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ORIGINAL ARTICLE

Bone metabolism in renal transplant patients treated with cyclosporine or sirolimus

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Summary

Sirolimus is a new immunosuppressive agent used as treatment to prevent acute renal allograft rejection. One of the complications of renal transplantation and subsequent long-term immunosuppression is bone loss associated with osteoporosis and consequent fracture. Two open-label, randomized, phase 2 studies comparing sirolimus versus cyclosporine (CsA) included indices of bone metabolism as secondary end-points. Markers of bone turnover, serum osteocalcin and urinary N-telopeptides, were measured over a 1-year period in 115 patients receiving either CsA or sirolimus as a primary therapy in combination with azathioprine and glucocorticoids (study A) or mycophenolate mofetil (MMF) and glucocorticoids (study B). Urinary excretion of N-telopeptides and the concentrations of serum osteocalcin were consistently higher in the CsA-treated patients and significantly different at week 24 for N-telopeptides and at weeks 12, 24, and 52 for osteocalcin. In conclusion, future trials are warranted to test whether a sirolimus-based regimen conserves bone mineral density compared with a CsA-based regimen.

Introduction

The use of potent immunosuppressive drugs represents a major advance in the field of organ transplantation. However, chronic complications such as post-transplantation bone disease are a major concern. In renal transplantation, osteoporosis is a clinically important problem affecting from 6 to 15% of all patients in the first year after transplantation [1,2]. Several investigations have documented a decline in bone mass and increased fracture rate [3,4]. Other bone abnormalities commonly observed after renal transplantation include avascular necrosis with an incidence of 8% in adults [2].

Decreases in bone mineral density are well documented to be especially rapid during the early post-transplantation period when the doses of all immunosuppressants are highest [4–6]. Because of the frequency of osteoporosis and pathologic fracture, new therapies are needed to minimize and treat this complication [7,8].

The cause of the decrease of bone mineral density in renal transplant recipients involves factors such as hyperparathyroidism and decreased vitamin D, but it is also secondary to the immunosuppressive therapy required to prevent rejection [3,9]. Glucocorticoids probably play a major role in this complication [2,10,11]. Indeed, glucocorticoid administration decreases bone formation rate and bone mineral density and, at the cellular level, increases apoptosis of mature osteoblasts and osteocytes [12]. Other immunosuppressive drugs commonly used in transplantation, such as cyclosporine (CsA) and tacrolimus (FK506), have also been shown to have a deleterious effect on bone mineral metabolism in the rat [13–16]. In humans, although there is some controversy in the literature, there is accumulating evidence that CsA has a negative impact on bone metabolism after renal transplantation [3,17,18].

Sirolimus (rapamycin, Rapamune®, Wyeth-Ayerst Research, Princeton, NJ, USA) is a macrocyclic lactone isolated from *Streptomyces hygroscopicus*, which has been shown to be a potent immunosuppressive drug in several phase 2 and phase 3 clinical trials either in combination with CsA or used as a primary therapy [19–22]. Although sirolimus is structurally related to tacrolimus and binds to the same immunophilin FK506-binding protein (FKBP)12, its mechanism of action is different. Unlike CsA or tacrolimus, sirolimus has no effect on calcineurin phosphatase, but in contrast it inhibits the activity of the mammalian target of rapamycin (mTOR). Sirolimus blocks T-cell proliferation at a later stage than calcineurin inhibitors by affecting interleukin (IL)-2- and IL-4-induced signal transduction pathways rather than having a direct effect on cytokine production, therefore conferring a different safety profile [23,24].

In rats, it has been demonstrated that treatment with CsA and tacrolimus leads to high-turnover osteoporosis with elevated serum osteocalcin, whereas sirolimus does not affect serum osteocalcin concentration [25]. Moreover, in contrast to CsA- and tacrolimus-treated rats, there were no observable alterations in trabecular bone volume in the sirolimus group. Another more recent study in rats confirmed earlier findings that high-dose CsA produced a high-turnover osteopenia with decreased bone mass and increased serum osteocalcin level and that combination of low-dose CsA and sirolimus prevented bone loss [26].

Cell culture work in human mature osteoblastic cells has also suggested that sirolimus could have a bone-sparing effect compared with CsA or tacrolimus [27]. Glucocorticoids, CsA, tacrolimus, and sirolimus all have negative effects in undifferentiated marrow stromal cells by inhibiting production of osteoprotegerin, an inhibitor of osteoclast differentiation and function. In mature osteoblastic cells, however, only sirolimus increased the production of osteoprotegerin.

The purpose of the present study was to assess the effect of sirolimus on bone metabolism, when compared with CsA, in human renal transplant recipients. N-telopeptide fragments (NTXs), which have been reported to be specific for bone collagen breakdown, are released into the circulation [28,29]. The majority of these fragments is relatively small and readily passes through the glomerulus; therefore they can be easily detected in the urine [30]. Osteocalcin is another marker of bone metabolism also known as bone Gla protein (BGP). Osteocalcin is produced by osteoblasts during bone formation and remodeling. It is released into the circulation and, although a product of osteoblasts, can be considered as a marker of bone turnover [30]. Both serum osteocalcin and urinary excretion of N-telopeptides were measured in sirolimus- and CsA-treated kidney transplant recipients over 1 year after transplantation in two clinical phase 2 studies. Because the design of these studies was very similar, a pooled data analysis are presented.

Patients and methods

Study design

Two clinical phase 2 randomized, open-label, parallel-group trials were conducted in 19 centers in Europe. Patients received triple therapy with either CsA or sirolimus, in combination with glucocorticoids and azathioprine (study A) [19], or glucocorticoids and mycophenolate mofetil (MMF; study B) [31]. Indices of bone metabolism were prospectively defined secondary end-points. Approvals were obtained from local ethics committees, and written informed consent was obtained

for each patient enrolled. The studies were carried out according to the Declaration of Helsinki.

Patient population

In both studies, patients were admitted to the hospital and underwent prestudy screening and baseline evaluations. Adults who had received a first cadaveric renal allograft were centrally randomized in a 1:1 ratio to receive CsA or sirolimus as primary therapy in association with azathioprine and glucocorticoids (study A) or MMF and glucocorticoids (study B). Among a total number of 161 renal transplant recipients enrolled in these two studies [19,31], the effect of CsA or sirolimus on bone metabolism was assessed in all patients in whom bone markers were obtained through at least 24 weeks (115 patients).

Immunosuppressive therapy

Control group patients in both trials received CsA microemulsion (Neoral®, Novartis, Basel, Switzerland) with dosage adjusted to maintain whole blood trough (predose) concentrations of 200–400 mg/ml for 2 months, and 100–200 ng/ml thereafter, by monoclonal immunoassay at the choice of the local center. Sirolimus oral solution (Rapamune®) was administered once daily in the morning after dilution with water or orange juice [19,31]. Sirolimus doses were adjusted to achieve steady-state whole blood trough concentrations of approximately 30 ng/ml for 2 months, and 15 ng/ml thereafter by high-performance liquid chromatography with ultraviolet detection [32]. All patients received glucocorticoids (500 mg intraoperatively, then 200 mg/day orally tapered to 10–30 mg/day by day 7 and to 10 mg/day by week 24). In study A, patients were treated with azathioprine (initially 2 mg/kg/day perioperatively, then adjusted if necessary according to leukocyte count). In study B, patients were treated with MMF (Cellcept®, Roche, Basel, Switzerland), 1.0 g twice daily for up to 6 months. MMF was then to be discontinued by tapering the dose over 1 month. At the discretion of the investigator, patients could then remain on double therapy or be converted to azathioprine when MMF was discontinued.

Renal function and laboratory measurements

Patients from both studies were followed daily during the first week, weekly through month 3, and then monthly through month 12. At each visit, fasting blood samples were collected for creatinine, calcium, and phosphorus levels, and glomerular filtration rate (GFR) was calculated by the method of Nankivell *et al.* [33].

Urinary telopeptides

Crosslinked N-telopeptides type I collagen in urine were measured in 115 patients at weeks 4, 12, 24, and 52 after transplantation as indicators of bone resorption. An aliquot of a morning spot urine sample was frozen at −20 °C, and all samples were kept frozen until week 52 samples had been received. All samples were then analyzed together to minimize interassay variability. NTXs concentration was measured at a central laboratory (Analytical Unit, St George's Hospital Medical School, London, UK) using the competitive-inhibition enzyme-linked immunosorbent assay (ELISA) Osteomark® obtained from Ostex International Inc. (Seattle, WA, USA). Assay values were corrected for urinary dilution by urinary creatinine analysis and expressed in nmols of bone collagen equivalents (BCE) per mmols creatinine. In healthy subjects, the mean N-telopeptides excretion is approximately 30 nmol BCE/mmol creatinine. The overall bias and coefficient of variation for the urinary N-telopeptides assays were ≤1.9% and ≤11.1%, respectively.

Serum osteocalcin measurement

Serum osteocalcin was measured in 115 patients at weeks 4, 12, 24, and 52 after renal transplantation. Blood samples were collected in the morning in a tube without anticoagulant and were allowed to clot. Following centrifugation, the serum was quickly frozen and stored at −20 °C for analysis. Serum osteocalcin was then measured at a central laboratory using the solid-phase sandwich immunoradiometric ELSA-OSTEO® assay purchased from CIS Ltd (High Wycombe, UK). The serum osteocalcin levels were expressed in ng/ml; the normal range given by the manufacturer is 15.7–24.4 ng/ml depending on age and sex. The overall bias and coefficient of variation for the serum osteocalcin assays were ≤−5.9% and ≤3.9%, respectively.

Statistical analysis

The statistical analyses used in this study were based on pooled data from all study centers. In this paper, the use of the word 'significant' in connection with the results of a pairwise comparison refers to *P*-values of ≤0.05. All tests of hypothesis were two-sided. Fisher's exact test was used for comparison of ethnic origin, sex, and concomitant medication. The number of human leukocyte antigen (HLA) matches was compared between groups using a Cochran-Mantel-Haenszel test.

Age, average daily glucocorticoid dose, cumulative total glucocorticoid dose, urinary N-telopeptides, serum osteocalcin, and renal function were compared between groups

by one-way ANOVA with treatment group as factor in the model. Laboratory data for calcium and phosphorus were compared by one-way ANCOVA with treatment group as factor in the model and baseline as a covariate, baseline being defined as the last record before transplantation.

Results

Patients characteristics

Fifty-nine (59) patients who had bone markers measured were randomized to sirolimus and 56 to CsA. The two treatment groups were well matched for age, sex, ethnic origin, and number of HLA matches (Table 1). Recipients ranged from 22 to 68 years of age, and patients in the sirolimus group were, on average, slightly older (45.6 vs. 42.0 years), although the difference was not significant. The incidence of biopsy-confirmed acute rejection was not significantly different between groups (25.4% for sirolimus and 28.6% for CsA). In addition, the percentage of patients from study B (initially receiving MMF rather than azathioprine) was also comparable in the two groups (55.9% for sirolimus and 51.8% for CsA).

All enrolled patients in both studies A and B were eligible for this substudy. Those that were ultimately not included were those that provided no data (discontinued prior to the first sampling at 4 weeks) or discontinued before 24 weeks, the minimum time felt necessary for patients to stabilize in the post-transplant maintenance period. Reasons for early discontinuation are provided in the original publications [19,31].

Mean glucocorticoid daily dose and total glucocorticoid cumulative dose for patients from both groups are shown in Fig. 1a,b. There was no significant difference in glucocorticoid intake between the sirolimus- and CsA-treated patients, although the mean cumulative glucocorticoid dose was on average slightly higher in the sirolimus-treated patient group. Moreover, there was no difference in the percentage of patients receiving calcium compounds, vitamin D analogs, thyroid hormones, or estrogens, all medications known to affect bone metabolism (Table 1). None of the patients received biphosphonates preoperatively or during the first year after transplantation.

Table 1. Descriptive characteristics of renal transplant population.

Characteristics	CsA ($n = 56$)	Sirolimus ($n = 59$)	*P*-value
Recipient age (years)			
Mean ± SD	42.0 ± 11.3	45.6 ± 10.8	0.083*
Range	22–65	23–68	
Recipient sex, *n* (%)			
Male	34 (60.7)	40 (67.8)	0.444†
Recipient ethnic origin, *n* (%)			
White	50 (89.3)	58 (98.3)	0.144†
Black	1 (1.8)	0 (0)	
Asian	3 (5.4)	1 (1.7)	
Other	2 (3.6)	0 (0)	
Donor age (years)			
Mean ± SD	38.7 ± 18.0	41.0 ± 15.2	0.452*
Range	7–71	12–75	
Donor sex, *n* (%)			
Male	31 (55.4)	40 (67.8)	0.185†
Number of HLA matches, *n* (%)			
0	2 (3.6)	3 (5.1)	0.224‡
1	10 (17.9)	6 (10.2)	
2	17 (30.4)	16 (27.1)	
3	18 (32.1)	16 (27.1)	
4	4 (7.1)	12 (20.3)	
5	3 (5.4)	6 (10.2)	
6	2 (3.6)	0	
Receiving calcium compounds, *n* (%)	47 (83.9)	53 (89.8)	0.413†
Receiving vitamin D and analogs, *n* (%)	30 (53.6)	32 (54.2)	1.000†
Receiving thyroid hormones, *n* (%)	1 (1.8)	3 (5.1)	0.619†
Receiving estrogens, *n* (%)	0	4 (6.8)	0.119†

*One-way analysis of variance.
†Fisher's exact test.
‡Cochran-Mantel-Haenszel test.
CsA, cyclosporine; HLA, human leukocyte antigen.

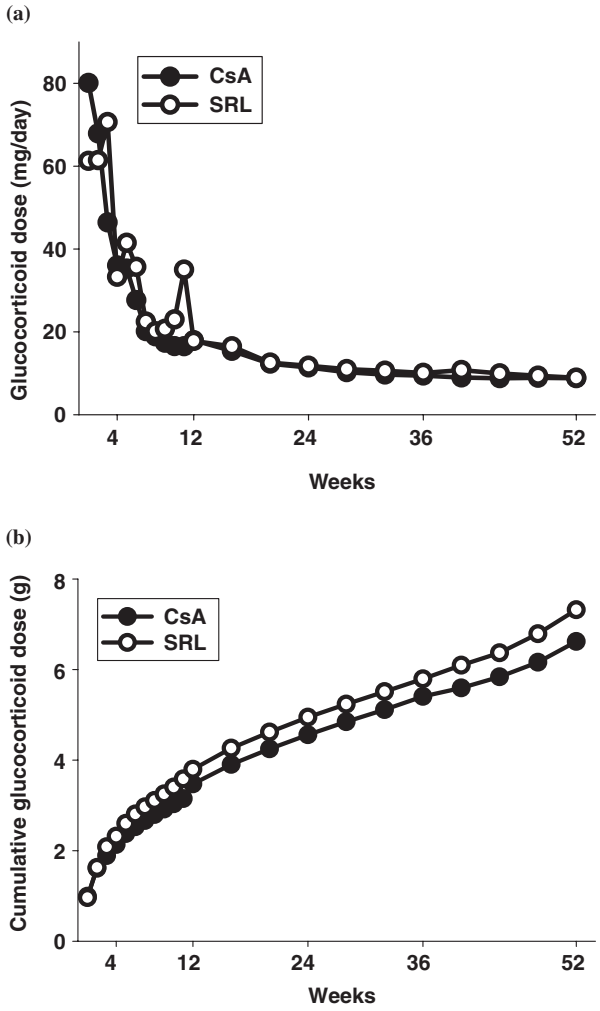

Figure 1 Mean daily dose of total glucocorticoids (a) and cumulative glucocorticoids dose (b) in cyclosporine (CsA)- (●) and sirolimus-treated (○) patients after renal transplantation.

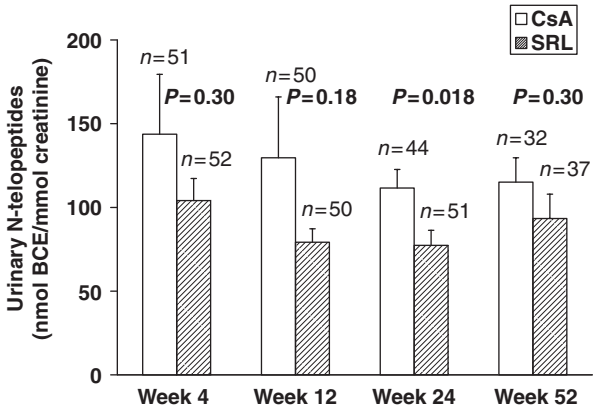

Figure 2 Measurement of urinary N-telopeptides excretion in cyclosporine (CsA)- (□) and sirolimus-treated (▨) patients after renal transplantation. Results are expressed in nmols of bone collagen equivalents per liter per mmols creatinine per liter (nmol BCE/mmol creatinine). The normal range is approximately 30 nmol BCE/mmol creatinine.

Renal function and laboratory measurements

The calculated GFR was significantly higher in the sirolimus-treated group (Table 2), and this increase was statistically significant compared with the CsA-treated group from week 12 after transplantation. In contrast, serum calcium and phosphorus concentrations were significantly lower in the sirolimus group, although these differences were small in magnitude (Table 2).

Bone markers

In both CsA- and sirolimus-treated patients, the mean values of NTXs excretion were higher than the normal range of ≤30 nmol BCE/mmol creatinine given by the manufacturer (Fig. 2) suggesting an elevated rate of bone resorption in renal transplant recipients [7]. From week 4 through week 52 after renal transplantation, however, the urinary excretion of NTXs in the sirolimus-treated patients was consistently lower than in the CsA group. This difference was statistically significant at week 24 ($P = 0.018$; Fig. 2).

Serum osteocalcin values increased over time from week 12 after transplantation in both groups (Fig. 3). In the CsA-treated patients, mean concentrations were higher than the normal range given by the manufacturer

Table 2. Mean (±SD) calculated GFR, serum calcium concentration, and serum phosphorus concentration in CsA- and sirolimus-treated patients at weeks 4, 12, 24, and 52.

	Calculated GFR (ml/min)		Calcium (mm)		Phosphorus (mm)	
Weeks	CsA	Sirolimus	CsA	Sirolimus	CsA	Sirolimus
4	50.68 ± 17.43	55.65 ± 21.27	2.39 ± 0.03	2.21** ± 0.03	0.76 ± 0.04	0.66 ± 0.04
12	56.08 ± 14.14	64.56** ± 16.62	2.46 ± 0.02	2.36** ± 0.02	0.95 ± 0.03	0.77** ± 0.03
24	58.56 ± 16.27	65.30* ± 15.88	2.49 ± 0.02	2.40* ± 0.02	0.96 ± 0.03	0.88* ± 0.03
52	60.16 ± 16.27	68.49* ± 16.18	2.45 ± 0.02	2.39* ± 0.02	1.04 ± 0.03	0.96 ± 0.03

*$P \leq 0.05$ vs. CsA group; **$P \leq 0.01$ vs. CsA group.
CsA, cyclosporine; GFR, glomerular filtration rate.

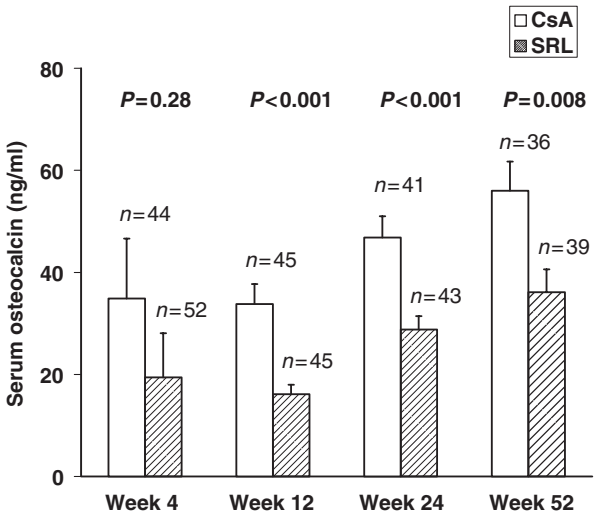

Figure 3 Measurement of serum osteocalcin levels in cyclosporine (CsA)- (□) and sirolimus- (▨) treated patients after renal transplantation. The normal range is 15.7–24.4 ng/ml, depending on age and sex.

(15.7–24.4 ng/ml) at each time-point studied. In contrast, sirolimus-treated patients showed elevated osteocalcin levels only from week 24. Serum osteocalcin was consistently lower in the sirolimus group, as for NTXs, and this diminution was statistically significant at weeks 12 ($P < 0.001$), 24 ($P < 0.001$), and 52 ($P = 0.008$) after transplantation.

Independently of whether studies A and B were analyzed individually or pooled, the same pattern of differences between the groups was observed for both NTXs and osteocalcin (data not shown).

Discussion

This study is the first to compare the effect of sirolimus and CsA on markers of bone metabolism in human renal transplant recipients. The two treatment groups were well matched and did not show any significant difference in medication that could affect bone metabolism, such as glucocorticoids, calcium compounds, vitamin D analogs, thyroid hormones, or estrogens. Enrolled patients that were not part of this substudy analysis were those that discontinued early because of graft loss, death, acute rejection or adverse event [19,31]. The incidence of acute rejection in patients enrolled in this substudy was similar between the groups, as was the percentage of patients initially receiving azathioprine rather than MMF. The choice of purine antagonist is probably not important with regard to bone loss as short-term studies in rats have indicated that neither azathioprine [34] nor MMF [35] affected bone histomorphometry compared with controls.

During the first year after transplantation, mean urinary excretion of NTXs and average serum osteocalcin were consistently lower in patients receiving sirolimus compared with those receiving CsA. This potential bone-sparing effect was observed when these two agents were combined with a purine antagonist (azathioprine or MMF) and glucocorticoids. Sirolimus can also be combined with calcineurin inhibitors (CsA or tacrolimus) and glucocorticoids [20,31,36]. It is not known whether any potential bone-sparing effects would be observed when sirolimus is combined with a calcineurin inhibitor in humans, although animal studies combining low-dose CsA and sirolimus showed bone sparing [26]. It should also be emphasized that while the bone marker profile was favorable for sirolimus in the present trial, it was abnormal in both groups, suggesting increased bone turnover and loss. Consequently, conservative measures to reduce bone loss such as calcium and vitamin D supplementation, hormone replacement therapy, and glucocorticoid dose reduction should be used whenever appropriate.

Calcium and phosphorus serum levels were usually slightly lower in the sirolimus group. For both groups, mean serum calcium concentrations remained within the normal range, but mean serum phosphorus concentrations were near the lower limit, as frequently observed after renal transplantation [37]. Although these differences are statistically significant at weeks 4–52 after transplantation for serum calcium and at weeks 12 and 24 for serum phosphorus, they are not clinically significant. Neither parathyroid hormone nor 1,25-dihydrocalciferol was measured in these patients and parathyroidectomy status was not obtained. Therefore, these differences in bone markers may also reflect indirect effects, such as secondary hyperparathyroidism.

Sirolimus treatment resulted in better renal function when compared with CsA. Calculated GFR was significantly higher (9.8–15.1%) in sirolimus-treated patients. There was, nevertheless, a wide range of renal function in both groups. For example, at week 24, the mean (median; range) of calculated GFR was 58.6 (60.2; 24.1–97.3) ml/min and 65.3 (64.7; 20.8–95.8) ml/min in the CsA and sirolimus groups respectively. Thus, both groups were quite heterogeneous, including patients with marked renal insufficiency and others with near normal renal function. Severe insufficiency can result in higher serum osteocalcin and NTX levels. It should be emphasized that the effects of variations in GFR on NTXs are minimzed by expressing them as nmol BCE/mmol creatinine in the urine. Although renal function is a confounding factor, it is unlikely that magnitude in differences of serum osteocalcin and urinary NTXs are due only to the difference in mean GFR between the groups.

In conclusion, urinary excretion of N-telopeptides and serum osteocalcin levels were consistently lower in the sirolimus-treated patients than in the CsA-treated group. These results are in accordance with preclinical data [15,16,25] and could suggest less bone turnover and less bone resorption in sirolimus-treated patients compared with CsA-treated patients. Sirolimus has previously been shown to have efficacy similar to CsA in preventing acute graft rejection, and a better safety profile with regard to renal function and hypertension [19,20,31]. Adequately powered studies including measurement of bone mineral density and fracture outcomes will be necessary to establish if sirolimus has bone-sparing properties compared with CsA.

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