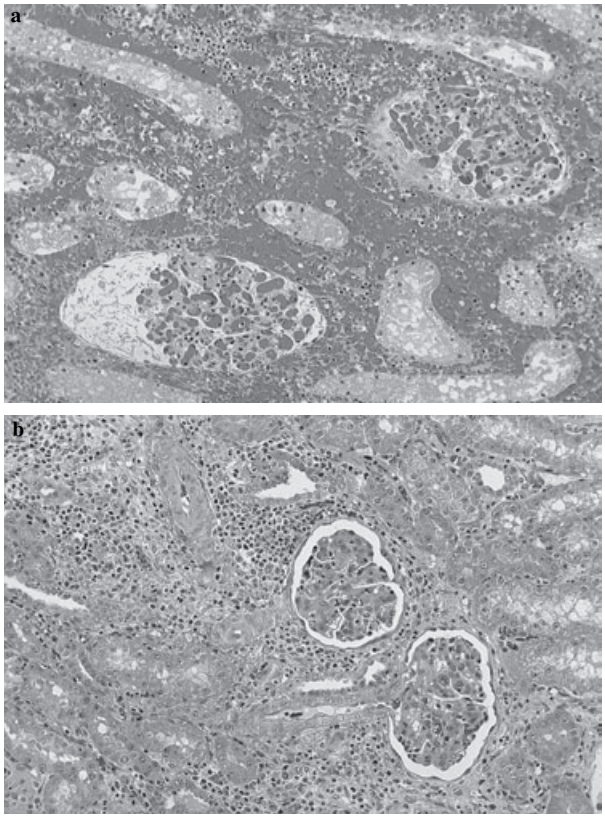

Figure 2 HE staining of excised grafts in groups 2 and 5. Graft specimen in group 2 with graft survival for 8 days (a). Resected graft on day 8 which shows severe acute humoral rejection with hemorrhagic necrosis (Banff grade III, t2 i3 g2 v3). Graft specimen in group 5 with graft survival for over 21 days (b). Rescue with 3 mg/kg of MZ was temporarily effective. However, the graft on day 21 in group 5 shows a marked mononuclear cell infiltration-like lymph follicle surrounding it (Banff grade IIA, t2 i3 g1 v1).

thickening, accumulation of mononuclear cells, fibrin covered parts of the luminal surface, mononuclear cell infiltration, hemorrhage, tubulitis and glomerular endocapillary hyperplasia (Fig. 3). The graft biopsy, which was conducted 14 days after rescue therapy with 30 mg/kg of MZ, revealed that vascular rejection was almost completely recovered. There remained mild interstitial mononulcear cell infiltration, edema and tubulitis. Mesangial cell hyperplasia with mild matrix expansion was considered to be caused by the reaction to recovery from acute rejection.

Mizoribine treatment at the dose of 10 mg/kg reversed acute humoral rejection in one of four in group 4 (Fig. 1c). The graft after MZ treatment showed recovery from acute vascular rejection, but included a trace of cellular rejection (data not shown). The remaining three pigs were dead 2, 3 and 5 days after initiation of the MZ rescue therapy. The rescue with 3 mg/kg of MZ reduced the serum creatinine level in three of four pigs in group 5 (Fig. 1d). However, one of the three pigs showed a re-elevation of serum creatinine because of refractory acute rejection. The remaining two pigs did not obtain a reduction of the elevated serum creatinine to the normal range (below 2 mg/dl). Histopathology of the graft biopsy on day 21 also revealed marked mononuclear cell infiltration like lymph follicle surrounding the arteriole (Fig. 2d).

The trough levels of serum MZ concentration in groups 3, 4 and 5 are also expressed in Fig. 1b–d, respectively. As MZ is mainly excreted from the kidney, its concentration is dependent on renal function. In three pigs with high creatinine levels caused by rejection episodes in group 3, MZ trough levels reached 30–70 μg/ml and were maintained around 10 μg/ml. Two of these three pigs showed leukocytopenia (below 3000/mm^3) and one pig died of infection on day 20. Another adverse effects such as gastrointestinal disturbance or hepatic dysfunction were not observed in any pigs.

Anti-donor antibody level was analyzed by flow cytometry crossmatch using donor lymphocytes. In one pig of group 1, one native kidney was left to examine the change of antibody level after graft removal. Immediately after the removal of the rejected graft on day 6, anti-donor antibody levels were suddenly increased (Fig. 4). This finding was attributed to the removal of the graft which had absorbed many anti-donor antibodies in spite of the active production of elicited antibodies. Most graft biopsies at the time of rejection revealed that vascular rejection was predominant. Anti-donor antibodies in sera were elevated at the time of rejection.

Figure 5 shows the changes in anti-donor antibodies (IgG and IgM) only in 21-day survivors of group 3 ($n = 3$), group 4 ($n = 1$) and group 5 ($n = 3$). MZ treatment with 3 mg/kg was able to extend graft survival. However, such a low dose of MZ could not suppress anti-donor antibody production, which was consistent with the findings of graft pathology showing marked cellular infiltration even after the MZ treatment. The production of anti-donor antibodies was completely suppressed in pigs with a high dose (30 mg/kg) of MZ treatment (group 3).

Consequently, the complete reversal of acute rejection, including the reduction of serum creatinine to a normal level, suppression of anti-donor antibody production, and recovery from rejection in graft specimen, was obtained in three of four cases in group 3, in one of four cases in group 4 and in zero of four cases in group 5.

Discussion

In the pig kidney allotransplantation model, a high dose of MZ at the dosage level of 30 mg/kg was found to reverse ongoing acute humoral rejection. Although rescue

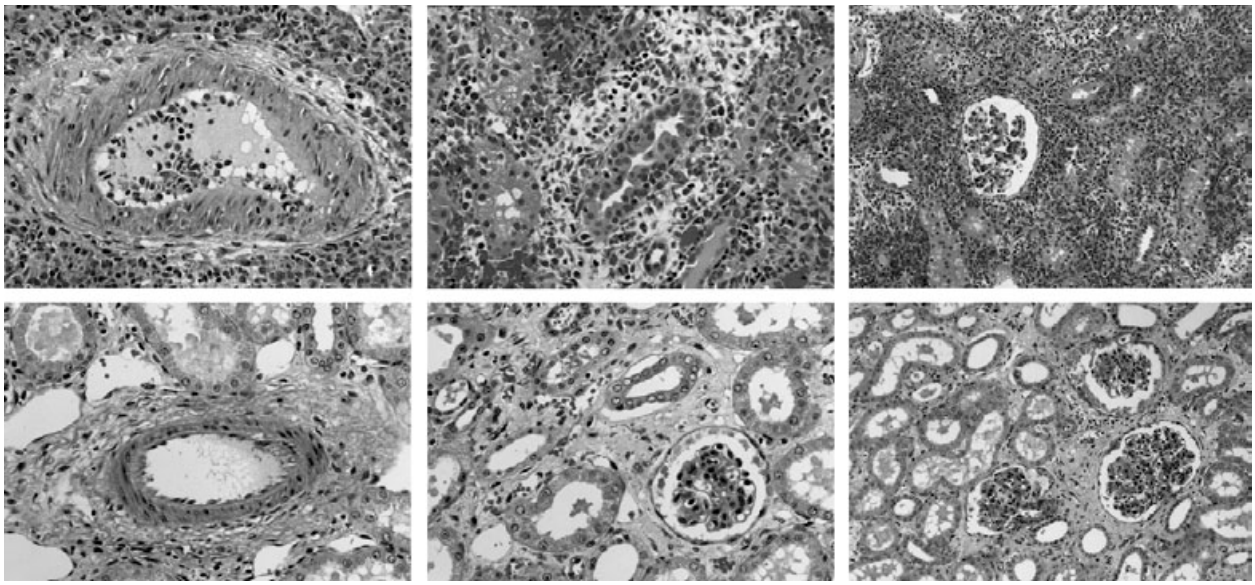

Figure 3 HE staining of graft specimens in group 3. Top panels are biopsy specimens when serum creatinine level was elevated (on day 7), which show typical humoral rejection characterized by vascular endothelial hyperplasia, intimal thickening, fibrin cover parts of luminal surface, mononuclear cell infiltration, hemorrhage and tubulitis (Banff grade III, t1 i3 g1 v3). Bottom panels show the recovery from vascular rejection 14 days after treatment with 30 mg/kg of MZ.

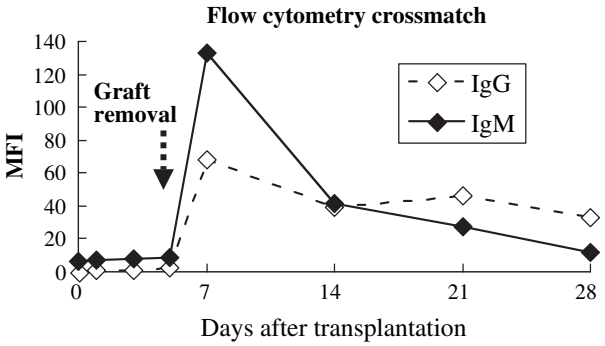

Figure 4 Changes in anti-donor antibodies (IgG and IgM) in group 1. Anti-donor antibodies were measured by flow cytometry using donor lymphocytes. One native kidney was not removed at transplantation, to enable the examination of the changes of anti-donor antibodies after graft removal. The graft was resected on day 6, which showed marked swelling and massive hemorrhage caused by severe acute rejection. Immediately after the graft removal, anti-donor antibodies were suddenly elevated. MFI, mean fluorescence intensity.

therapy with 3 mg/kg of MZ, which is the same as the clinically approved dosage, also showed effectiveness against acute rejection to some extent, anti-donor antibodies could not be suppressed completely and marked mononuclear cell infiltration was observed in the graft biopsy at the end of the experiment (21 days after transplantation). When MZ rescue therapy was initiated, as all recipient pigs had renal dysfunction due to severe allograft rejection, the MZ trough level in serum might have been maintained at a high level. This may be the reason as even 3 mg/kg of MZ was effective in reducing serum creatinine levels.

At a relatively high dose of MZ (30 mg/kg), recipient pigs which reached a very high trough level >30 μg/ml and maintained over 10 μg/ml, showed adverse effects such as leukocytopenia. Attention should be paid to the toxicity caused by high concentrations of MZ when MZ is to be used for rescue therapy in renal transplantation.

Mizoribine was reported to suppress humoral and cellular immunity by the inhibition of both T-cell and B-cell proliferation. Most experimental work on MZ examined its inhibitory effect on T-cell activation and T-cell-mediated immune response [10,11]. The efficacy of prophylactic treatment with MZ, single or combined with calcineurin inhibitors, on graft survival has been shown [12–14].

In a large animal model using dogs, the main obstacle was heavy toxicity [12,15]. Over 5 mg/kg of MZ in renal transplantation made all dogs develop gastroenteritis such as vomiting, anorexia and bloody diarrhea. Dogs seem to be much more sensitive to gastrointestinal disturbance than other animals, and this sensitivity may be species-specific because of low levels of IMPDH in the canine gut [16]. It was reported that mean plasma concentrations <1.0 μg/ml would be prone to rejection, while plasma concentrations >1.5 μg/ml would probably

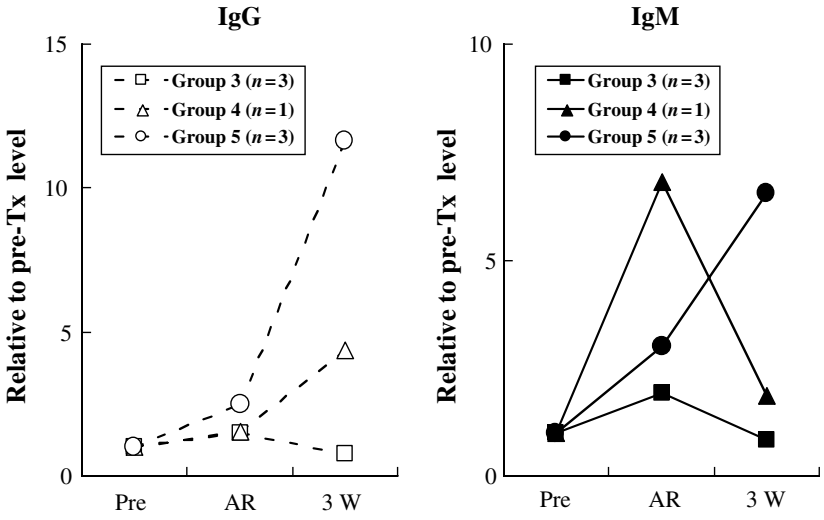

Figure 5 Changes in anti-donor antibodies (IgG and IgM) in 21-day survivors. The value was expressed relative to pretransplantation level. Thirty milligrams per kilograms of MZ treatment suppressed anti-donor IgG and IgM antibodies, while 3 mg/kg of MZ did not inhibit the development of anti-donor antibodies. Pre, pretransplantation; AR, acute rejection; 3W, 21 days after transplantation.

cause symptoms from toxicity [17]. The optimal target range of MZ blood level is very narrow in dogs.

In contrast, pigs were able to tolerate a much higher dosage of MZ. However, pigs appeared to show lower incidence of kidney allograft rejection than dogs even between different pure-bred animals [18]. It was also reported that different major histocompatibility complex, namely, different swine leukocyte antigen (SLA), would cause kidney allograft rejection [19]. In the present study, transplantation between Pitman-Moore/Taiwan-Small Ear (NIBS miniature) pigs and Landrace/Yorkshire crossbred pigs certainly caused acute rejection. Moreover, elicitation of anti-donor antibodies and the histopathological findings with predominant vascular change were observed. Although a detailed genetic disparity has not been determined yet, this combination of strains might be considered sufficiently different to develop acute allograft rejection, particularly humoral rejection. The drawback of this article is that SLA has not been identified and that target epitopes (binding site) of anti-donor antibodies have not been elucidated. Nomenclature of SLA based on DNA typing will be established soon. Genetic analysis of donor–recipient combination is an important subject of future investigation.

Clinical studies showed that no gastrointestinal disturbance was observed in MZ-treated recipients at the dose of 2–5 mg/kg [20–23]. The therapeutic range for serum concentration has been reported to be 0.1–3 μg/ml [24]. Liver dysfunction, hyperuricaemia and bone marrow suppression as adverse reactions were observed in patients with trough level >5 μg/ml. The pig model would be considered to mimic clinical transplantation more closely than a dog model which shows an adverse effect even at a low dose of MZ. MMF has been widely used for the prevention and treatment of acute rejection in organ transplantation [4–7]. The dosage of MMF is 20–60 mg/kg, which is much higher than the clinically approved dosage of MZ. As MMF has successfully reversed ongoing acute rejection [25], a high dose of MZ would be also expected to show similar effectiveness.

Mizoribine and MMF have almost the same function of inhibiting IMPDH. The differences are as follows [26–30]. MMF is changed into an active form, mycophenolic acid, by esterase and then metabolized in liver. An inactive mycophenolic acid glucuronide is mainly eliminated from the kidney. Its binding rate of plasma protein is very high (97.5%) and half-life is long (17.9 h). MZ is metabolized into an active form, MZ 5′monophosphate by adenosine kinase and is excreted mainly by the kidney, making the serum concentration greatly dependent on renal function. However, the half-life is very short (1.62 h) and MZ is dialyzable because the binding rate of plasma protein is low (2.3%).

Immunosuppressive regimen is chosen according to the characteristics of these drugs. The optimal level with maximum efficacy and minimum adverse effect of MZ, should be re-examined. Although the issue of dose-limiting toxicity must be cleared, when high-dose MZ can be proven to be effective and tolerable in a clinical study, options for treatment of rejection, particularly humoral rejection, could become widely available.

In conclusion, (i) treatment with high-dose MZ (30 mg/kg/day) reversed ongoing acute humoral rejection in a porcine allotransplantation model. (ii) Pigs were able to tolerate this high dosage of MZ, with the exception that high serum concentration of MZ (trough level >10 μg/ml) caused leukocytopenia. (iii) High-dose MZ would be applicable to clinical trials if blood level is carefully monitored.

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References

1. Djamali A, Premasathian N, Pirsch JD. Outcomes in kidney transplantation. *Sem Nephrol* 2003; **23**: 306.
2. Bohmig GA, Regele H, Exner M, *et al.* C4d-positive acute humoral renal allograft rejection: effective treatment by immunoadsorption. *J Am Soc Nephrol* 2001; **12**: 2482.
3. Crespo M, Pascual M, Tolkoff-Rubin N, *et al.* Acute humoral rejection in renal allograft recipients: I. Incidence, serology and clinical characteristics. *Transplantation* 2001; **71**: 652.
4. Pascual M, Saidman S, Tolkoff-Rubin N, *et al.* Plasma exchange and tacrolimus-mycophenolate rescue for acute humoral rejection in kidney transplantation. *Transplantation* 1998; **66**: 1460.
5. Chardot C, Nicoluzzi JE, Janssen M, *et al.* Use of mycophenolate mofetil as rescue therapy after pediatric liver transplantation. *Transplantation* 2001; **71**: 224.
6. Sollinger HW and US Renal Transplant Mycophenolate Mofetil Study Group. Mycophenolate mofetil for the prevention of acute rejection in primary cadaveric renal allograft recipients. *Transplantation* 1995; **60**: 225.
7. European Mycophenolate Mofetil Cooperative Study Group. Placebo-controlled study of mycophenolate mofetil combined with cyclosporine and corticosteroids for prevention of acute rejection. *Lancet* 1995; **345**: 1321.
8. Katayama A, Kobayashi T, Uchida K, *et al.* Beneficial effect of antibody removal and enhanced immunosuppression in flow cytometry crossmatch-positive and ABO-incompatible renal transplantation. *Transplant Proc* 2002; **34**: 2771.
9. Takada K, Nakae H, Asada S, *et al.* Rapid method for the high-performance liquid chromatographic determination of bredinin in human serum. *J Chromatogr* 1981; **222**: 156.
10. Kamada H, Inoue N, Takaoka Y, Nakagami K, Mori H, Nagai H. Effect of mizoribine on effector T cell-mediated immune responses in mice. *Biol Pharm Bull* 1996; **19**: 1136.
11. Turka LA, Dayton J, Sinclair G, Thompson CB, Mitchell BS. Guanine ribonucleotide depletion inhibits T cell activation. *J Clin Invest* 1991; **87**: 940.
12. Gregory CR, Gourley IM, Haskins SC, *et al.* Effects of mizoribine on canine renal allograft recipients. *Am J Vet Res* 1988; **49**: 305.
13. Gregory CR, Gourley IM, Cain GR, *et al.* Effects of combination cyclosporine/mizoribine immunosuppression on canine renal allograft recipients. *Transplantation* 1988; **45**: 856.
14. Amemiya H, Suzuki S, Niiya S, Watanabe H, Kotake T. Synergistic effect of cyclosporine and mizoribine on survival of dog renal allografts. *Transplantation* 1988; **46**: 768.
15. Gregory CR, Gourley IM, Cain GR, Patz JD, Imondi KA, Martin JA. Mizoribine serum levels associated with enterotoxicity in the dog. *Transplantation* 1991; **51**: 877.
16. Platz KP, Sollinger HW, Hullett DA, Eckhoff DE, Eugui EM, Allison AC. RS-61443-a new, potent immunosuppressive agent. *Transplantation* 1991; **51**: 27.
17. Gruber SA, Erdmann GR, Burke BA, *et al.* Mizoribine pharmacokinetics and pharmacodynamics in a canine renal allograft model of local immunosuppression. *Transplantation* 1992; **53**: 12.
18. Terblanche J, Hickman R, Uys CJ. Renal transplantation in the unimmunosuppressed pig: an abnormal response. *Br J Surg* 1975; **62**: 474.
19. Kirkman RL, Colvin RB, Flye MW, *et al.* Transplantation in miniature swine. VI. Factors influencing survival of renal allografts. *Transplantation* 1979; **28**: 18.
20. Aso K, Uchida H, Sato K, *et al.* Immunosuppression with low-dose cyclosporine combined with bredinin and prednisolone. *Transplant Proc* 1987; **19**: 1955.
21. Tajima A, Hata M, Ohta N, Ohtawara Y, Suzuki K, Aso Y. Bredinin treatment in clinical kidney allografting. *Transplantation* 1984; **38**: 116.
22. Cho DK, Kim JH, Park SH, *et al.* The efficacy and safety of mizoribine in living donor kidney transplantation: a 24-week, open-label, prospective study (phase III clinical trial). *Transplant Proc* 2001; **33**: 3256.
23. Tanabe K, Tokumoto T, Ishikawa N, *et al.* Long-term results in mizoribine-treated renal transplant recipients: a prospective, randomized trial of mizoribine and azathioprine under cyclosporine-based immunosuppression. *Transplant Proc* 1999; **31**: 2877.
24. Sonda K, Takahashi K, Tanabe K, *et al.* Clinical pharmacokinetic study of mizoribine in renal transplantation patients. *Transplant Proc* 1996; **28**: 3643.
25. Platz KP, Bechstein WO, Eckhoff DE, Suzuki Y, Sollinger HW. RS-61443 reverses acute allograft rejection in dogs. *Surgery* 1991; **110**: 736.
26. Ishikawa H. Mizoribine and mycophenolate mofetil. *Curr Med Chem* 1999; **6**: 575.
27. Bullingham RES, Nicholls AJ, Kamm BR. Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 1998; **34**: 429.
28. Kusumi T, Tsuda M, Katsunuma T, Yamamura M. Dual inhibitory effect of bredinin. *Cell Biochem Funct* 1988; **7**: 201.
29. Koshikawa S, Sato M, Narita M, Sakai O, *et al.* Clinical study of HE-69 (mizoribine) on patients with incurable nephritic syndrome – A multicenter open study. *Kidney Dialysis* 1987; **23**: 971 (in Japanese).
30. Kusaba R, Otubo O, Inou T, *et al.* Studies on pharmacokinetics of bredinin. *Jpn J Transplant* 1982; **17**: 585 (in Japanese).