Effect of Calcitriol on Bone Loss After Cardiac or Lung Transplantation

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ABSTRACT

Rapid bone loss after cardiac and lung transplantation results in an increased risk of osteoporotic fracture. This study examined the efficacy of treatment with calcitriol (1,25-dihydroxyvitamin D3) in preventing bone loss in patients undergoing cardiac or lung transplantation. In this 2-year double-blind, stratified study, 65 patients undergoing cardiac or single lung transplantation were randomly allocated to receive either placebo or calcitriol (0.5–0.75 mg/day), the latter for either 12 months or 24 months. All patients received 600 mg calcium/day. Bone mineral density (BMD) was measured every 6 months for 2 years by dual-energy X-ray absorptiometry. There was no significant difference between groups with respect to age or cumulative dose of prednis(ol)one or cyclosporine over the 2 years. Bone loss at the proximal femur was significantly reduced or prevented at all three sites by treatment with calcitriol for 2 years compared with treatment with calcium alone. Treatment with calcitriol for 12 months followed by calcium for 12 months resulted in similar proximal femoral bone loss to that seen in those patients treated with calcium for 24 months, suggesting calcitriol prophylaxis needs to be continued beyond 12 months. At the lumbar spine, there were no significant differences in BMD between groups. Over a period of 2 years, 22 new vertebral fractures/deformities occurred in 4 patients treated with calcium alone compared with one new vertebral fracture in 1 patient treated with calcitriol. Because the sample size was too low to provide reliable interpretation of vertebral fracture rates, this difference is likely a chance result. Mild hypercalcemia was common with calcitriol therapy, as was mild hypercalciuria (59% of patients vs. 10% controls), but there were no significant differences between groups in serum creatinine after 2 years. These data suggest calcitriol has a role in reducing proximal femur bone loss after cardiac or lung transplantation but treatment needs to be continued beyond 1 year.

(J Bone Miner Res 2000;15:1818–1824)

Key words: transplantation, corticosteroids, vitamin D, osteoporosis, calcium

INTRODUCTION

Organ and tissue transplantation are being performed more often worldwide since the introduction of newer immunosuppressive agents such as cyclosporine. Cardiac transplantation and lung transplantation, in particular, produce long-term survival. However, with longer survival, patients undergoing organ transplantation are increasingly recognized to be at risk of osteoporotic fractures. Vertebral fractures have been reported in up to 35% of patients. One contributing factor could be reduced mobility but cardiac transplant patients, although suffering from a life threatening illness, usually have a relatively short prior history and often show a dramatic response to transplantation with a
return to a normal lifestyle afterward. Moreover, the majority of these patients do not suffer from any confounding underlying disease that will cause bone loss. Unlike patients undergoing renal and liver transplantation, most patients undergoing cardiac or lung transplantation do not have significant hepatic or renal dysfunction that may cause metabolic bone disease by other mechanisms. The rapid bone loss in patients undergoing cardiac and lung transplant has been attributed to the use of drugs such as corticosteroids; however, serum osteocalcin, a marker of bone turnover, is increased after transplantation\(^{(2,3)}\) suggesting cyclosporine also is a contributing factor.

Because bone loss after transplantation appears to be most marked in the first 12 months after transplantation\(^{(4)}\) and active vitamin D metabolites such as calcitriol and 1-alpha-hydroxylvitamin D have been shown to be effective in preventing bone loss in rheumatic disease patients starting corticosteroids\(^{(5,6)}\), the objective of this study was to evaluate the prophylactic effect of calcitriol in patients undergoing cardiac or single lung transplantation and for how long such therapy is needed.

**MATERIALS AND METHODS**

**Patients**

Patients aged 20–70 years were recruited within 4 weeks of undergoing cardiac or single lung transplantation. Exclusion criteria were before treatment with chronic corticosteroids, calcitonin, calcitriol, fluoride, vitamin D > 50,000 U/week, diseases that may affect bone metabolism, and significant renal impairment or liver disease. Patients were recruited at two centers in Sydney and Melbourne, Australia.

**Study design**

The present study was a randomized, double-blind, parallel group study in which patients were assigned to treatment with calcitriol (group D) or placebo (group C) in a 2:1 ratio with stratification according to age (≤40 years or >40 years), sex, and type of transplant. After 12 months, half the calcitriol patients switched to placebo (group D1) while the remainder continued calcitriol for 2 years (group D2). All patients also received 600 mg of elemental calcium daily as calcium carbonate (Caltrate; Whitehall Laboratories, Sydney, Australia) for the 2 years of the study. Rocaltrol (0.25-μg capsules) and identical placebo capsules were provided by Roche Pharmaceuticals (Sydney, Australia). Calcitriol was commenced at a dose of 2 capsules/day (0.5 μg) for 2 weeks and, in the absence of hypercalcemia, increased by 1 capsule/day after 2 weeks to 3 capsules/day. However, in patients receiving <10 mg prednis(ol)one/day at any time, the dose of calcitriol was limited to 2 capsules/day. Dietary calcium\(^{(7)}\) and physical activity\(^{(8)}\) also were assessed at entry. Corticosteroid and cyclosporine doses were recorded in a daily diary. In total, 64 patients underwent randomization (Sydney, \(n = 60\); Melbourne, \(n = 5\)).

**Immunosuppression**

After transplantation all patients received corticosteroids and cyclosporine A. The corticosteroid regimen involved intravenous methylprednisolone, 1–2 g perioperatively and 125 mg every 8 h for 3 doses followed by oral prednisolone at 1 mg/kg per day reducing to 0.15–0.18 mg/kg per day by day 14 and 0.10–0.15 mg/kg per day by 6 months. Rejection was managed by high-dose intravenous or oral corticosteroids followed by a rapid tapering; the precise regimen depended on time since transplant and rejection severity. Cyclosporine A was administered at a dose of 2–3 mg/kg intravenously preoperatively and oral cyclosporine was commenced 24 h after surgery with a dosage adjusted usually according to whole blood levels. Azathioprine also was given to all patients at a dose of 2 mg/kg after surgery with the exception of 22 patients transplanted between April 1994 and July 1995 who were enrolled in a double-blind, placebo-controlled 3-year trial comparing the efficacy and safety of mycophenolate mofetil with azathioprine. In these 22 patients, immunosuppression was the same as usual except that 11 patients were randomized to receive mycophenolate mofetil, 1500 mg twice a day (bd), instead of azathioprine.

Before September 1996, the form of cyclosporine A used was SANDIMMUNE (Sandoz, Novartis Pharma, Basle, Switzerland) capsules or liquid. From September to December 1996, all Sydney recipients were switched to microemulsified cyclosporine (NEORAL, Novartis Pharma, Basle, Switzerland). Cyclosporine levels and serum creatinine were monitored during the changeover and cyclosporine dosage was adjusted where necessary.

**Bone density measurements**

Bone mineral density (BMD) was measured at the lumbar spine and proximal femur every 6 months for 24 months. BMD (g/cm\(^2\)) in the lumbar spine (L2–L4) and proximal femur was measured using a Lunar DPX-L dual-energy X-ray absorptiometer (LUNAR Corp., Madison, WI, U.S.A.) in Sydney and a Hologic 2000 (Hologic, Inc., Waltham, MA, U.S.A.) in Melbourne. Duplicate measurements were made at baseline: 12 months and 24 months. A detector replacement in the Lunar DPX-L machine at approximately the midpoint of the study was recognized to have caused a systematic shift in BMD values for Sydney patients measured after the change. Subsequently, a uniform correction was applied to all patients scanned in Sydney to allow for this effect. The correction was determined from measurements performed pre- and postdetector change using both Lunar and Hologic phantoms. Because the systematic correction was applied to patients in all groups equally, this did not affect comparisons between groups from the preliminary presentation of these data before the correction.\(^{(3)}\)

**Radiographic assessment**

Lateral radiographs of the thoracic and lumbar spine obtained at entry and after 12 months and 2 years were...
analyzed blinded to treatment group by an experienced investigator (P.S.). X-rays of the thoracic and lumbar spine were secondary measurements of efficacy. Vertebral fracture was assessed semiquantitatively as 0, a normal vertebra; 1, a mild fracture with a 25% reduction in anterior, middle, or posterior height (or all three); 2, a moderate fracture with a 25–40% reduction in any height; and 3, a severe deformity with a reduction of more than 40% in any height. A new vertebral fracture was deemed to have occurred when any grade progressed to a higher grade between visits.

**Biochemical analysis**

Serum for measurement of osteocalcin, testosterone, or estradiol; procollagen I peptide and collagen type I–carboxy telopeptide, and a second voided urine specimen (2 h test) were collected after an overnight fast at baseline and each 6 monthly visit for 2 years. Total plasma calcium was measured at baseline, 4 weeks, 3 months, and every 3 months thereafter. If hypercalcemia occurred, both the calcium supplement and the calcitriol were ceased. The calcitriol was subsequently reintroduced, after the plasma calcium had returned to normal, at a lower dose without the calcium supplement. Serum chemistry analyses were determined by automated methods. Baseline samples were collected where possible before transplant and repeated in the first week after transplant. Serum osteocalcin (reference range, 3–18 ng/ml) was determined as previously described. Serum procollagen type I C-terminal peptide (PICP), a marker of collagen deposition, and collagen type I C-terminal telopeptide (ICTP), a marker of collagen breakdown, were measured by radioimmunoassay (Farmos Diagnostica, Oulu, Finland). Urinary calcium was measured by titration using a Corning Calcium Analyzer 940 (Halstead, Essex, U.K.) and hydroxyproline was quantified using a Technicon autoanalyzer-AAI (Technicon, Tarrytown, NY, U.S.A.). Hydroxyproline was expressed as a ratio relative to the urinary creatinine (Astra autoanalyser; Beckman Instruments, Inc., Brea, CA, U.S.A.).

**Statistical analysis**

The primary analysis was based on an intention-to-treat model. Our intention-to-treat analysis included all available subject data up to 2 years. For 6, 12, 18, and 24 months the BMD data were analyzed by repeated measurements analysis of covariance with baseline as the covariate. Treatment means at 6, 12, 18, and 24 months were adjusted for baseline differences, if present. The least significant difference (LSD) was used to assess the pairwise differences when effects were significant in the analysis of covariance.

**RESULTS**

There were 47 cardiac and 18 single lung transplants. Differences between treatment groups with regard to baseline data are shown in Table 1. Of the 65 patients initially enrolled, 6 patients had no measurements after baseline leaving 59 patients who could be evaluated. Five participants died in the first year and one in the second year. The six deaths were considered unrelated to the trial medications: pneumonia and myocardial infarction. Five others withdrew because of underlying illnesses in the first 12 months. Three women commenced estrogen during the study, but excluding them made no difference to the results described below.

**Corticosteroid and cyclosporine doses**

Cumulative doses of corticosteroid and cyclosporine for patients who could be evaluated are shown in Table 2. There were no significant differences between groups with respect to cumulative corticosteroid or cyclosporine dose in either the first or second 12 months posttransplant, respectively. Neither corticosteroid nor cyclosporine dose was a significant predictor of bone loss at any site.
Bone densitometry

The mean lumbar spine and femoral neck T scores for all patients combined was $-0.88$ and $-0.98$, respectively, indicating that the study population was mildly osteopenic at baseline. There were no significant differences in proximal femur BMD at any site between groups (Table 1). Baseline lumbar spine BMD was significantly lower in controls (group C) than the two calcitriol groups (Table 1); however, baseline values were included as a covariate in the repeated measures analysis, as noted above.

For femoral neck BMD the overall mean difference at 2 years between groups D2 and C was significant, with a value of $0.038$ g/cm$^2$, a 95% CI (0.001 and 0.075) and $p = 0.044$. The overall mean differences of groups D2 and D1 and groups D1 and C were not significantly different. Mean femoral neck BMD values are shown in Fig. 1A for each group at each time. Figure 1A shows groups D2 and C were significantly different at the 0.05 level at month 6 and month 12, because the outer error bars do not overlap. Figure 1A shows significant falls for group D2 from 6 to 18 months and 24 months, for group D1 from 12 to 18 months and 24 months after the withdrawal of calcitriol at 12 months, and for group C from 6 to 24 months; the within treatment falls are significant because the inner error bars do not overlap.

Mean percentage change (SE) from baseline to 12 months and 24 months, respectively, were $-1.2\% (1.7)$ and $-5.0\% (1.9)$ for D2, $-6.6\% (2.3)$ and $-8.2\% (2.2)$ for C, and $-3.9\% (1.4)$ and $-7.4\% (1.5)$ for D1.

For Ward’s Triangle the overall mean difference of groups D2 and C at 2 years was significant, with a value of $0.067$ g/cm$^2$, a 95% CI (0.017 and 0.117), and $p = 0.009$. The overall mean differences of groups D2 and D1, and groups D1 and C were not significantly different. Figure 1B shows mean Ward’s Triangle BMD values at each time.
Figure 1B shows groups D2 and C were significantly different at the 0.05 level at month 6 and month 12 and shows groups D1 and C were significantly different at 12 months. There were significant falls for group D2 from 6 to 18 months and 24 months, for group D1 from 12 to 18 months and 24 months after the withdrawal of calcitriol at 12 months, and for group C from 6 to 24 months. Percentage change from baseline to 12 months and 24 months, respectively, were +0.8% (2.8) and −5.0% (2.4) for group D2, −11.7% (2.9) and −12.8% (2.6) for C, and −3.4% (3.4) and −10.7% (2.1) for group D1.

For the trochanteric site, the overall mean difference of groups D2 and C at 2 years was significant, with a value of 0.038 g/cm², a 95% CI (0.004 and 0.172), and \( p = 0.033 \). The overall mean differences of groups D2 and D1 and groups D1 and C were not significantly different. Figure 1C shows mean trochanteric BMD values at each time. Figure 1c shows groups D2 and C were significantly different at the 0.05 level at month 12 and month 24 and shows groups D2 and D1 were significantly different at the 0.05 level at month 18 and month 24 after the withdrawal of calcitriol at 12 months. Figure 1C shows no significant falls for group D2 but shows significant falls for group D1 from 12 to 18 months and 24 months after the withdrawal of calcitriol at 12 months and for group C from 6 to 24 months. Percentage change from baseline to 12 months and 24 months, respectively, were +1.1% (1.4) and −0.18% (1.4) for group D2, −4.6% (2.1) and −5.4% (2.1) for group C, and −1.8% (1.7) and −5.2% (1.9) for group D1.

For the lumbar spine, the overall mean differences of groups D1, D2, and C were not significantly different after 2 years. Figure 1D shows mean lumbar spine BMD values at each time. Figure 1D shows no significant differences between the means of groups D1, D2, and C at months 6, 12, 18, and 24. Figure 1D shows significant falls for group D2 from 6 to 18 months and 24 months and for group D1 from 12 to 18 months and 24 months after the withdrawal of calcitriol at 12 months, but no significant falls for group C. Percentage change from baseline to 24 months, respectively, were: −1.4% (1.1) and −2.7% (1.1) for group D2, −2.9% (1.0) and −3.0% (1.2) for group C, and −2.3% (1.3) and −5.6% (1.4) for group D1.

The rates of change in BMD in different groups were analyzed in relation to corticosteroid dose, cyclosporine dose, sex, and biochemical markers of bone turnover. There was no relationship between rates of change and gender, corticosteroid, or cyclosporine dose. There was no difference in rates of loss in mycophenolate-treated patients versus azathioprine-treated subjects.

**Vertebral fractures**

In those randomized to placebo (calcium alone) for 2 years (group C), 4 patients sustained a total of 22 new vertebral fractures whereas in the calcitriol treated patients, one patient in group D2 sustained one new vertebral fracture over 2 years (\( p < 0.04 \)).

**Biochemical measurements**

The mean serum calcium of each group did not change significantly during the study but 8 patients developed mild hypercalcemia (defined as a total serum calcium above the 95% CI for the upper level of the laboratory range but below 2.90 mmol/liter): 5 in group D2 and 3 in group D1. Hypercalcemia was common, occurring in 13/22 patients in group D1, 13/22 in group D2, and 2/21 in group C. This usually occurred between 3 and 6 months and settled with cessation of the calcium supplement and/or reduction in the calcitriol dose. There were no significant differences between the groups in dose (mean ± SD) of calcitriol (0.65 ± 0.1 μg/day). Serum creatinine rose significantly in all groups over 2 years, but there were no significant differences between groups (Table 3). Urinary hydroxyproline/creatinine values were increased at baseline in all groups but returned to normal by 12 months and there were no significant differences between groups. Serum testosterone was reduced in males but rose significantly by 12 months in all three groups. Mean serum osteocalcin gradually rose in all three groups, although this rise was only significant for the calcium group (\( p = 0.003 \) in group C and \( p = 0.07 \) in group D1), possibly because of a lower baseline. Serum PICP levels also showed a modest rise in all three groups, but this was only significant for the calcium group (\( p = 0.05 \)). Serum ICTP levels showed a modest decline in all groups but again was only significant in the calcium group (\( p = 0.04 \) in group C and \( p = 0.06 \) in group D1). There was no relationship between biochemical markers at any time point and change in BMD over 12 or 24 months.

**DISCUSSION**

In this double-blind prospective study, bone loss from all three sites in the proximal femur was reduced or prevented by prophylactic treatment with calcitriol over 24 months, in patients undergoing cardiac or single lung transplantation. Proximal femoral bone loss was greatest in the group treated with calcium alone and, with respect to calcitriol, greater in the group who had been treated for the first 12 months only compared with the group who had been treated with calcitriol for 24 months. There were no significant differences in BMD trends between groups at the lumbar spine; however, the mean loss observed was considerably less than in our earlier studies of untreated patients.\(^{13,14}\) However, mild hypercalcemia was common with calcitriol, given in conjunction with calcium, and needs to be monitored for and the dosage adjusted accordingly.

There have been a small number of previous studies of therapeutic agents to prevent bone loss after cardiac transplantation. In one open study of 54 patients who received calcium and calcidiol, lumbar spine bone density did not decline over 12 months but this treatment was started at a mean duration of 8 months posttransplant\(^{15}\) and other studies have shown that major loss occurs in the first 6 months posttransplant.\(^{13,14}\) Another open study reported treatment with calcium and alphacalcidol reduced bone loss in the spine and femoral neck relative to cyclical etidronate
in 48 patients after cardiac transplantation. There was no control or “untreated” group but spinal loss at 6 months averaged 4.6% with alfacalcidol and 7.7% with etidronate. Two vertebral deformities occurred in the alfacalcidol group over 2 years compared with eight in the etidronate group. By contrast, lumbar spine bone loss averaging 4.5% in the first 3 months after liver transplantation was reported despite treatment with cyclical etidronate and alfacalcidol. New vertebral fracture rates were 25% in the first 12 months after transplant, our data suggest such protection is unlikely to explain our lumbar spine trends.

In our previous prospective study of 25 patients undergoing cardiac transplantation we observed significant loss of bone density in the first 12 months averaging 8.8% from the lumbar spine. The degree of bone loss from the lumbar spine observed in the present study in the control group was therefore somewhat surprising and suggests calcium may attenuate posttransplant bone loss. Recent longitudinal studies of patients losing weight have suggested BMD may be calculated erroneously because of errors in measuring bone area and lead to overestimates of bone loss, especially for Hologic machines. These effects are thought to be mainly evident with total body scans and minimal for spine and hip BMD measurements; weight gain posttransplantation is unlikely to explain our lumbar spine trends.

The sample size of our study is too low to provide reliable interpretations of the vertebral fracture rate results. For example, if the true vertebral fracture rate for placebo was 15%, with n = 22, and for calcitriol was 5%, with n = 44, then the power to detect this difference at the 0.05 level with a two-sided test is only 18%. Thus, our observed borderline significant difference is likely a chance result. The vertebral fracture rate results are reported for inclusion in possible future m-analyses. Hip fractures also are increased posttransplant and in corticosteroid users; so our findings in regard to the proximal femur are likely to have clinical importance. Reductions in BMD before transplantation have been reported to be generally more severe in lung transplant patients than cardiac transplant patients, but because lung transplant patients who had had prior treatment with chronic corticosteroids were excluded from this study, this is not a relevant consideration in our study.

These results have important therapeutic implications for patients undergoing cardiac or lung transplantation. Fractures are a common and important complication of high-dose corticosteroid therapy and bone density has been shown to predict fracture incidence. Thus, our findings that bone loss can be reduced by prophylactic treatment with calcitriol plus calcium suggest that transplantation-related fractures could be reduced by this treatment. Although bone loss after transplantation appears to be most marked in the first 12 months after transplant, our data suggest such prophylactic therapy needs to be continued at least to 2 years.

ACKNOWLEDGMENTS

We gratefully acknowledge the expert assistance of Sisters Sheila Hunt, Stella Yeung, and Gai Marshall, Harriet McCathie, Elizabeth Downs, Louise Tchan, and Philip Mc-
Cloud of Roche Australia and the Departments of Chemical Pathology, Hematology and Nuclear Medicine at St. Vincent’s Hospital. This study was supported by grants from the National Health and Medical Research Council of Australia and Roche Australia.

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