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ORIGINAL ARTICLE

Randomized trial of cyclosporine and tacrolimus therapy with steroid withdrawal in living-donor renal transplantation: 5-year follow-up

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Summary

The aim of this study was to compare the long-term safety and efficacy of immunosuppressive regimens consisting of cyclosporine (CsA) plus mycophenolate mofetil (MMF) or tacrolimus (TAC) plus MMF after steroid withdrawal 6 months after kidney transplantation in low-risk patients. One hundred and thirty-one patients were randomized to receive either CsA ($n = 63$) or TAC ($n = 68$). Of these, 117 patients satisfied the criteria for steroid withdrawal (no biopsy-proven rejection episode and serum creatinine level <2.0 mg/dl 6 months after transplantation). Fifty-five recipients were of the CsA group, and 62 were of the TAC group. The 5-year graft survival rate did not differ between groups (90.5% vs. 93.3% respectively; $P = 0.55$). The cumulative incidence of acute rejection 5 years after transplantation was 16.4% and 8.1% for the CsA and TAC groups respectively ($P = 0.15$). Post-transplantation diabetes mellitus was more frequent in the TAC group than in the CsA group ($P = 0.05$), but the incidence of other side-effects did not differ between groups. In conclusion, CsA- and TAC-based regimens in conjunction with MMF have similar patient- and graft survival rates in low-risk patients who underwent steroid withdrawal 6 months after kidney transplantation.

Introduction

Steroids are the mainstay of immunosuppressive therapies for solid-organ transplants. However, the well-known adverse effects of long-term therapy with steroids have prompted many trials on possible steroid-withdrawal immunosuppressants for kidney transplant recipients [1,2]. In the cyclosporine (CsA)/azathioprine era, withdrawal of steroids from immunosuppressive regimens was considered to promote long-term graft loss as well as acute rejection [3,4]. After the introduction of mycophenolate mofetil (MMF)-based immunosuppression, several randomized prospective studies showed that the use of CsA/MMF or tacrolimus (TAC)/MMF in steroid-withdrawal regimens does not affect graft survival in low-risk patients [5–7].

To compare the long-term efficacy and safety of CsA- versus TAC-based immunosuppressive therapy in steroid withdrawal regimens, we conducted a prospective, randomized, single-center clinical trial comparing the safety and efficacy of immunosuppressive regimens containing CsA plus MMF or TAC plus MMF after steroids were withdrawn 6 months after kidney transplantation in low-risk patients. We have reported the results of the first year of this study. The study revealed that, after 1 year of patient follow up, there were no significant differences between the regimens in the incidence of acute rejection episodes as proven by biopsy, and there was no graft

failure or patient death during the follow-up period [8]. The aim of this report was to present results obtained after 5 years of follow up.

Patients and methods

Study design and population

The study was a prospective, randomized, open-labeled, single-center trial. The study was approved by the Institutional Review Board of the Samsung Medical Center. Patients older than 15 years who had undergone their first living-donor renal transplantation at Samsung Medical Center between September 2000 and August 2003 were included in the study. The exclusion criteria were congestive heart failure (ejection fraction <35%), chronic liver disease, diabetes mellitus, systemic infection, malignant disease, multiple organ transplantation and positive serologic evidence of human immunodeficiency virus. A total of 131 patients were enrolled and randomized to receive either CsA ($n = 63$) or TAC ($n = 68$) concomitantly with steroids and MMF after their informed consent was obtained. Steroids were withdrawn if acute rejection (assessed from a biopsy sample) did not occur during the first 6 months after transplantation and the serum creatinine level was <2.0 mg/dl.

Immunosuppressive protocol

On the first postoperative day, 4 mg/kg CsA (Cipol inj.®; Chong Kun Dang, Seoul, Korea) was administered intravenously; on the second postoperative day, 12 mg/kg/day CsA (Cipol-N soft cap.®; Chong Kun Dang) was administered orally as a microemulsion in two doses. The target trough levels of CsA were 300–350 ng/ml during the first postoperative week, 200–300 ng/ml within 1 month of transplantation, 150–250 ng/ml within 3 months of transplantation and 100–200 ng/ml thereafter. TAC (Prograft®; Fujisawa, Osaka, Japan) was administered orally as two separate doses, starting at 0.15 mg/kg/day. Target trough levels of TAC were 12–15 ng/ml during the first postoperative week, 10–12 ng/ml within 1 month of transplantation, 8–10 ng/ml within 3 months of transplantation and 6–8 ng/ml thereafter. The mean trough levels of CsA and TAC were maintained within the target range during the postoperative periods (data not shown). The percentage of patients in which the trough levels of CsA and TAC were out of target range was not significantly different between the groups during the postoperative periods [median, 5.8% (range, 4.6–18.3%) in the CsA group; median, 13.0% (range, 6.5–21.4%) in the TAC group].

Mycophenolate mofetil (MMF) (Cellcept®; Roche, Basel, Switzerland) was administered at a dose of 1500 mg/day to patients of both groups except for those in whom it provoked side-effects. Five hundred milligrams of methylprednisolone (MPD) was administered intravenously before and during surgery. One dose of 500 mg of MPD was administered on the first postoperative day, and thereafter the dose was tapered by 50% daily. From the eighth postoperative day, 30 mg/day of prednisolone was administered orally; thereafter the dose was gradually tapered and ultimately withdrawn from patients who satisfied the steroid withdrawal criteria 6 months after transplantation.

Treatment of acute rejection

After steroid withdrawal, all acute rejections were treated with an intravenous bolus of 1000 mg of MPD for 3 days. Patients who underwent acute rejection episodes were maintained on steroid medication after steroid pulse therapy.

Endpoints

Patients were followed up for 5 years or until death. The primary endpoints were patient survival and graft survival 5 years after transplantation. The secondary endpoints were the cumulative incidence of acute rejection and estimated glomerular filtration rate (eGFR) 5 years after transplantation, the presence of new-onset diabetes mellitus, the use of lipid-lowering agents and the presence of hypertension. We compared the annual change in serum levels of total cholesterol and low-density lipoprotein (LDL) cholesterol between groups during the 5 years after transplantation. We also recorded the incidence of adverse events during this period. Graft loss was defined as a return to long-term dialysis. We treated death with a functioning graft as censored data. Acute rejection was suspected when there was an unexplained rise in serum creatinine concentration and was confirmed by graft biopsy. The estimated GFR was calculated using the abbreviated Modification of Diet in Renal Disease Study (aMDRD) formula, $\text{eGFR} = 1.86 \times (\text{serum creatinine, mg/dl})^{-1.154} \times (\text{age})^{-0.203}$ for men, and the result of the same formula multiplied by 0.742 for women. Hypertension was defined as a condition requiring the use of antihypertensive drugs. Post-transplant diabetes mellitus (PTDM) was defined as a fasting plasma glucose level of more than 126 mg/dl on at least two different days, or a condition requiring oral hypoglycemic agents or insulin for glycemic control.

Statistical analysis

This study was designed to determine which of the two regimens was better in terms of long-term graft survival.

The prestudy power analysis was done using data on biopsy-proven acute rejection because there were few data on long-term graft survival in steroid withdrawal regimens. The effect of acute rejection on long-term graft outcome was taken into consideration [9]. Based on the randomized trial reported by Mayer *et al.* [10], we expected that a difference in acute rejection rate >20% would affect long-term graft survival. Assuming a 6% biopsy-proven acute rejection rate after steroid withdrawal in the TAC group, 52 patients per group would be required to detect a 20% increase in the CsA group for an alpha error of 5% (two-tailed) and a statistical power of 80%.

We analysed data from patients who successfully discontinued steroid treatment 6 months after kidney transplantation. All data were analysed on an intention-to-treat basis. For comparison of baseline characteristics, we used Student's *t*-test for independent samples; the Mann–Whitney test was used for nonparametric distributions. Pearson's Chi-squared test was used for comparison of categorical variables. A logistic regression analysis was used to identify significant risk factors for the development of PTDM. Kaplan–Meier survival analysis was used to compare patient survival, graft survival and the cumulative incidence of acute rejection. Differences between groups were analysed using the log-rank test. A multivariate Cox regression model was used to analyse any confounding influence of the following variables on the incidence of acute rejection: age, gender, human leukocyte antigen (HLA) mismatch, donor type, body mass index (BMI) (≥24 kg/m^2 vs. <24 kg/m^2), trough levels of CsA and TAC (within the target range versus out of the target range), dose of MMF and type of calcineurin inhibitor (CsA versus TAC). Mixed-model analysis was used to compare the annual changes in serum levels of total cholesterol and LDL cholesterol during follow up between groups. *P* values <0.05 were considered statistically significant.

Results

Patient population

A total of 131 participants were enrolled in the study. Fourteen patients were excluded because of acute rejection as proven by biopsy within 6 months of transplantation or because of protocol violation. In the CsA group, four patients experienced acute rejections within 6 months, and four patients violated the protocol; in the TAC group, five patients experienced acute rejections within 6 months, and one patient violated the protocol. Therefore, steroid treatment was tapered off and withdrawn 6 months after transplantation for 117 patients (55 of the CsA group vs. 62 of the TAC group). The baseline characteristics of both groups are shown in Table 1. Although the mean body weight of recipients was greater in the TAC group when compared to the CsA group, BMI was not significantly different between groups. There appeared to be a predominance of males in the TAC group, although it was not statistically significant (male:female = 47.3%:52.7% in the CsA group vs. 62.9%:37.1% in the TAC group). However, there were no significant differences between groups in donor gender, donor age, HLA mismatch or panel reactive antibody (PRA) factors that could have affected long-term graft survival.

Patient- and graft survival and kidney function

The death-censored graft survival 5 years after transplantation was 90.5% for the CsA group and 93.3% for the TAC group, but the difference was not statistically significant ($P = 0.55$). Kaplan–Meier estimates of graft survival are presented in Fig. 1. The 5-year patient survival was

Table 1. Baseline characteristics of patients receiving cyclosporine or tacrolimus.

	Cyclosporine (*n* = 55)	Tacrolimus (*n* = 62)	*P* value
Recipient gender (%)			
Male	26 (47.3%)	39 (62.9%)	0.09
Female	29 (52.7%)	23 (37.1%)	
Donor gender (%)			
Male	30 (54.5%)	36 (58.1%)	0.69
Female	25 (45.5%)	26 (41.9%)	
Recipient age (years)*	38.5 ± 9.5	38.8 ± 9.2	0.67
Donor age (years)*	39.7 ± 10.2	38.9 ± 11.5	0.70
Donor source			
Living, related	35 (63.6%)	36 (58.1%)	0.79
Living, unrelated	20 (36.4%)	26 (41.9%)	
Family history of diabetes	8 (14.5%)	8 (12.9%)	0.80
Number of HLA mismatches			
0	10 (18.2%)	7 (11.3%)	0.10
1	5 (9.1%)	5 (8.1%)	
2	12 (21.8%)	9 (14.5%)	
3	17 (30.9%)	22 (35.5%)	
4	8 (14.6%)	14 (22.6%)	
5	1 (1.8%)	2 (3.2%)	
6	2 (3.6%)	3 (4.8%)	
Median (IQR)†	3.0 (1.0–3.0)	3.0 (2.0–4.0)	0.13
Panel reactive antibody			
0%	53 (96.4%)	59 (95.2%)	0.56
<50%	2 (3.6%)	2 (3.2%)	
>50%	0 (0%)	1 (1.6%)	
Body weight (kg)	58.0 ± 7.6	62.6 ± 11.0	0.01
Body mass index (kg/m^2)	22.0 ± 2.4	23.0 ± 3.2	0.06

HLA, human leukocyte antigen; NS, not significant.
*Mean ± SD. †Median (interquartile range).

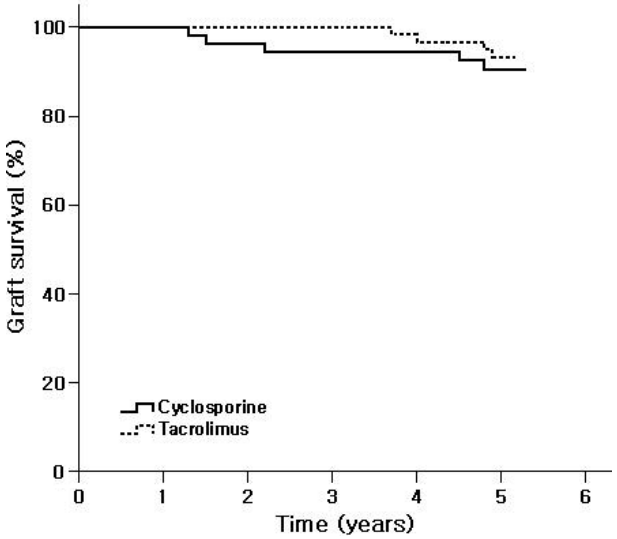

Figure 1 Death-censored graft survival curves for patients receiving cyclosporine or tacrolimus ($P = 0.55$ according to the log-rank test).

98.2% (54/55) in the CsA group and 98.4% (61/62) in the TAC group ($P = 0.90$). Death was caused by suicide in one instance and by colon cancer in another. The renal function of all patients who did not receive dialysis was assessed using eGFR. The eGFR 5 years after transplantation did not differ significantly between groups (61.5 ± 14.6 ml/min/1.73m^2 for the CsA group vs. 62.9 ± 20.3 ml/min/1.73m^2 for the TAC group; $P = 0.69$). The median rate of change in eGFR also did not differ significantly between groups (Table 2).

Acute rejection

The cumulative incidence of acute rejection during the 5-year interval after steroid withdrawal was estimated using the Kaplan–Meier method (Fig. 2). The incidence of acute rejection was more frequent in the CsA group (nine episodes) than in the TAC group (five episodes) (16.4% vs. 8.1% respectively; $P = 0.15$). The median time at which the first rejection after steroid withdrawal occurred was 11.5 months (range, 9–18 months). Of the recipients who experienced acute rejection, seven patients in the CsA group and four patients in the TAC group were completely treated with pulse steroid therapy. On multivariate Cox regression analysis, trough level and type of calcineurin inhibitor were associated with the development of acute rejection. When the level of CsA or TAC was out of target range, the risk of acute rejection was significantly increased (relative risk = 4.4, $P = 0.05$). The TAC group had a significantly reduced risk of acute rejection compared with the CsA group (relative risk = 0.15, $P = 0.02$).

Table 2. eGFR (ml/min/1.73m^2) at baseline, 6 months and 1 year after transplantation and the annual rate of change in eGFR in both groups.

	Cyclosporine	Tacrolimus	*P* value
Baseline*	69.6 ± 13.1	71.6 ± 12.7	0.41
6 months*	59.6 ± 9.1	62.9 ± 11.0	0.08
1 year*	58.6 ± 13.8	64.7 ± 12.6	0.01
5 years*	61.5 ± 14.6	62.9 ± 20.3	0.69
Rate of change in eGFR†	−1.92 (−4.10 to 0.42)	−1.30 (−3.25 to 1.11)	0.29

*Mean ± SD. †Median (interquartile range).

Adverse events

The incidence of PTDM 5 years after transplantation was significantly greater in the TAC group than in the CsA group (19.4% vs. 7.3% respectively; $P = 0.05$). On multivariate analysis, elderly recipients older than 50 years appeared to have a significantly higher risk for PTDM, after adjusting for age, gender, BMI and family history of diabetes (relative risk = 5.0, $P = 0.01$). The incidence of patients requiring lipid-lowering agents did not differ between the CsA and TAC groups (32.7% vs. 27.0% respectively; $P = 0.32$). The annual change in total cholesterol levels did not differ significantly between groups ($P = 0.82$) (Fig. 3). Similarly, the annual change in LDL cholesterol levels did not differ significantly between

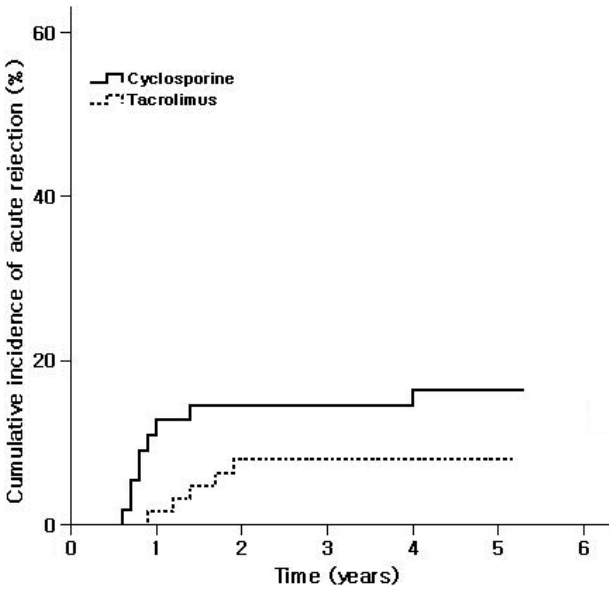

Figure 2 Kaplan–Meier estimates of the cumulative incidence of acute rejection in patients receiving cyclosporine or tacrolimus during a 5-year follow-up period (16.4% in cyclosporine vs. 8.1% in tacrolimus, $P = 0.15$ according to the log-rank test).

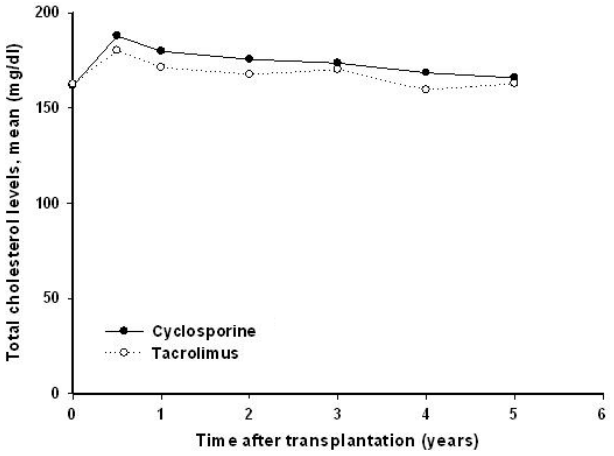

Figure 3 The annual change in total cholesterol level did not differ between the two groups ($P = 0.82$).

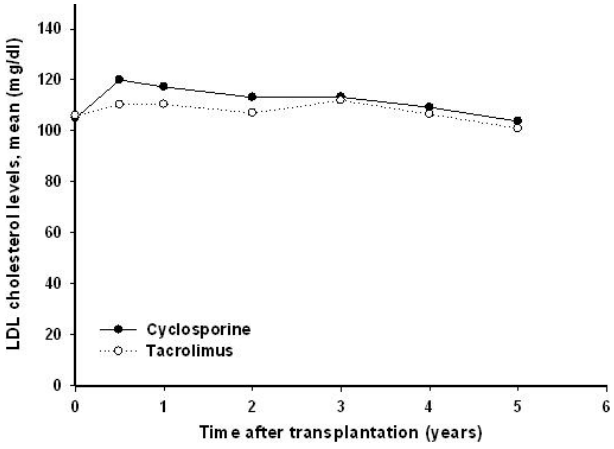

Figure 4 The annual change in LDL cholesterol level did not differ between the two groups ($P = 0.40$).

groups ($P = 0.40$) (Fig. 4). The incidence of hypertension, bacterial infection, leukopenia, liver function test abnormalities, opportunistic infection, gingival hypertrophy and avascular necrosis (AVN) did not differ significantly between groups (Table 3).

Discussion

When interpreting the effect of steroid-sparing protocols on kidney transplant outcomes, it is important to take into consideration the sample size, study design and duration of follow up. Although the sample size in this study was relatively small, it was a long-term, randomized parallel clinical trial. To our knowledge, no studies have compared the long-term efficacy and safety of CsA- versus TAC-based immunosuppressive therapy in steroid withdrawal regimens [11].

This study shows that there is no difference in the long-term graft- or patient survival between patients treated with CsA or TAC after steroid withdrawal 6 months after transplantation. Five years after transplantation, the CsA and TAC groups had graft survival rates of 90.5% and 93.3%, respectively, and patient survival rates of 98.2% and 98.4% respectively. These results are consistent with those of previous steroid withdrawal studies on low-risk patients [5–7].

The current trend is to withdraw steroid treatment at an early stage or use steroid-free protocols in which anti-CD25 antibodies are administered for induction [12–14]. We did not use anti-CD25 antibodies in this study because basiliximab and daclizumab were not available in Korea when the study was designed. At that time, it was customary to use OKT3 or ALG for induction in high-risk patients, but we decided not to use these antibodies in the study because of their adverse side-effects [15,16]. Some studies have shown that early steroid withdrawal may affect the incidence of acute rejection [17,18]. However, many studies have shown that late steroid withdrawal (6 months or more after transplantation) does not increase the risk of acute rejection or graft loss [5,14,17,19–21]. Therefore, instead of using induction antibodies, we withdrew steroids 6 months after transplantation.

Several studies have been conducted to compare the use of TAC versus CsA in conventional protocols involving maintenance of steroid treatment. Many of these studies showed that the incidence of graft loss is less with TAC treatment than with CsA treatment [22–24]. However, contrary to previous results, there was no difference in our study in graft or patient survival between patients treated with CsA or TAC. The cumulative incidence of

Table 3. Long-term adverse events in patients receiving cyclosporine or tacrolimus.

	Cyclosporine ($n = 55$)	Tacrolimus ($n = 62$)	*P* value
PTDM	4 (7.3)	12 (19.4)	0.05
Use of lipid-lowering agents	18 (32.7)	16 (25.8)	0.41
Hypertension	33 (60.0)	38 (60.3)	0.89
Bacterial infection	12 (21.8)	12 (19.4)	0.74
Leukopenia	18 (32.7)	17 (27.4)	0.53
Liver function test abnormality	7 (12.7)	5 (8.1)	0.41
Opportunistic infection	5 (9.1)	7 (11.3)	0.70
Gingival hypertrophy	4 (7.4)	1 (1.6)	0.14
Avascular necrosis	3 (5.5)	1 (1.6)	0.27

Values given in parenthesis are given in percentage. PTDM, post-transplantation diabetes mellitus; NS, not significant.

acute rejection, which is a predisposing factor for graft failure, differed between the groups (16.4% in the CsA group vs. 8.1% in the TAC group). The authors of several previous studies concluded that the risk of acute rejection is less with TAC therapy than with CsA therapy [10,22,25–27]. A meta-analysis comparing the efficacy of TAC and CsA showed that TAC therapy reduces the incidence of acute rejection 1 year after transplantation by 31% compared with CsA therapy [22]. In our study, TAC therapy reduced the incidence of acute rejection 5 years after transplantation by 51% when compared to CsA therapy, but the difference was not statistically significant (relative risk = 0.49, 95% confidence interval = 0.18–1.38). However, after adjusting for the confounding effects of several variables using multivariate Cox regression analysis, the TAC group had a significantly reduced risk of acute rejection compared with the CsA group. This difference may have resulted from the relatively small sample size.

Despite this favorable effect of TAC therapy on acute rejection, both groups had comparable graft survival. The long-term outcome of transplantation may be affected by nonimmunologic factors such as hyperlipidemia, hypertension and infection as well as immunologic factors [28,29]. In particular, hypertension and hyperlipidemia may contribute to chronic allograft nephropathy [30,31]. Although maintenance therapy with TAC increases the rate of development of PTDM, it has been suggested that TAC causes less hypertension and hyperlipidemia than CsA, which may contribute to graft survival [23,26,32,33]. However, in our study, the incidence of hypertension did not differ between the groups. Furthermore, the annual changes in the levels of total cholesterol and LDL cholesterol and the numbers of patients who received lipid-lowering agents did not differ significantly between the CsA and TAC groups. There is no obvious explanation for the dissimilarity between these results and those of previous studies. However, steroid withdrawal might have attenuated the hypertensive and hyperlipidemic response to CsA in our study [34,35]. Factors such as the absence of significant differences in the incidence of hypertension and hyperlipidemia and the higher incidence of PTDM in the TAC group compared with the CsA group may have attenuated the favorable effect of TAC therapy on graft survival.

Registry data show that the overall incidence of PTDM within the first year after renal transplantation is 15–20% [36] and that it is greater in TAC-treated patients than in CsA-treated patients [37]. Some reports showed a significant improvement in glucose metabolism after conversion from TAC to CsA in TAC-treated renal transplant recipients with PTDM [38]. In our study, although steroids were withdrawn 6 months after transplantation, TAC treatment was associated with a higher rate of PTDM than CsA treatment (19.4% vs. 7.3% respectively; $P = 0.05$). This suggests that the effect of TAC on the glycemic status of patients persists after steroid withdrawal, probably because TAC affects insulin secretion [39]. Although age over 50 years was a significant risk factor for PTDM after adjusting for the confounding effects of factors such as age, gender, BMI and family history of diabetes, the mean age of recipients was not significantly different between the CsA and TAC groups. Recently, several reports were published on the association between hepatitis C infection and PTDM, especially in patients treated with TAC [40,41]. Because we initially excluded recipients with hepatitis C infection from this study, we investigated the prevalence of newly developed hepatitis C infection after transplantation in both groups. However, none of the recipients in either group had newly developed hepatitis C infection during follow up (data not shown).

The prevalence of avascular necrosis (AVN) was 10–20% before the introduction of cyclosporine [42]. The use of cyclosporine reduced the cumulative steroid dosage and consequently reduced the incidence of AVN to 5% [43]. AVN is more common in patients receiving CsA than in patients receiving TAC [44]. In our study, 5.5% (3/55) of patients in the CsA group and 1.6% (1/62) of those in the TAC group experienced AVN. Although the incidence of AVN was greater in the CsA group, the difference between groups was not statistically significant. We could not investigate subclinical AVN abnormalities because imaging was limited to recipients who displayed clinical signs and symptoms of AVN.

In conclusion, in low-risk patients, there was no difference in long-term graft or patient survival between CsA- and TAC-based steroid withdrawal regimens that included MMF treatment. However, after steroid withdrawal, some patients of both groups developed acute rejection, which predisposes towards graft failure [45]. Therefore, further studies on means of identifying recipients of steroid withdrawal regimens who have a low risk of acute rejection are needed.

Authorship

YJL: collected the data, performed the analyses and wrote the manuscript. BK: contributed to the data collection and writing of the manuscript. JEL: contributed to the collection and interpretation of the data. YGK: contributed to the collection of data. DJK: contributed to the collection of data. SJK: designed the study and collected the data. JWJ: contributed to the study design. HYO: contributed to the study design and the data collection. WH: designed the study, performed the analyses and

contributed to the interpretation of the data and writing of the manuscript.

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