Conversion from Calcineurin Inhibitors to mTOR Inhibitors in Renal Transplantation: A Single Centre Experience

Renal Transplantasyonda Kalsinörin İnhibitörlerinden mTOR İnhibitörlerine Geçiş: Tek Merkez Deneyimi

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ABSTRACT

OBJECTIVE: Mammalian target of rapamycin (mTOR) inhibitors are among the immunosuppressive drugs used in renal transplantation. The aim of this study was to reveal our experiences in conversion from calcineurin inhibitors (CNI) to mTOR inhibitors in 20 renal transplant patients.

MATERIAL and METHODS: Various protocols were used in the conversion from CNIs to mTOR inhibitors. CNIs were discontinued and mTOR inhibitors were initiated in patients with malignancy. In cases of CNI toxicity and in cases in which conversion was performed for other causes, reduced doses of CNIs were administered for three days in combination with mTOR inhibitors.

RESULTS: The study included 20 renal transplant patients, of whom 14 were male and 6 were female. The mean age of the patients was 43.5±8.8 years. The reason for conversion was CNI toxicity in 16 patients (80%), malignancy in 3 patients (15%), and premalignant lesion in 1 patient (5%). Conversion to mTOR inhibitors [(sirolimus (n=14) and everolimus (n=6)] was performed at 62.6±45.7 months after transplantation. The mean follow-up period after administration of mTOR inhibitors was 50.5±29.9 months. The mean proteinuria at the time of conversion was 227.5±147.9 mgr/day and increased to 636.5±388.2 mgr/day after treatment with mTOR inhibitors ($p<0.001$). There was an increase in the creatinine levels after treatment with mTOR inhibitors, though it was not significant ($p=0.126$). Everolimus was not discontinued in any patient, but sirolimus had to be discontinued in three patients that was due to proteinuria in two patients and pneumonia in one patient.

CONCLUSION: Conversion from CNIs to mTOR inhibitors may cause side-effects. When basal renal functions are good and when proteinuria is mild, conversion may result in fewer side effects.

KEY WORDS: Calcineurin inhibitors, Everolimus, mTOR inhibitors, Renal transplantation, Sirolimus

ÖZ

AMAÇ: mTOR inhibitörleri (mTOR i) renal transplantasyonda tercih edilen immünsupresiflerdendir. Bu çalışmada, kalsinörin inhibitörleri (KNI) kesilerek mTOR i'nin başlandığı, 20 olgudan oluşan, böbrek nakilli hastalardan elde ettiğimiz deneyimler sunulacaktır.

GEREÇ ve YÖNTEMLER: KNI'dan mTOR i'ye geçiş protokolünde farklı yöntemler uygulanmıştır. Maligniteli hastalarda KNI birden kesilerek mTOR i'ye başlanırken KNI toksisitesi olan ve diğer nedenlerle dönüşüm yapılan olgularda ise KNI dozu azaltılarak mTOR i ile birlikte 3 gün verilmiştir.

BULGULAR: Renal transplantlı 20 hastanın yaş ortalaması 43,5±8,8 yıl olup; 14 erkek 6 kadın hastadan oluşuyordu. Geçiş nedenleri; maligniteler (n=3, %15), KNI toksitesi (n=16, %80) ve premalign lezyon (n=1 %5) idi. mTOR i'ye geçiş (14 hasta sirolimus, 6 hasta everolimus) transplantasyondan ortalama 62,6±45,7 ay sonra yapıldı. mTOR i sonrası ortalama takip süresi 50,5±29,9 aydır. Tedavi değişimi sırasında proteinüri miktarı ortalama 227,5±147,9 mgr/gün iken, mTOR i tedavisi sonrası bu değer artarak ortalama 636,5±338,2 mgr/gün oldu ($p<0,001$). mTOR i tedavisi ile kreatinin düzeylerinde artış olmasına rağmen artış istatistiksel olarak anlamlı değildi ($p=0,126$). Sirolimus tedavisi 3 hastada kesilmek zorunda kalındı. Nedenleri 2 hastada proteinüri, 1 hastada sirolimusa bağlı interstisyel pnömoni şeklindeydi.

SONUÇ: KNI tedavisinden mTOR i tedavisine konversiyonda yan etkiler görülebilmektedir. Konversiyon yapılacak hasta seçiminde bazal renal fonksiyonların iyi olması ve proteinüri miktarının az olması yan etki sıklığını azaltabilir.

ANAHTAR SÖZCÜKLER: Everolimus, mTOR inhibitörleri, Kalsinörin inhibitörleri, Renal transplantasyon, Sirolimus

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INTRODUCTION

Currently the most frequently preferred immunosuppressive treatment regimen after renal transplantation includes calcineurin inhibitors (CNI), mycophenolate mophetil (MMF) and steroids. This regimen has positive effects on early graft survival, but the long term graft functions are not satisfactory (1). Primary causes of long-term graft losses are immunological factors, non-immunological factors (post-transplant diabetes, dyslipidemia, hypertension and obesity), opportunistic infections due to excessive immunosuppression and malignancies (2). CNIs cause chronic vasculopathy and interstitial fibrosis that result in chronic renal failure (3). Due to these untoward effects; CNI avoidance, CNI withdrawal or low-dose CNI maintenance regimens have been introduced. Combination of antiproliferative agents with mammalian target of rapamycin (mTOR) inhibitors is used as a CNI-sparing regimen.

mTOR inhibitors prevent progression of the cell cycle from the G1 phase to the S phase and exert an anti-proliferative effect. Sirolimus is a macrocyclic lactone antibiotic derived from *Streptomyces hygroscopicus*. Similar to tacrolimus; both everolimus and sirolimus bind to immunophilin protein. The drug-receptor complex then binds to mTOR. This causes serine-threonine kinase inhibition and cessation of the cell cycle, stopping DNA and protein syntheses (4). Sirolimus has in vitro antiproliferative effects on smooth muscle cells, fibroblasts and endothelial cells and has been shown to reduce hyperplasia in vascular damage models (5). Sirolimus has been observed to prevent myointimal proliferation in experimental studies using coronary angioplasty models (6). Similarly, MMF exhibits an anti-proliferative effect by inhibiting *de novo* purine synthesis and thus inhibits proliferation of smooth muscle cells, mesenchymal cells, fibroblast and collagen deposition. In one study, MMF was shown to reduce TGF expression and pathological effects of cyclosporine on arterial walls (7-8). Inhibition of smooth muscle and fibroblast proliferation by mTOR inhibitors and MMF suggests that they can have complimentary immunosuppressive effects when used in combination. The aim of this study was to reveal our experiences in conversion from CNIs to mTOR inhibitors in 20 renal transplant patients.

MATERIAL and METHODS

This was a retrospective study. The study population included all renal transplant recipients that were regularly followed at Ankara Diskapi Yildirim Beyazit Training and Research Hospital and in whom CNI treatment was switched to mTOR inhibitors during routine transplant care. There were no exclusion criteria. Demographic characteristics, primary kidney diseases, transplantation-related parameters (donor type, transplantation duration and doses and types of immunosuppressive drugs) and laboratory results were recorded from patient files. Various protocols were used in the conversion from CNIs to mTOR inhibitors. CNIs were discontinued and mTOR inhibitors were initiated in patients with malignancy. In cases of CNI toxicity and in cases in which conversion was performed for other causes, reduced doses of CNIs were administered for three days in combination with mTOR inhibitors. CNI toxicity was diagnosed based on clinical symptoms, drug levels and allograft biopsy. When used together with CNIs, blood levels of mTOR inhibitors were targeted as 4-12 ng/ml for sirolimus and 3-8 ng/ml for everolimus. All patients were informed about the potential side effects of mTOR inhibitors before the switch in immunosuppressive regimen.

Statistical analysis

For statistical analysis, SPSS (Statistical Package for Social Sciences) for Windows 16.0 was used. The Kolmogorov-Smirnov/Shapiro-Wilk tests were used to determine whether obtained data were normally distributed. Results of descriptive statistics were expressed as mean ± SD. The paired sample t test was used to determine differences between measurements before and after the change in treatment regimen.

RESULTS

The study included 20 renal transplant patients, of whom 14 were male and 6 were female. The mean age of the patients was 43.5±8.8 years. The main causes of end stage renal disease were hypertension (5 patients), nephrolithiasis (4 patients) and glomerulonephritis (4 patients). The etiology of renal failure was unknown in 7 patients. Transplantations had been performed from cadaveric donors in four cases and from live donors in 16 cases. Initial immunosuppressive regimens were as follows: cyclosporine A (CsA)+ azatioprine (AZA)+ prednisolone (PRD) (n=6), CsA+ MMF +PRD (n=5), tacrolimus (TAC) + AZA +PRD (n=2), TAC+MMF+PRD (n=7). The dose of MMF was 2 gr/day in all patients. There was no patient under statin or angiotensin converting enzyme inhibitor (ACEI) treatment.

The reason for conversion was CNI toxicity in 16 patients (80%), malignancy in 3 patients (15%), and premalignant lesion in 1 patient (5%). CNI toxicity was diagnosed by allograft biopsy in 12 patients and on clinical grounds in 4 patients. Conversion to mTOR inhibitors [(sirolimus (n=14) and everolimus (n=6)] was performed at 62.6±45.7 months after transplantation. The mean follow-up period after administration of mTOR inhibitors was 50.5±29.9 months. The mean proteinuria at the time of conversion was 227.5±147.9 mgr/day and increased to 636.5±388.2 mgr/day after treatment with mTOR inhibitors ($p<0.001$). During a mean follow-up of 50.5 months, 10 patients developed *de novo* proteinuria. The mean creatinine level was 1.96±0.43 gr/dL at the time of conversion and 2.48±1.73 gr/dL after treatment with mTOR inhibitors. There was an increase in the creatinine levels after treatment with mTOR inhibitors, though it was not significant (p=0.126) (Table I).

There was an increase in serum cholesterol and triglyceride levels after conversion but this was not statistically significant (p=0.130 and p=0.134 respectively). There was no difference in leukocyte count, hemoglobin and platelet levels before and

Table I: Laboratory parameters before and after mTOR i.

Parameter	Before-mTOR inhibitor	After-mTOR inhibitor	p
Creatinine (mgr/dl)	1.96±0.43	2.48±1.73	p=0.126
Urea (mg/dL)	62.1±26.9	68.2±32.6	p=0.440
Glucose (mg/dL)	92.5±12.7	81.7±12.8	p=0.007
Protein (gr/dl)	7.1±0.5	6.8±0,6	p=0.052
Albumin (gr/dl)	4.1±0.3	4.1±0.4	p=0.433
Cholesterol (mgr/dl)	183.4±40.5	200.1±38.0	p=0.130
Triglycerides (mgr/dl)	137.4±62.5	163.4±72.0	p=0.134
Wbc (x$10^3/\mu$L)	7.4±2.7	7.2±1.6	p=0.839
Hgb (g/dL)	12.4±1.6	11.9±2.7	p=0.415
Platelet (x$10^3/\mu$L)	251.4±64.7	252.5±84.6	p=0.958
Proteinuria (mgr/day)	227.5±147.9	636.5±388.2	p<0.001

Wbc: White blood cells, **Hgb:** Hemoglobin.

after conversion (p=0.839, p=0.415 and p=0.958 respectively). No patient developed lymphedema after the switch.

Everolimus was not discontinued in any patient but sirolimus had to be discontinued in three patients. The discontinuation reason was proteinuria in two patients (3734 mg/day and 2129 mg/day respectively) that appeared in the 5th month and 14th month respectively. Pneumonia was the reason in the other patient. No drug-related adverse effect was observed. One patient died and one patient returned to dialysis under everolimus treatment during the follow-up period.

DISCUSSION

The most common use of mTOR inhibitors in renal transplantation is in patients with chronic allograft nephropathy or in patients with stable renal functions to prevent calcineurin toxicity.

It is recommended that conversion to mTOR inhibitors should be performed 6-12 months after transplantation (9). In our study, the conversion from CNIs to mTOR inhibitors in 20 renal transplant patients was carried out 62.6 months after transplantation. The reason for conversion was CNI toxicity in 16 patients (80%), malignancy in 3 patients (15%), and premalignant lesion in 1 patient (5%). Renal cell carcinoma, gastric lymphoma and angiosarcoma were the malignancies that resulted in conversion. The patient with angiosarcoma died eighth months after conversion and the other two patients are still on mTOR inhibitor treatment. The premalignant lesion for which conversion to mTOR inhibitors was performed was a BIRADS3 cystic lesion in the breast of a female patient.

Common adverse effects of mTOR inhibitors are bone marrow suppression and hyperlipidemia. It is thought that the mTOR pathway is important in terms of erythroid cell replication and that mTOR inhibitors create resistance at erythropoietin receptor levels. Studies on immunosuppressive therapy with sirolimus/MMF have shown that 43%-68% of the patients have anemia and that 20%-45% of the patients have leucopenia and thrombocytopenia (10-11). In the present study, the mean follow-up period after treatment with mTOR inhibitors was 50.5±29.9 months and no hyperlipidemia or bone marrow suppression was observed. Conversion to mTOR inhibitors was mostly performed due to CNI toxicity (16 of 20 patients). There was a progressive deterioration in the renal functions of most patients before conversion. We can speculate that mTOR inhibitors caused stabilization and prevented further deterioration in renal function since there was no significant difference between pre and post switch creatinine levels (p=0.126).

Everolimus was not discontinued in any patient. A single patient returned to hemodialysis and died in the eighth month of everolimus treatment. However sirolimus had to be discontinued in three patients due to proteinuria in two patients and pneumonia in one patient. Direct or indirect proximal tubular cell damage due to loss of vasoconstriction caused by CNIs, increased glomerular filtration and binding of sirolimus to albumin (12) are implicated as the causes of increase in proteinuria after conversion from CNIs to sirolimus. It has been shown that proteinuria considerably increases in patients with chronic allograft damage and already increased protein excretion when CNIs are discontinued and sirolimus is initiated (13). It has also been reported that the rate of *de novo* proteinuria due to mTOR inhibitors may reach 30% and proteinuria can be at nephrotic levels (14). Letavernier et al. have described focal segmental glomerulosclerosis-like lesions in some patients receiving *de novo* sirolimus (15).

It is recommended that mTOR inhibitors should not be used in patients with chronic allograft damage accompanied by proteinuria since they increase renal damage and reduce survival when proteinuria is above 800 mg/day (16). In the present study, the mean proteinuria was 210 mg/day initially.

Renin-angiotensin-aldosterone system (RAAS) blockers were prescribed to patients who developed proteinuria and mTOR inhibitors were discontinued in patients with persistent proteinuria >800 mg/day under RAAS blockade. Two patients developed proteinuria 1200 (mg/day) at the 5th and 14th months respectively. After transient proteinuria was excluded, ACEIs were started to both patients. However persistent proteinuria (3734 mg/day and 2129 mg/day respectively) necessitated stopping mTOR inhibitors in these patients.

Another cause of discontinuation of sirolimus was interstitial pneumonia in a patient at the 66th month of treatment in this study. CsA had been stopped and sirolimus had been prescribed due to

nephrotoxicity one month after transplantation in this patient. The patient had fever and nonproductive cough lasting for 10 days and was not found to have an infection focus and did not respond to empirical antibiotic therapy. There was peribronchial thickening in the superior and medial pulmonary zones and bilateral infiltration on high-resolution computed tomography (HRCT). The patient's complaints disappeared one week after discontinuation of sirolimus. There was also an improvement on HRTC.

Interstitial pneumonia is a rare but an important complication of sirolimus (17). It was first reported by Morelon in 2000 (18). Pneumonia due to sirolimus is more frequently seen in elderly patients who were initiated sirolimus after a CNI was discontinued in the presence of impaired renal functions (19). In the present study, no impairment in liver function tests or a skin lesion due to treatment with mTOR inhibitors was observed. During the follow-up period, one patient receiving everolimus died and another patient receiving everolimus resumed dialysis. The cause of death was complications due to malignity (angiosarcoma) in the eighth month of treatment with everolimus. Another patient on everolimus started to receive renal replacement therapy and hemodialysis.

A study on mTOR inhibitors with a large sample size (CONVERT) has reported that graft functions were better but proteinuria was higher in renal transplant patients administered sirolimus as a maintenance therapy compared to those whose treatment did not change from CNI to sirolimus during the 24-month follow-up; however, the rates of acute rejection, disease and survival were similar in both groups (20). In another study (SYMPHONY), comprising 1645 patients at 83 centers from 15 countries including Turkey, a CsA-MMF-steroid protocol, a standard immunosuppressive treatment, was compared with daclizumab induction-steroids-low dose of CsA, tacrolimus and sirolimus combinations. The patients on low dose of tacrolimus had the highest glomerular filtration rate and the lowest rate of rejection demonstrated on biopsy during one-year follow-up. There was no difference in survival rates between the groups (21). Results of these two studies suggested that mTOR inhibitors were not superior to CNIs and could not be primary drugs. In the present study, we did not observe side effects except for proteinuria and pneumonia during approximately 50 months of follow-up.

In conclusion mTOR inhibitors can be preferred especially in patients with CNI toxicity. Conversion from CNIs to mTOR inhibitors may cause side effects. When basal renal functions are good and proteinuria is mild, conversion may cause lower rates of side effects. Further studies are needed to determine outcomes of the sirolimus/MMF combination more clearly.

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