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Renal calcium handling after rapamycin conversion in chronic allograft dysfunction

Mai-Szu Wu, Cheng-Chieh Hung and Chiz-Tzung Chang

Department of Nephrology, School of Medicine, Chang-Gung University, Chang Gung Memorial Hospital, Keelung, Taiwan

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Correspondence
Dr Mai-Szu Wu, Division of Nephrology, School of Medicine, Chang-Gung University, Chang Gung Memorial Hospital, 222, Mai-Chin Road, Keelung, 204, Taiwan. Tel.: 886 2 24313131 (ext. 3169); fax: 886 2 24335342; e-mails: maxwu1@adm.cgmh.org.tw, maisuwu@yahoo.com

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Summary

To study the effect of rapamycin on calcium balance, we conducted a prospective study on transplant recipients. The patients were converted to rapamycin and observed for 6 months (C). Urinary Ca and P, ALK-p, Ca, P, and intact parathyroid hormone (iPTH) were examined before and 6 months after conversion. A nonconversion group (N) was found for comparison. Sixteen patients entered the study. There were increases of ALK-p (C: 67.4 ± 32.9 to 79.6 ± 37.0*; N: 67.3 ± 25.1 to 67.8 ± 14.7 IU) (*$P < 0.05$), Ca^{2+} (C: 8.7 ± 0.3 to 9.5 ± 0.2*; N: 8.8 ± 0.4 to 8.7 ± 0.5 mg/dl), urinary P excretion (C: 287.6 ± 257.1 to 439.4 ± 260.9*; N: 233.9 ± 117.2 to 264.8 ± 143.4 mg/day) and iPTH (C: 133.7 ± 149.6 to 200.6 ± 171.5*; N: 128.4 ± 57.1 to 136.3 ± 40.4 pg/ml). Serum P (C: 5.3 ± 1.4 to 3.6 ± 0.6*; N: 5.2 ± 0.8 to 5.1 ± 0.9 mg/dl) and urinary Ca^{2+} (C: 93.9 ± 52.6 to 31.8 ± 45.1*; N: 84.6 ± 38.3 to 75.9 ± 38.4 mg/day) were decrease. Rapamycin was associated with decreased urinary Ca^{2+} and increased P excretion. The alteration might come from the increased parathyroid hormone.

Introduction

Successful kidney transplantation corrects the abnormalities of mineral metabolism that lead to renal osteodystrophy. This includes correction of uremia, normalization of serum calcium and phosphorus levels, and restoration of calcitriol production. However, the renal function recovery might not be complete. In addition, the immunosuppressive drugs used to prevent graft rejection exert profound effects on bone metabolism [1]. For these reasons, disturbances of mineral metabolism and skeletal alterations are common causes of morbidity after kidney transplant [2]. The major clinical problems include hypercalcemia and persistent hyperparathyroidism, hypophosphatemia, post-transplantation bone loss and fractures, osteonecrosis, and bone pain syndromes [3].

Evaluating and managing post-transplant bone disease is an integral part of post-transplant medical care. Immunosuppression is a major cause of post-transplant bone disorders. Glucocorticoids lead to decreased bone formation [4], whereas the calcineurin inhibitors appear to cause the increased bone turnover [5]. New immunosuppressant drugs, which are more potent and less deleterious than cyclosporine (CsA), are being developed [6]. The effect of these agents on the calcium metabolism is still unclear.

Rapamycin (sirolimus) is a macrolide antibiotic produced by *Streptomyces hygroscopicus* [7]. Rapamycin is an effective immunosuppressant in preventing the acute allograft rejection [7]. The most attracting characteristic of the immunosuppressant is free of known nephrotoxicity [8]. Large-scale studies had suggested that rapamycin is effective in reducing the incidence of acute rejection in *de novo* therapy in kidney transplant either with or without Calcineurin inhibitor (CNI) in combination [9,10]. Dominguez [11] also reported a smooth conversion of CNI to rapamycin with a better subsequent allograft function in patients with frank CNI nephrotoxicity. Previous experiments have shown that sirolimus is relatively bone sparing [12] in rat. However, the exact effect of rapamycin on renal calcium handling is still unknown. It is interesting to know if rapamycin has an effect on renal calcium handling in kidney transplant recipients.

To answer this question, we conducted a prospective study in patients receiving cadaveric kidney transplant.

Rapamycin was given to replace CNI in patients under classic CNI, mycophenolate mofetil, and prednisolone triple therapy. Urinary excretion of calcium (Ca^{2+}) and phosphate (P), serum alkaline phosphatase (ALK-p), serum Ca^{2+}, serum P, and intact parathyroid hormone (iPTH) were examined before and 6 months after the rapamycin conversion.

Patients and methods

To define the renal calcium-handling effect of rapamycin in post-transplant patients, we conducted a prospective study of kidney transplant recipients in a university hospital (Chang-Gung Memorial Hospital, Taiwan). All of the included renal transplant recipients had a functional graft for more than 24 months. Twenty-four months were set to indicate a stable graft function. All of the included patients were initially under classic CNI, mycophenolate mofetil, and prednisolone triple therapy. All of them were converted from CNI to rapamycin directly and observed for 6 months in this study. The reasons for the conversion were because of CNI toxicity, including tremor, diabetes, gum hypertrophy, hirsutism, and hypertension. The patients with obvious CNI nephropathy were not included in the study for excluding the effect of graft function in the renal calcium handling. We converted the CNI directly to 2 mg/day rapamycin without overlapping period [13]. All of them had a trough-specific whole blood CsA level within 50–150 ng/ml or trough tacrolimus level within 5–10 ng/ml before converting to rapamycin. Patients with elevated CNI serum level were excluded from the study to exclude the obvious CNI nephropathy. The doses of mycophenolate mofetil and prednisolone were not changed. Serum creatinine levels, serum calcium, serum phosphate, ALK-p level, urine calcium (Ca^{2+}), urine phosphate, iPTH by radioimmunoassay (RIA), and adverse effects were evaluated before and after the rapamycin conversion. All the blood samples were fasting for 12 h. An experienced nurse instructed the 24 h urine collection.

An age, gender, transplantation duration and graft function-matched nonconversion group without converting to rapamycin was found to compare the effect of rapamycin conversion. All the studied patients were not taking the calcium supplement or VitD3 during the study period.

All the included patients had given their consensus to enter the study after throughout the explanation of the study.

Analytical Methods

Continuous variables are expressed as mean ± SD. For normally distributed continuous variables, ANOVA tests were employed to evaluate the difference between means of three groups. Paired student *t*-test was used to evaluate the difference before and after intervention in the same group. The major statistics was calculated on a personal computer using StatView 5.0 with Survival Tool (Abacus Concept Inc., Berkeley, CA, USA).

Results

Sixteen patients were studied in rapamycin conversion group. There are eight female and eight male in the study. The mean age at the start of study was 47.4 ± 8.4 year old (33–62 year old). The mean transplant duration was 8.0 ± 4.5 years (2.5–15 years). The underlying renal disease was chronic glomerulonephritis in all these 16 patients. Eight patients were on tacrolimus-based and the other eight patients were on CsA-based immunosuppressant regimens (Table 1). The mean CsA trough level was 89.5 ± 28.5 ng/ml (51.0–126.3 ng/ml) and the mean tacrolimus trough level was 6.9 ± 2.0 ng/ml (3.4–8.9 ng/ml) before the conversion. The trough levels of CNI were all within the recommended level [14]. An age, gender and graft function-matched controlled group was selected for comparison. Sixteen patients were found in the nonconversion group. The mean age was 47.6 ± 13.3 year old (27–72 year old) and the mean transplant duration was 8.2 ± 4.2 years (2.5–15.3 years) in nonconversion group. The underlying renal disease was chronic glomerulonephritis in all these controlled patients. Eight patients were on tacrolimus-based and the other eight patients were on cyclosporine-based immunosuppressant regimens in nonconversion group. The trough levels of CNI in control group were all within the recommended levels (CsA: 89.5 ± 28.5; tacrolimus 7.0 ± 1.9 ng/ml). There was no significant difference in age, gender, CNI serum levels and transplantation duration between conversion and nonconversion groups.

Serum creatinine levels were measured before and 6 months after rapamycin conversion. The serum creatinine dropped in patients with successful rapamycin conversion (creatinine: before conversion: 3.15 ± 0.56; 6 months after conversion: 2.59 ± 0.83 mg/dl). The mean reduction of the serum creatinine was 18.8% postrapamy-

Table 1. Patient characteristics of rapamycin conversion group and nonrapamycin conversion.

	Conversion group	Nonconversion group
Mean age (years)	47.4 ± 8.4	47.6 ± 13.3
Male:female	8:8	8:8
Transplant duration	8.0 ± 4.5	8.2 ± 4.2
CsA/tacolimus treatment	8/8	8/8

cin conversion ($P < 0.001$). In contrast, there is a significant increase in serum creatinine before and after the observation period (before: 2.84 ± 0.54; after: 3.28 ± 0.52 mg/dl, $P < 0.05$) in the nonconversion group. The results indicated the possible renal rescue effect of rapamycin conversion (Fig. 1).

The serum albumin levels did not change significantly before and after observation period in conversion and nonconversion groups (conversion: 3.9 ± 0.5 to 4.1 ± 0.3 g/dl, $n = 10$, NS; nonconversion: 3.8 ± 0.3 to 3.9 ± 0.2 g/dl, $n = 16$, NS). The serum ALT levels did not change in conversion (from 15.6 ± 10.6 to 19.3 ± 7.7 IU, $n = 10$, NS) and nonconversion groups (from 18.3 ± 9.2 to 18.3 ± 5.1 IU, $n = 16$, NS). There was increase of ALK-p (from 67.4 ± 32.9 to 79.6 ± 37.0 IU, $n = 10$, $P < 0.05$) in conversion group, but not in nonconversion group (from 67.3 ± 25.1 to 67.8 ± 14.7 IU, $n = 16$, NS). The serum Ca^{2+} increased (from 8.7 ± 0.3 to 9.5 ± 0.2 mg/dl, $n = 10$, $P < 0.01$) in conversion group, but remained unchanged in nonconversion group (from 8.8 ± 0.4 to 8.7 ± 0.5 mg/dl, $n = 16$, NS). Serum P (from 5.3 ± 1.4 to 3.6 ± 0.6 mg/dl, $n = 10$, $P < 0.05$) decreased in conversion group, but not in nonconversion group (from 5.2 ± 0.8 to 5.1 ± 0.9 mg/dl, $n = 16$, NS). Daily urinary Ca^{2+} excretion (from 93.9 ± 52.6 to 31.8 ± 45.1 mg/day, $n = 10$, $P < 0.01$) decreased after rapamycin conversion, but not in nonconversion group (from 84.6 ± 38.3 to 75.9 ± 38.4 mg/day, $n = 16$, NS). Daily urinary phosphate excretion increased (from 287.6 ± 257.1 to 439.4 ± 260.9 mg/day, $n = 10$, $P < 0.05$) in conversion group, but not in nonconversion group (from 233.9 ± 117.2 to 264.8 ± 143.4v mg/day, $n = 16$, NS) (Table 2). The improvement of the graft function could increase phosphate excretion and calcium reabsorption, which might indicate the increase of the glomerular filtration rate (GFR). Using creatinine clearance (Ccr) to estimate GFR, our patients also presented the clinical picture (from 35.3 ± 16.3 to 41.1 ± 16.9 ml/min, $P < 0.05$). To ensure that the increased phosphaturia was not solely a manifestation of increased GFR, we corrected the phosphaturia with GFR, estimated by Ccr. The phosphaturia remained significant increased after correction of Ccr (from 720.8 ± 455.3 to 1036.3 ± 484.8 mg/day/100 ml Ccr, $P < 0.05$). Acid–base status plays a role in calcium handling of renal tubules. The total CO_2 levels were 20.8 ± 5.4 mmol/l and 21.8 ± 5.4 mmol/l before and after rapamycin conversion. There was no significant change on the total CO_2 levels before and after conversion.

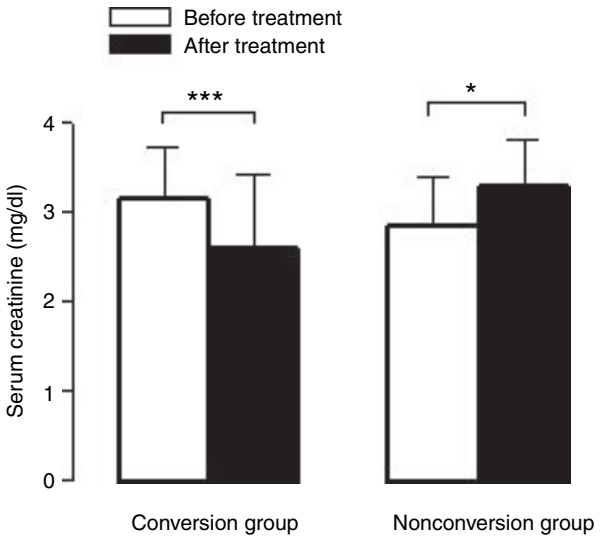

Figure 1 Rapamycin significantly decreased serum creatinine from 3.15 ± 0.56 to 2.59 ± 0.83 mg/dl after 6 months treatment in conversion group (***$P < 0.001$, $n = 10$), but in nonconversion group serum creatinine significantly increased from 2.84 ± 0.54 to 3.28 ± 0.52 mg/dl (*$P < 0.05$, $n = 16$).

Table 2. Biochemical parameters of serum and urine before rapamycin conversion and 6 months after rapamycin conversion or nonrapamycin-based therapy.

	Conversion group		Nonconversion group	
	Before	After	Before	After
Serum albumin (g/dl)	3.9 ± 0.5	4.1 ± 0.3	3.8 ± 0.3	3.9 ± 0.2
Serum Cr (mg/dl)	3.15 ± 0.56	2.59 ± 0.83*	2.84 ± 0.54	3.28 ± 0.52*
Ccr	35.3 ± 16.3	41.1 ± 16.9*	37.8 ± 18.4	30.1 ± 15.9*
ALT(IU)	15.6 ± 10.6	19.3 ± 7.7	18.3 ± 9.2	18.3 ± 5.1
ALK-p(IU)	67.4 ± 32.9	79.6 ± 37.0*	67.3 ± 25.1	67.8 ± 14.7
Serum Ca^{2+} (mg/dl)	8.7 ± 0.3	9.5 ± 0.2**	8.8 ± 0.4	8.7 ± 0.5
Serum P (mg/dl)	5.3 ± 1.4	3.6 ± 0.6*	5.2 ± 0.8	5.1 ± 0.9
Urine Ca^{2+} excretion (mg/day)	93.9 ± 52.6	31.8 ± 45.1**	84.6 ± 38.3	75.9 ± 38.4
Urine P excretion (mg/day)	287.6 ± 257.1	439.4 ± 260.9*	233.9 ± 117.2	264.8 ± 143.4
iPTH (pg/ml)	133.7 ± 149.6	200.6 ± 171.5*	128.4 ± 57.1	136.3 ± 40.4

Cr, creatinine; Ccr, clearance of creatinine.
*$P < 0.05$; **$P < 0.01$ (paired *t*-test).

The increase of the serum Ca^{2+} levels and urinary phosphate excretion, together with decreased serum phosphate and urinary Ca^{2+} excretion, suggested that there might be an increase in serum parathyroid hormone (PTH) in rapamycin conversion group. Intact PTH measurement revealed an increase (from 133.7 ± 149.6 to 200.6 ± 171.5 pg/ml, $n = 10$, $P < 0.05$; normal range 10–65 pg/ml) in conversion group (Fig. 2). To exclude that the observed significance of iPTH increases was just caused by a few individuals spontaneously developing into more overt tertiary hyperparathyroidism, individual data were plotted (Fig. 3). The range of the iPTH before conversion was from 21 to 378 pg/ml and 70.2–515 pg/ml. There were only two patients who had iPTH levels above 200 pg/ml before conversion (210 and 478 pg/ml). The elevated iPTH levels remained significant even after excluding these patients. On the other hands, there was no significant change of iPTH levels in nonconversion group (from 128.4 ± 57.1 to 136.3 ± 40.4 pg/ml, $n = 16$, NS). There were three patients who had iPTH levels higher than 200 pg/ml. The results suggested that the change was probably not from few individuals, who had tertiary hyperparathyroidism. VitD3 plays an important role in the calcium homeostasis. We had checked 25(OH)VitD3 levels in some of the patients in each group by RIA. There was no significant difference between patients with or without rapamycin conversion (conversion: 55.9 ± 8.3; nonconversion: 56.6 ± 18.3, NS).

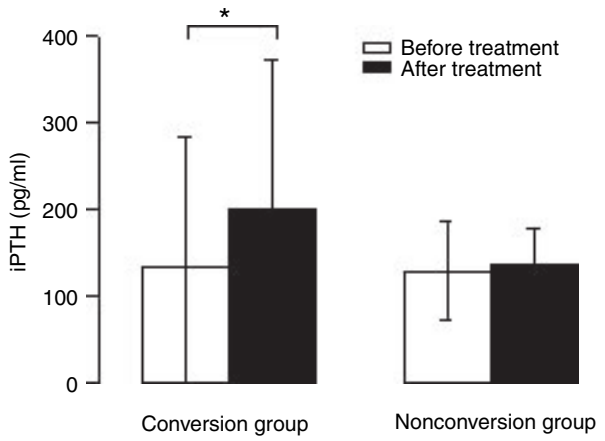

Figure 2 Serum iPTH significantly increased from 133.7 ± 149.6 pg/ml to 200.6 ± 171.5 pg/ml in rapamycin conversion group (*$P < 0.05$, $n = 10$). There was no significant change of serum iPTH before and after 6 months observation in nonconversion (128.4 ± 57.1 vs. 136.3 ± 40.4 pg/ml, NS, $n = 16$) group.

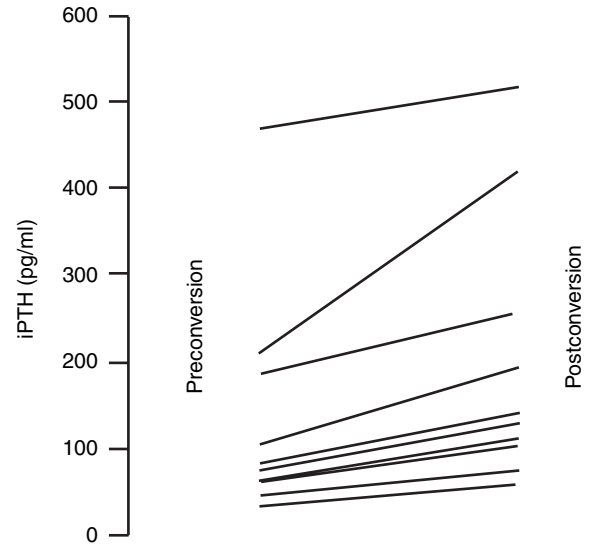

Figure 3 The iPTH levels before (preconversion) and after (postconversion) rapamycin conversion.

Discussion

Our study revealed that there were increased serum Ca^{2+}, decreased serum phosphate, increased iPTH, decreased urinary Ca^{2+}, increased urinary phosphate, and improved graft function after the rapamycin conversion. Adding nephrotoxicity-free rapamycin in the immunosuppressive regimen might offer some promise in patients with chronic allograft dysfunction [15]. Our previous study suggested that there were 62.5% successful conversion rates in patients with chronic allograft dysfunction [13]. The present study also revealed the rescue effect of rapamycin in chronic allograft dysfunction. Previous experiments in rats had shown that sirolimus is relatively bone sparing [12]. However, the effect of rapamycin in calcium metabolism in human is still unclear. The improvement of the graft function could increase phosphate excretion and calcium reabsorption, which might indicate the increase of the GFR. To ensure that the increased phosphaturia was not solely a manifestation of increased GFR, we corrected the phosphaturia with GFR, estimated by Ccr. The phosphaturia remained significantly increased after the correction of GFR. The finding suggested an additional effect of rapamycin conversion other than improved graft function on phosphate excretion. Intact PTH should decrease in improving graft function. The iPTH level increased instead of decrease in rapamycin conversion group. The finding suggested the possible direct effect of rapamycin in inducing the release of iPTH. Recent study of Bumbea *et al.* [16] found that the calcium level was decreased in rapamycin conversion patients. The conflicting results might come from the difference in study population. Our patients had a worse graft function comparing with the Bumbea's patients (Ccr: 35.3 ± 16.3 vs. 49.4 ± 14.9 ml/min) at the point of conversion. The graft function might

play a role in the reverse results. More study on calcium levels postrapamycin conversion with different graft function is necessary to clarify the finding.

Parathyroid hormone is synthesized as a 110-amino acid polypeptide called pre-pro-PTH, which is cleaved within parathyroid cells to pro-PTH (90 amino acids) and then to PTH (84 amino acids), which is the major storage, secreted, and biologically active form of the hormone. With respect to renal calcium handling, PTH is responsible for maintaining serum-ionized calcium concentrations within a narrow range, through its actions to stimulate renal tubular calcium reabsorption and reduce phosphate reabsorption. Filtrated calcium is reabsorbed passively in the proximal tubule and the loop of Henle downs the favorable electrochemical gradients created by sodium and water reabsorption. Calcium transport is also actively regulated according to changes in calcium balance in the distal tubule and adjacent connecting segment. PTH acts at the latter site to stimulate calcium reabsorption. PTH also inhibits proximal tubular reabsorption of phosphorus. This effect is primarily mediated by decreased activity of the sodium phosphate cotransporter in the luminal membrane of the proximal tubules. The net result is decreasing urinary calcium and increasing urinary phosphate. The possible effect of elevated PTH in our patients might lead to the observed increasing serum calcium and decreasing serum phosphate. It is very interesting to know if this altered iPTH levels might cause any clinical problem. We did not find the bone pain and proximal muscle weakness in our patients during the follow-up period of 6 months. However, it is important to follow up the clinical course in these patients.

The regulation of the PTH secretion is mainly through serum-free calcium level, vitamin D3, and calcium-sensing receptor (CaR) [17]. Recent study had indicated that rapamycin did not alter the bone metabolism and vitamin D3 concentration [18]. We had checked 25(OH)VitD3 levels in some of the patients in each group by RIA. There was no significant difference between patients with or without rapamycin conversion. The results suggested that the VitD3 might play a minimal role in the observed clinical findings. The effect of the rapamycin on CaR is still unclear. Activation of CaR is associated with inhibition of PTH synthesis and secretion [19]. The down stream signals of CaR are increased intracellular calcium and activated protein kinase A [20]. Rapamycin was known to inhibit the release of intracellular calcium and activation of protein kinase A [21] through the inhibition of mammalian target of rapamycin (mTOR). Rapamycin might act through the pathway to prevent the inhibitory effect of CaR signals to PTH production. The mechanism of the rapamycin-altered PTH regulation requires further study. On the other hand, rapamycin might also have direct tubular effect. Schwarz *et al.* [22] found that rapamycin-based immunosuppression prolongs the phosphate leak of the allograft kidney. Urinary phosphate loss could be mediated by an effect of rapamycin on type IIb sodium phosphate cotransporter. Study on patients taking rapamycin with or without CNI withdraw is beneficial to clear the question.

Our study suggested that conversion from CNI to rapamycin was associated with the increased renal calcium reabsorption, phosphate excretion, and increased PTH in kidney transplant recipients. An iPTH-related bone metabolism alteration might be chronic and take long time to clinical appearance. Regular monitoring of the iPTH and calcium homeostasis is suggested in patients taking rapamycin. The long-term outcome of the observed clinical manifestation requires further observation.

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