Effectiveness of the Combination of Everolimus and Tacrolimus With High Dosage of Mizoribine for Living Donor–Related Kidney Transplantation

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ABSTRACT

Background. Everolimus (EVR) has been used widely for the purpose of reducing the dosage of calcineurin inhibitor (CNI), leading to decreasing CNI nephrotoxicity. In Japan, high-dose mizoribine (MZR) (6 mg/kg/day) has been increasingly used because of incidences of virus infection and gastrointestinal disorder in kidney transplant recipients. However, the efficacy and safety of EVR and MZR combination therapy is still uncertain.

Methods. A total of 29 living kidney transplant recipients from October 2012 to June 2014 were analyzed. Tacrolimus (TAC), MZR, basiliximab, and prednisolone were administered to all recipients. EVR was added to the regimen for 10 recipients from postoperative day 10 to 14; TAC trough levels were minimized simultaneously (EVR group). The remaining 19 recipients were defined as the control group. We evaluated the outcomes between the 2 groups.

Results. The mean TAC trough level was 5.17 ng/mL at 1 month after transplantation in the EVR group, and 7.89 ng/mL in the control group ($P = .007$), respectively. The mean TAC trough level was 4.0 ng/mL at 18 months after transplantation in the EVR group, and 6.97 ng/mL in the control group ($P = .003$) respectively. There were no differences in the rate of acute rejection and serum creatinine level. There was no significant difference in the incidence of histological nephrotoxicity between the 2 groups in the 1-year biopsy results.

Conclusions. We succeeded in reducing TAC trough level immediately after transplantation by adding EVR. Our study results suggest that this combination therapy is effective for kidney transplantation recipients.

OWING to the introduction of calcineurin inhibitor (CNI), acute rejection has been well controlled in the context of organ transplantation. Nevertheless, it has been reported that CNI toxicity is a major obstacle in terms of longer acceptance of kidney allografts [1]. Therefore, introduction of the mammalian target of rapamycin (mTOR) inhibitor everolimus (EVR) seems to be effective in minimizing CNI toxicity [2,3]. Furthermore, histopathological CNI toxicity might be reversed due to administration of EVR [4]. Interestingly, we have previously shown that the mTOR inhibitor rapamycin has the potential to induce myeloid-derived suppressor cells that might have a protective role for transplanted organs: namely, by restoring histopathological changes associated with CNI toxicity [5]. Moreover, mizoribine (MZR) has been used increasingly in kidney transplant recipients as a substitute for mycophenolate mofetil (MMF) because of its superiority in terms of cytomegalovirus (CMV) infection [6]. However, investigations into the combination therapy of TAC, MZR, and EVR have rarely been performed. Thus, in this study we

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evaluated the effectiveness, interaction, and safety of these medicines for living donor–related kidney transplant recipients.

MATERIALS AND METHODS

Patient Status and Immunosuppressive Regimen

CNIs were administered for all living donor–related kidney transplant (LDKT) recipients in our hospital. Transplant recipients who used cyclosporine were excluded from this study. A total of 29 LDKT recipients from October 2012 to June 2014 were enrolled in this study. Two days before transplantation, we started administering TAC to recipients (target trough levels: 8–12 ng/mL). TAC (0.2 mg/kg C0: 8–12 ng/mL), MZR (6 mg/kg/day C0: 1–2 μg/mL), basiliximab (20 mg/body on postoperative days 0 and 4), and prednisolone (PSL; initial dosage 50 mg/day and maintenance dosage 5 mg/day) were used according to our immunosuppressive protocol [7]. They were observed by trough level and area under the blood concentration time curve strictly for TAC and MZR.

In 10 recipients in the EVR group, EVR (2.0 mg/body/day) was administered on postoperative days 10 to 14. At that time, the TAC trough value was reduced to 4 to 6 ng/mL (Fig 1). Target rough levels of EVR were 2 to 6 ng/mL. A total of 19 recipients were not administered EVR and were defined as the control group (Table 1).

Protocol biopsies were performed 1 month and 1 year after LDKT as usual. Some recipients did not undergo biopsy for reasons such as obesity and use of antiplatelet medications.

Evaluation of Patient Status and Adverse Effects

We evaluated sex, age, serum creatinine level (at 1 month, 12 months, and 18 months), tacrolimus (TAC) trough value (at 1 month, 12 months, and 18 months), patient survival, and graft survival between 2 groups. In addition, we evaluated adverse effects such as insufficient wound healing, acute rejection, histological nephrotoxicity 1 year after transplantation, increase in proteinuria 1 year after transplantation, infection (defined as use of additional antibiotic and antiviral medications), neutropenia (defined as <1500, cytomegalovirus [CMV]–Ag positivity), posttransplantation lymphoproliferative disorder (PTLD), stomatitis, ileus, and loss of hair between the 2 groups (Table 2).

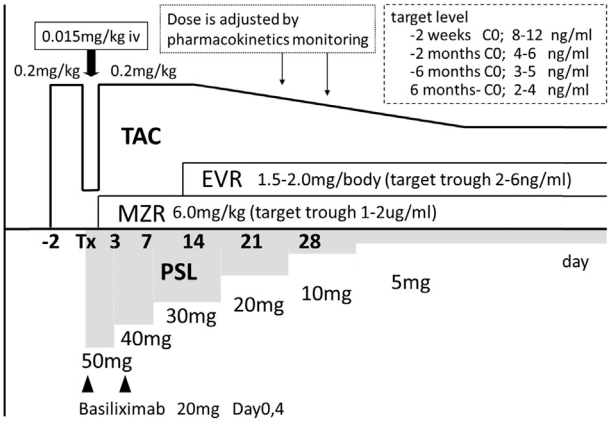

Fig 1. Immunosuppressive regimens: TAC + MZR + PSL + basiliximab + EVR. Tx, transplant; TAC, tacrolimus; MZR, mizoribine; PSL, prednisolone; EVR, everolimus.

Table 1. Comparison of Patient Demographics, Complications, and Immunosuppressive Conditions Between the Everolimus (EVR) Group and Control Group

Characteristic	EVR(+) (n = 10)	EVR(−) (n = 19)	*P* Value
Sex (male:female)	7:3	12: 7	.711
Age	45.9 ± 13.0	44.3 ± 18.5	.827
Serum creatinine (1 mo) (mg/dL)	1.08 ± 0.54	1.20 ± 1.10	.77
Serum creatinine (12 mo) (mg/dL)	1.31 ± 0.38 (n = 10)	1.24 ± 0.76 (n = 17)	.798
Serum creatinine (18 mo) (mg/dL)	1.18 ± 0.42 (n = 6)	1.54 ± 1.29 (n = 13)	.364
TAC trough (1 mo) (ng/mL)	5.17 ± 1.45 (n = 9)	7.89 ± 2.50 (n = 19)	.0074
TAC trough (12 mo) (ng/mL)	5.42 ± 1.72 (n = 9)	6.81 ± 2.17 (n = 14)	.14
TAC trough (18 mo) (ng/mL)	4.00 ± 0.74 (n = 6)	6.97 ± 2.86 (n = 11)	.00332
Patient survival (1 y) (%)	100	100	–
Graft survival (1 y) (%)	100	94.7	.352

Abbreviation: TAC, tacrolimus.

Statistical Analysis

The 2 groups were compared using the Student *t* test. Tukey tests were used for multiple comparisons. *P* values less than .05 were considered statistically significant. All statistical analyses were performed using JMP Software 11 version (SAS Institute Inc., Cary, NC).

RESULTS

Patient Demographics, Serum Creatinine Levels, Tacrolimus Trough Levels, and Patient and Graft Survival

In the EVR group, 7 recipients were male and 3 were female; in the control group, 12 recipients were male and 7 were female. The mean age was 45.9 ± 13.0 years (range 20–67 years) in the EVR group and 44.1 ± 18.5 years (range 6–70 years) in the control group ($P = .827$). The mean serum creatinine (s-Cr) level was 1.08 ± 0.54 mg/dL (range 0.37–2.46 mg/dL) 1 month after transplantation in the EVR group and 1.20 ± 1.10 mg/dL (range 0.49–5.67 mg/dL) in

Table 2. Comparison of Adverse Effects Between Everolimus (EVR) Group and Control Group

Adverse Effect	EVR(+) (n = 10)	EVR(−) (n = 19)	*P* Value
Insufficient wound healing (n)	0	0	–
Acute rejection (n, %)	1, 10	3, 15.8	.0729
Histological nephrotoxicity (1 y) (n, %)	1, 10	1, 5.3	.365
Increase of proteinuria (1 y) (n, %)	3, 30	3, 15.8	.461
Infection (n, %)	1, 10	2, 10.8	.94646
Neutropenia (n)	0	0	–
CMV-Ag positive (n, %)	3, 30	7, 36.8	.711
PTLD (n, %)	1, 10	0, 0	.1382
Stomatitis (n)	0	0	–
Ileus (n, %)	1, 10	0, 0	.1382
Loss of hair (n, %)	0, 0	1, 5.3	.3525

Abbreviations: CMV, cytomegalovirus; PTLD, posttransplantation lymphoproliferative disorder.

the control group ($P = .77$). The s-Cr level was 1.31 ± 0.38 mg/dL (range 0.65–1.83 mg/dL) 12 months after transplantation in the EVR group and 1.24 ± 0.76 mg/dL (range 0.64–4.14 mg/dL) in the control group ($P = .798$). The s-Cr level was 1.17 ± 0.42 mg/dL (range 0.65–1.83 mg/dL) 18 months after transplantation in the EVR group, and 1.54 ± 1.29 mg/dL (range 0.64–4.14 mg/dL) in the control group ($P = .364$).

The mean TAC trough value was 5.17 ± 1.45 ng/mL (range 3.2–8.1 ng/mL) 1 month after transplantation in the EVR group, and 7.89 ± 2.50 ng/mL (range 3.5–12.9 ng/mL) in the control group ($P = .0074$). The mean TAC trough was 4.00 ± 0.74 ng/mL (range 2.2–6.8 ng/mL) in the EVR group, and 6.80 ± 2.18 ng/mL (range 3.7–9.9 ng/mL) in the control group ($P = .0332$) 18 months after transplantation.

The cumulative 1-year patient survival was 100% in the both groups. Cumulative graft survival was 100% in the EVR group, and was 95% in the control group ($P = .352$) because 1 patient lost graft function due to arterial thrombosis immediately after transplantation.

Adverse Effects

No insufficient wound healing was found in the EVR or control group. One patient developed acute rejection in the EVR group, as did 3 recipients in the control group ($P = .0729$). One patient experienced histological nephrotoxicity 1 year after transplantation in the EVR group, as did 1 patient in the control group ($P = .365$). Three recipients developed increase of proteinuria 1 year after transplantation in the EVR group, as well as 3 patient in the control group ($P = .461$). One recipient developed infection in the EVR group, along with 2 recipients in the control group ($P = .94646$). No patient developed neutropenia in either group. Three transplant recipients developed CMV-Ag positivity in the EVR group, as did 7 recipients in the control group ($P = .711$). One patient developed PTLD in the EVR group only ($P = .1382$). No stomatitis was found in the EVR or control group. One patient developed ileus in the EVR group ($P = .1382$). One patient developed loss of hair in the control group ($P = .3525$).

DISCUSSION

It has often been reported that introduction of EVR has advantages in both clinical and histological studies [2,4]. Recently, a combination of high-dose mizoribine (MZR) (6 mg/kg/day) with cyclosporine, basiliximab, and corticosteroids has been reported to be associated not only with satisfactory immunosuppression but also with low incidences of viral infection and gastrointestinal disorders in kidney transplant recipients [6]. In Japan, MZR has been used increasingly in kidney transplant recipients as a substitute for MMF because of superiority in terms of CMV infection [6]. However, there is almost no report that investigates the combination therapy of TAC, MZR, and EVR in the context of living donor–related kidney transplantation. We therefore evaluated the efficacy of this combination therapy.

In this study, the results indicated that patients in the EVR group had outcomes equivalent to those of the control group in terms of patient survival, graft survival, and renal function during this study period early after transplantation. We also did not find notable differences in serum creatinine levels and acute rejection rates 1 year after transplantation. Decreasing the dose of TAC and adding EVR, as in this study, seems to result in satisfactory immunosuppression.

For administration of EVR, it is essential to minimize proteinuria and to avoid delayed wound healing because of the mTOR effects of delaying cell synthesis, which leads to precipitating proteinuria and insufficient wound healing. Therefore, we added EVR on days 10 to 14 following transplantation after confirming minimized proteinuria and absence of delayed wound healing. These results indicated that 10 to 14 days following transplantation was appropriate timing to resolve that obstacle. However, although the addition of EVR to the usual TAC and MZR combination therapy seemed to suggest a high risk of infection because of over-immunosuppression, there was no significant difference in infectious complications compared with that in the control group (reduced TAC and MZR therapy). It is probable that decreasing the dose of TAC prevented an increase in the risk of infection. Moreover, combination therapy with MMF and EVR has been reported to result in tonsillitis and stomatitis [8]. However, in this study, no stomatitis was found at any kidney transplant recipients with MZR combination therapy, regardless of the addition of EVR. The reason for this is unknown and may be associated with lower risk of gastrointestinal disorder with MZR than with MMF [6]. Thus, it is reasonable that EVR is added onto a reduced dosage of TAC and MZR around 2 weeks after kidney transplantation.

In this study, unexpectedly, there was no significant difference in histologically confirmed CNI nephrotoxicity, primarily due to the limitation on the available number of biopsies: two in the EVR group and six in the control group, respectively. Nevertheless, it is clear that administration of EVR can reduce the dosage of TAC; subsequent TAC trough levels in the EVR group were clearly lower than those in the control group. Given the protective effects of mTOR inhibitors on transplanted organs [1,4,5], it can be considered that the combination therapy of TAC, MZR, and EVR is useful in general, not only for CNI-intolerant patients. Some effects of transforming growth factor–β of EVR [1] are expected to contribute to preventing the precipitation of fibrosis for transplant recipients.

In conclusion, we confirmed that combination therapy consisting of TAC, MZR, and EVR was effective and safe over the short term. However, it is necessary to observe these patient groups for a longer period in terms of patient survival, graft survival, serum creatinine levels, adverse effects, and histological examination.

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