Clinical Experience of Late Conversion From Antimetabolites With Standard Exposure Calcineurin Inhibitors to Everolimus With Calcineurin Inhibitor Minimization in Stable Kidney Transplant Recipients With Good Renal Function

J. Uchida, T. Iwai, N. Kuwabara, K. Kabei, S. Nishide, T. Yamasaki, T. Naganuma, N. Kumada, Y. Takemoto, T. Nakatani

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Clinical experience of late conversion from antimetabolites with standard exposure calcineurin inhibitors to everolimus with calcineurin inhibitor minimization in stable kidney transplant recipients with good renal function

Junji **Uchida**¹, Tomoaki **Iwai**¹, Nobuyuki **Kuwabara**¹, Kazuya **Kabei**¹, Shunji **Nishide**¹, Takeshi **Yamasaki**¹, Toshihide **Naganuma**¹, Norihiko **Kumada**², Yoshiaki **Takemoto**¹, Tatsuya **Nakatani**¹

¹Department of Urology, Osaka City University Graduate School of Medicine, Osaka, Japan

²Department of Urology, Suita Municipal Hospital, Suita, Japan

E-mail addresses: Junji Uchida: m9492120@msic.med.osaka-cu.ac.jp, Tomoaki Iwai: iwai@med.osaka-cu.ac.jp, Nobuyuki Kuwabara: m0738487@msic.med.osaka-cu.ac.jp, Kazuhiro Kabei: a03ma017@hotmail.co.jp, Shunji Nishide: nishide115s@yahoo.co.jp, Takeshi Yamasaki: m2049447@med.osaka-cu.ac.jp, Toshihide Naganuma: m9643361@msic.med.osaka-cu.ac.jp, Norihiko Kumada: cacaz904@hcn.zaq.ne.jp, Yoshiaki Takemoto: ytakemoto@msic.med.osaka-cu.ac.jp, Tatsuya Nakatani: nakatani@med.osaka-cu.ac.jp

Correspondence to: Junji **Uchida**, MD, PhD, Department of Urology, Osaka City University Graduate School of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka, 545-8585, Japan
Phone: +81-6-6645-3857 Fax: +81-6-6647-4426 E-mail: m9492120@msic.med.osaka-cu.ac.jp

Abstract

Introduction

The aim of this study was to describe our clinical experience of late conversion from antimetabolites with standard exposure calcineurin inhibitors (CNIs) to everolimus with CNI minimization in stable kidney transplant recipients with good graft function.

Patients and Methods

A 1-year retrospective pilot study of 26 kidney recipients converted from antimetabolites with standard exposure CNIs to everolimus with CNI minimization was performed. The recipients enrolled in this study had normal or slightly impaired renal function defined as a serum creatinine (S-Cr) value <2.0 mg/dl, and normal or slightly increased albuminuria defined as a urinary albumin excretion rate <100 mg/g Cr.

Results

The median time from transplant to conversion was 39.5 months, with a range from 3 to 275 months posttransplant. Treatment with everolimus was stopped due to adverse events in 11 patients (42.3%). In the analysis of the patients in whom everolimus was maintained, the mean estimated glomerular filtration rate (eGFR) significantly increased from 50.7 ± 11.9 ml/min 1.73m^2 at baseline to 53.6 ± 13.9 ml/min 1.73m^2 at 1 year after conversion. In the patients in whom everolimus was stopped during the observation period, there was no difference in eGFR

between baseline and 1 year after conversion.

Conclusions

This study demonstrated that among the patients converted to everolimus at a late stage, there was no deterioration in renal function whether everolimus was maintained or stopped within 1 year after conversion.

Introduction

Despite improvements in immunosuppressive therapy, long-term allograft survival is a major challenge in kidney transplantation [1]. Calcineurin inhibitors (CNIs), cyclosporin (CsA) and tacrolimus (Tac), are the cornerstones of immunosuppressive management in kidney transplant recipients even though their nephrotoxicity may induce chronic allograft nephropathy, which is histologically described as interstitial fibrosis and tubular atrophy. Chronic allograft nephropathy is the main cause of graft failure [2][3], while CNIs are known to predispose to the development of malignancies and cardiovascular disease [4][5]. Efforts to reduce CNI exposure are therefore necessary in order to achieve long-term allograft survival.

Everolimus is a mammalian target of rapamycin (mTOR) inhibitor, providing safe and effective immunosuppression after kidney transplantation. Everolimus has been extensively evaluated in conversion strategies to improve renal function and reduce CNI-associated long-term toxicities [6]. Previous reports have suggested that mTOR inhibitor plus CNI minimization may be quite effective in preventing rejection and obtaining satisfactory renal function while reducing the number and severity of side effects [7]. It can be speculated that everolimus may be especially useful in long-term immunosuppressive maintenance protocols in combination with low-dose CNIs. In addition, everolimus has pleiotropic effects including antiproliferative [8], antineoplastic [9], antiviral [10], and antiatherosclerotic [11] properties,

which may provide benefits in transplant recipients.

Previously, the introduction of everolimus with the elimination of CNIs or a marked reduction of CNIs was found to have no overall benefit on renal function at a late posttransplant stage [12]. On the other hand, it has been reported that late conversion of kidney transplant recipients with good renal function from mycophenolate mofetil (MMF) with standard exposure CNIs to everolimus with very low exposure CNIs may induce an improvement in renal function [12][13].

The aim of this study was to describe our clinical experience with late conversion of stable kidney transplant recipients from antimetabolites [MMF or mizoribine (MZ)] with standard exposure CNIs to everolimus with CNI minimization during a 1-year period.

Patients and methods

Patients

This study analyzed retrospectively the efficacy and safety of late conversion of stable kidney transplant recipients from antimetabolites with standard exposure CNIs to everolimus with very low exposure CNIs as a 1-year pilot study. A total of 26 recipients of kidney transplantation at our institution were converted from antimetabolites to everolimus for 1 year ending in March 2013. The inclusion criteria for conversion were as follows: (1) at least 3

months after transplantation, (2) follow-up period ≥ 1 year (2) normal or slightly impaired renal function defined as a serum creatinine (S-Cr) value <2.0 mg/dl, (3) no acute rejection episodes for more than 3 months, and (4) normal or slightly increased albuminuria defined as a urinary albumin excretion rate (the ratio of spot urine albumin to Cr) <100 mg/g Cr. All patients were required to be receiving CNIs with antimetabolites and steroids. CsA or Tac was administered at a dose level that resulted in a blood trough level of 100-120 ng/ml (CsA) or 4-6 ng/ml (Tac). The dosage of MMF, MZ, and methylprednisolone was 1 g/day, 6 mg/kg body weight/day, and 4 mg/day, respectively, in all patients.

Protocols

The patients were switched from MMF 1 g/day to everolimus 1.5 mg/day in the patients who received CsA (CsA group) or everolimus 3.0 mg/day in the patients who received Tac (Tac group) with dose adjustments from 1 week onward to target an everolimus trough level of 3 to 8 ng/ml. Everolimus trough levels were assessed at 1 week and every month until 1 year after conversion. The CNI dose was reduced to 40-60% below baseline values with dose adjustments from 1 week onward to a target trough level of 25-50 ng/ml (CsA) or 2-4 ng/ml (Tac). Baseline doses of methylprednisolone were continued unaltered in all patients.

All adverse events and rejection episodes were collected. At baseline, clinical parameters including age, gender, cause of end-stage renal disease, duration of dialysis, time to

transplantation, donor type, and ABO-compatibility were collected. At baseline and at 1, 3, 6, and 12 months after conversion, fasting blood samples were obtained in the early morning for biochemical studies, including S-Cr, total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol, and trough levels of CNi. Estimated glomerular filtration rate (eGFR) was calculated using the modified Modification of Diet in Renal Disease equation using the new Japanese coefficient [14]. Urinary albumin excretion rate (the ratio of spot urine concentrations of albumin to creatinine) was measured at baseline and at 1, 3, 6, and 12 months after conversion. The effects of everolimus with CNi minimization on renal function, urinary albumin excretion, and lipid profiles were examined. All subjects provided informed consent prior to enrollment in this study, which was approved by the Human Ethics Committee of Osaka City University Hospital. All procedures were in accordance with the Helsinki Declaration of 1975.

Statistical analysis

All analyses and calculations were performed using the Stat View V Statistical System. The results were presented as median and ranges or mean values $\pm$ standard deviations for continuous variables and as the proportion for the categorical variables. Differences between the groups were examined with Student's t-test or Mann-Whitney U-test. Categorical variables were

compared using chi-squared analysis or Fisher's exact test. Changes were evaluated using paired t test or Wilcoxon test. Statistical significance was defined as $p < 0.05$.

Results

Baseline characteristics

The median age at transplant was 53 years, with a range between 23 and 74 years, and the median time from transplant to conversion was 39.5 months, with a range between 3 and 275 months posttransplant. Nineteen patients were under CsA treatment and 7 under Tac treatment. Twenty-five patients were receiving MMF and 1 was receiving MZ. At baseline before conversion, the mean total daily CsA and Tac doses were 128.5 ± 33.8 mg/day and 2.9 ± 1.3 mg/day, respectively. The mean baseline values of S-Cr and eGFR were 1.18 ± 0.31 mg/dl and 48.5 ± 10.9 ml/min/1.73m², respectively. Other clinical characteristics of the participants at baseline are reported in Table 1.

Adverse effects and discontinuation of everolimus

Treatment with everolimus was stopped due to adverse events in 11 patients (42.3%). Median time from conversion to discontinuation was 124 days, with a range between 77 and 271 days (Figure 1). Adverse events were categorized as drug related when they occurred after conversion, the most frequent of which were hypercholesterolemia (65.4%), peripheral edema

(38.5%), albuminuria (30.8%), aphthous stomatitis (30.8%), general fatigue (30.8%), anemia (3.8%), menoxenia (11.5%), interstitial pneumonitis (7.7%), and acne (3.8%). Seven patients with general fatigue, two with interstitial pneumonitis, 1 with peripheral edema, and 1 with menoxenia were led to discontinuation of everolimus and reversion to MMF or MZ. In all cases, no apparent cellular or humoral rejection was observed during the observation period after conversion to everolimus. None of the cases received episode biopsies.

Comparison between the everolimus continuation and discontinuation group

1) Baseline data

The patients in whom everolimus was stopped (everolimus discontinuation group) were significantly older than those in whom everolimus was maintained (everolimus continuation group) (62.6 ± 13.4 vs 49.4 ± 11.5 years old, $p=0.0124$). Urinary albumin excretion in the everolimus discontinuation group was significantly greater than that in the everolimus continuation group. However, the average urinary albumin excretion rate was in the range of normoalbuminuria. There were no significant differences in gender ratio, dialysis duration, baseline renal function, cyclosporine/tacrolimus ratio, dose of CNIs, trough levels of CNIs, donor type, ABO compatible/incompatible ratio, and posttransplant duration between the two groups (Table 2).

2) Renal function (Figure 2)

In the analysis of the patients in the everolimus continuation group, the mean S-Cr level significantly decreased from 1.15 ± 0.29 mg/dl at baseline to 1.03 ± 0.26 mg/dl at month 1, 1.06 ± 0.28 mg/dl at month 3, 1.08 ± 0.28 mg/dl at month 6, and 1.09 ± 0.29 ml/dl at month 12. The mean eGFR value significantly increased from 50.7 ± 11.9 ml/min 1.73m^2 at baseline to 56.8 ± 12.9 ml/min 1.73m^2 at month 1, 55.8 ± 15.7 ml/min 1.73m^2 at month 3, 54.4 ± 14.9 ml/min at month 6, and 53.6 ± 13.9 ml ml/min 1.73m^2 at month 12. In the patients in the everolimus discontinuation group, the mean S-Cr level significantly decreased from 1.22 ± 0.33 mg/dl at baseline to 1.11 ± 0.33 mg/dl at month 1 and 1.12 ± 0.32 mg/dl at month 3. However, there were no differences in S-Cr levels between baseline and month 6 or month 12 after conversion. The mean eGFR value significantly increased from 45.5 ± 9.2 ml/min 1.73m^2 at baseline to 50.5 ± 10.0 ml/min 1.73m^2 at month 1 and 49.9 ± 10.7 ml/min 1.73m^2 at month 3. There were no differences in eGFR between baseline and month 6 or month 12 after conversion.

3) *Albuminuria*

In the analysis of the patients in the everolimus continuation group, there were no differences in urinary albumin excretion between baseline and month 1, 3, 6, and 12 after conversion. In the patients in the everolimus discontinuation group, the mean urinary albumin excretion rate significantly increased from 24.2 ± 14.8 mg/dl at baseline to 38.7 ± 33.8 mg/dl at month 1 and 46.4 ± 35.8 mg/dl at month 3. However, there were no differences in urinary

albumin excretion between baseline and month 6 and 12 after conversion.

4) Changes in doses and trough concentrations of everolimus and CNIs

In the everolimus continuation group, the mean everolimus dose in the CsA group and Tac group was 1.75 ± 0.68 and 1.90 ± 0.65 mg/day, respectively, at month 1, 1.80 ± 0.67 and 1.90 ± 0.68 mg/day, respectively, at month 3, 1.70 ± 0.75 and 1.80 ± 0.76 mg/day, respectively, at month 6, and 1.50 ± 0.67 and 1.70 ± 0.84 mg/day, respectively at month 12. The mean everolimus concentration in the CsA group and Tac group was 4.97 ± 1.24 and 5.60 ± 2.11 ng/ml, respectively, at month 1, 5.44 ± 1.38 and 5.29 ± 1.83 ng/ml, respectively, at month 3, 5.03 ± 1.40 and 4.86 ± 1.09 ng/ml, respectively, at month 6, and 4.60 ± 0.80 and 4.68 ± 1.13 ng/ml, respectively, at month 12. The mean CsA and Tac trough concentration was 102.5 ± 28.5 and 3.9 ± 1.3 ng/ml, respectively, at baseline, 42.4 ± 11.9 and 3.0 ± 0.7 ng/ml, respectively, at month 1, 43.8 ± 16.2 and 2.7 ± 0.6 ng/ml, respectively, at month 3, 31.9 ± 7.4 and 3.7 ± 1.4 , respectively, at month 6, and 33.6 ± 6.3 and 3.04 ± 0.6 ng/ml, respectively, at month 12. The mean CsA and Tac trough concentration decreased by 32.8% and 77.9%, respectively, from baseline to at month 12.

In the everolimus discontinuation group, the mean everolimus dose in the CsA group and Tac group was 1.75 ± 0.60 and 2.67 ± 0.58 mg/day, respectively, at month 1, and 1.86 ± 0.67 and 2.67 ± 0.58 mg/day, respectively, at month 3. The mean everolimus concentration in the CsA group and Tac group was 5.11 ± 1.78 and 4.61 ± 0.96 ng/ml, respectively, at month 1, and

4.89±1.27 and 4.40±1.17 ng/ml, respectively, at month 3. The mean CsA and Tac trough concentration was 100.1±20.7 and 4.9±1.5 ng/ml, respectively, at baseline, 38.1±17.0 and 3.4±1.0 ng/ml, respectively, at month 1, and 40.9±17.0 and 2.0±0.2 ng/ml, respectively, at month 3.

There were no significant differences in everolimus trough concentrations and CNI trough concentrations between the everolimus continuation group and discontinuation group.

Discussion

Our study showed that late conversion to everolimus with CNI minimization at a median of 39.5 months after kidney transplantation induced discontinuation of everolimus due to adverse events in 42.3% of the recipients. However, there was no deterioration in renal function in the patients whether everolimus was maintained or stopped within 1 year after conversion. Moreover, no apparent cellular or humoral rejection was observed during the observation period after conversion to everolimus. This study suggested that there may be no disadvantage to the graft kidney even if the kidney transplant recipient with good graft function is converted from antimetabolites to everolimus at a late posttransplant stage in expectation of avoiding the adverse effects of MMF, such as gastrointestinal complications, or reducing CNI-associated long-term toxicities.

Previous reports have demonstrated the possibility that late conversion (≥ 3 years after transplantation) to everolimus and conversion of patients with high baseline proteinuria induces a decline in graft function and a poor prognosis for graft survival [15]. The main objective of conversion to everolimus with CNI minimization or CNI elimination in patients with deteriorating renal function is stabilization, rather than a significant improvement in renal function for kidneys with established chronic irreversible lesions [15]. The ASCERTAIN study revealed that in kidney transplant recipients who were, on average, 5 years posttransplant, introduction of everolimus with elimination of CNIs or a marked reduction of CNIs had no overall benefit on renal function and was associated with more frequent adverse events and discontinuations. However, that study identified that patients with a creatinine clearance of more than 50 ml/min may benefit from a change in therapy more than 6 months after transplantation [12]. In our present study, the graft function in the patients in whom everolimus was maintained at late conversion was significantly improved compared with the baseline graft function. Moreover, the graft function of even those recipients who later experienced discontinuation of everolimus improved temporarily during the administration of everolimus. Our study is a pilot trial. Because of the small sample size, we could not show the effects of everolimus discontinuation on renal function by multivariate analysis. However, in cases of grafts that have normal renal function with considerably less proteinuria and are considered to receive little

chronic allograft damage, late conversion to everolimus might be speculated to improve graft function if everolimus is not discontinued.

It was previously reported that the adverse effects of mTOR inhibitors accounted for 20-40% of the drop-out rate in a clinical Phase III trial [16]. By late conversion to everolimus even in recipients with good graft function, some adverse events of mTOR inhibitors may occur at the same rate as previous reports. In this study, the patients in whom everolimus was later discontinued due to everolimus-induced adverse effects were significantly older than those in whom everolimus was continued. However, there are no available reports on the application of everolimus in elderly recipients older than 60 years of age, as conversion from antimetabolites with standard exposure CNIs to everolimus with CNI minimization in elderly patients have not been well studied yet. Late conversion to everolimus and CNI minimization in elderly recipients may be associated with more frequent adverse events and discontinuations, although we could not identify this by multivariate analysis. ABO-incompatible kidney transplantation may be a viable treatment option for elderly patients with end-stage renal disease [17].

There is a genuine proteinuria-causing effect of mTOR inhibition [18]. In this study, although urinary albumin excretion was slightly elevated after conversion to everolimus in the patients in whom everolimus was stopped within 1 year, the average urinary albumin excretion rate was in the range of microalbuminuria (the lower limit of microalbuminuria). In the patients

in whom everolimus was maintained, there was no difference in urinary albumin excretion during the observation period. Late conversion to everolimus and CNI minimization may therefore be associated with very little elevation of albuminuria in recipients who have good graft function with normoalbuminuria.

Apart from the immunosuppressive effects such as prevention of T cell proliferation and increased expression of T regulation, the beneficial effects of mTOR inhibitors in kidney transplantation are antioncogenic activity [9], antiviral activity [10], and cardioprotective effects [11]. Moreover, it has been identified that everolimus can prevent allograft vasculopathy caused by smooth muscle cell proliferation in the arterial intima [8][19]. To achieve long-term patient and graft survivals, everolimus may be helpful in protecting transplant recipients from the risk of cancer and cardiovascular disease, and from progression of chronic allograft damage. Before chronic and irreversible damage occur in the transplanted kidneys, conversion to everolimus and CNI minimization may lead to a favorable prognosis of long-term patient and graft survivals.

For the application of everolimus in kidney transplantation at a late phase, two acceptable approaches have been proposed: everolimus with CNI minimization and everolimus with an antimetabolite and CNI elimination. A CNI elimination regimen may probably allow a better renal function, but the need of maintaining relatively high doses of mTOR inhibitors and the association with MMF may induce a number of adverse events [19]. Moreover, this strategy

may also increase the risk of developing donor-specific antigen and antibody-mediated rejection [20]. A previous report showed that everolimus with CNI minimization may be quite effective in preventing rejection and obtaining satisfactory renal function while reducing the number and severity of side effects [7]. In this study, although late conversion of the recipients with good graft function to everolimus induced discontinuation of everolimus due to adverse events in about 40% of recipients, there was at least no deterioration in graft function or clinical rejection.

The present study might have limitations because of the small sample size and the fact that it is a retrospective study. Because of the sample size, multivariate analysis could not be performed. However, although this is a pilot study, it is the first demonstration of late conversion of recipients with good graft function from antimetabolites with standard CNI exposure to everolimus with CNI minimization. Moreover, the possibility that late conversion to everolimus and CNI minimization in elderly recipients may be associated with more frequent adverse events and discontinuations was identified.

In conclusion, although late conversion to everolimus with CNI minimization is associated with a high incidence of discontinuation of everolimus due to adverse events, there was no deterioration in renal function or clinical rejection in our patients whether everolimus was maintained or stopped within 1 year after conversion. Our results suggested that there may be no disadvantage to the graft kidney even if an attempt is made to convert the kidney

transplant recipient with good graft function from antimetabolites to everolimus at a late posttransplant stage in expectation of avoiding MMF toxicity or reducing CNI-associated long-term toxicities.

There are no financial or commercial interests to disclose.

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Table 1. Patient characteristics

Number of patients	26
Gender (male/female)	15/11
Cause of ESRD	Chronic glomerular nephritis; 7, IgA nephropathy; 3, Diabetes nephropathy; 3, Renal sclerosis; 2, Others; 11.
Age at transplant (year)	53 (23-74)
Dialysis duration (months)	45 (0-340)
Posttransplant duration (months)	39.5 (3-275)
Age at conversion to everolimus (year)	59 (29-76)
Donor type (living/deceased)	22/4
ABO-compatibility (compatible/incompatible)	9/17
Calcineurin inhibitor (cyclosporin/tacrolimus)	19/7
Antimetabolite (mycophenolate mofetil/mizoribine)	25/1
Serum creatinine (mg/dl)	1.18±0.31
Estimated glomerular filtration rate (ml/min/1.73m ²)	48.5±10.9
Urinary albumin excretion (mg/g Cr)	17.9±11.8
Total cholesterol (mg/dl)	199.3±29.5
Low density lipoprotein cholesterol (mg/dl)	103.2±21.4
High density lipoprotein cholesterol (mg/dl)	66.3±16.9
Triglyceride (mg/dl)	115.5±43.1

ESRD; end-stage renal disease.

Table 2. Comparison between everolimus continuation and discontinuation group

	Everolimus continuation group	Everolimus discontinuation group
Number of patients	15	11
Gender (male/female)	8/7	7/4
Age at transplant (year)	48 (28-59)	59 (23-74) *
Dialysis duration (months)	40 (2-340)	45 (0-244)
Posttransplant duration (months)	32 (3-275)	46.5 (15-76)
Age at conversion to everolimus (year)	52 (28-64)	64 (29-76)
Donor type (living/deceased)	12/3	10/1
ABO-compatibility (compatible/incompatible)	6/9	3/8
Serum creatinine at conversion (mg/dl)	1.15±0.29	1.22±0.34
Estimated glomerular filtration rate (ml/min/1.73m ²)	50.7±11.9	45.5±9.2
Urinary albumin excretion (mg/g Cr)	13.3±6.3	24.2±14.8 *
Dose of CNI at conversion (mg/day)	Cyclosporin; 137.5±39.7	Cyclosporin; 121.3±23.6
	Tacrolimus; 2.6±1.1	Tacrolimus; 3.2±1.8
	Cyclosporin; 102.5±20.7	Cyclosporin; 102.5±28.5
Trough level at conversion (ng/ml)	Tacrolimus; 3.9±1.3	Tacrolimus; 3.9±1.3

*p<0.05 Everolimus continuous group vs Everolimus discontinuous group

Figure legends

1. Kaplan-Meier curve showing probability of freedom from discontinuation of everolimus administration

2. Change in renal function. (a) %change in serum creatinine (b) %change in estimated glomerular filtration rate