Kidney International, Vol. 63 (2003), pp. 2275–2279

Long-term effects on bone mineral density of pamidronate given at the time of renal transplantation

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Background. Fracture rate after renal transplantation is substantially increased, is a source of morbidity and mortality, and correlates with osteopenia. The rate of bone loss after transplantation is time dependent. While we recorded marked bone loss during the first year after renal transplantation, bone loss in long-term recipients (>24 months) was found to be similar to expected age-related decline. We have previously shown that treatment with pamidronate at the time of transplantation protected the skeleton over a 1-year study period.

Methods. We have reexamined patients who participated in our original study, all of whom had been randomized to receive either placebo or pamidronate (0.5 mg/kg) at the time of transplantation and 1 month later. We now report 4-year data from 17 of the 26 original cohort. All patients received immunosuppression, comprising prednisolone, cyclosporine, and azathioprine.

Results. We found that without prophylaxis bone loss at 4 years was substantial and significant at the femoral neck (mean loss was −12.3%) but was not significant at the lumbar spine (mean loss was −4.64%). Patients who received two doses of pamidronate experienced no statistically significant bone loss at either the femoral neck or the lumbar spine. Patient characteristics of the placebo and treatment groups were similar with the exception of serum parathyroid hormone concentrations, which remained higher at 4 years in the pamidronate-treated patients (15.8 ± 3.7 pmol/L vs. 9.8 ± 1.8 pmol/L, $P < 0.05$).

Conclusion. Without prophylaxis, most patients who continue to receive low dose glucocorticoids as part of maintenance immunosuppression manifest a substantial deficit in bone mineral density (BMD) at the femoral neck. In contrast, two doses of pamidronate given at the time of transplantation and 1 month later protected the skeleton from significant bone loss over the 4 years after transplantation.

Recipients of renal and other solid organ transplants experience rapid bone loss during the first year after transplantation [1, 2] and we have previously shown that the degree of bone loss and the site at which bone is lost is affected by gender [3]. Renal transplant patients have also been documented to have bone fracture rates three times higher than comparable dialysis patients [4]. As many as 40% of patients with type I diabetes mellitus suffer a bone fracture within 3 years of renal transplantation [5]. While bone mineral density (BMD) is a good predictor of fracture risk in postmenopausal women, this is less clear in other causes of secondary osteoporosis. However, Grotz et al [4] have found that this correlation exists in renal transplant patients. The cause of bone loss in the immediate posttransplant period is likely to be multifactorial, including the effects of immunosuppression (particularly glucocorticoids and calcineurin inhibitors [6]) and immobility superimposed on preexisting renal osteodystrophy [2]. Moreover, even in patients with excellent graft function, hyperparathyroidism has been found to persist for a considerable time and sometimes indefinitely.

In a prospective randomized trial [7], we found that in male transplant recipients without prophylaxis, bone loss 12 months after transplantation was 9.0% at the femoral neck and 6.4% at the lumbar spine. Patients randomized to receive two doses of pamidronate (0.5 mg/kg), given intravenously at the time of transplantation and at 1 month, experienced no statistically significant bone loss at 12 months at either the femoral neck or the lumbar spine. Similar protection against early bone loss has since been shown with ibandronate [8]. Moreover, Grotz et al [9] have documented that bone loss 24 months after transplantation is no greater than the expected age- and gender-dependent decline. It is, therefore, possible that early bone protection can provide sufficient long-term protection against osteoporosis in many transplant patients.

In an attempt to define the extent of long-term protection provided by treatment with pamidronate at the time of transplantation, we now report the skeletal status of the patients 4 years after they were randomized to receive pamidronate or no treatment.

Key words: transplantation, osteoporosis, bisphosphonates, pamidronate, renal, control trial.

Received for publication July 22, 2002
and in revised form November 18, 2002
Accepted for publication January 17, 2003

Table 1. Clinical details

	Pamidronate	Control
Number of patients	9	8
Age at time of transplantation *range*	46.2 (21.1–67.1)	41.5 (21.3–65.0)
Modality at time of transplantation (hemodialysis: continuous ambulatory peritoneal dialysis)	2:7	5:3
Time on dialysis *years*	3.6 (±1.7)	4.0 (±2.1)
Number with diabetes mellitus	0	0
Etiology of renal failure		
Chronic glomerulonephritis	1	2
APKD	4	4
Hypertension	1	0
Vasculitis	1	1
Dysplastic kidneys	1	0
Unknown/others	1	1

METHODS

We originally studied 26 male patients admitted for renal transplantation. Of the 14 patients who were randomized to receive pamidronate prophylaxis, nine underwent a dual energy x-ray absorpiometry (DXA) scan 4 years after transplantation. One patient returned to hemodialysis within 12 months, three died, and one declined to have a repeat DXA scan. Of the original 12 patients who were randomized to receive placebo (500 mL of 0.9% saline), eight patients underwent a DXA at 4 years. Again, one patient died, one was transferred to another hospital, one returned to hemodialysis, and one patient declined to have a repeat DXA scan. The details of the enrolled patients are listed in Tables 1 and 2.

Study protocol

Patients assigned to receive pamidronate had an intravenous infusion of 0.5 mg/kg in 500 mL 0.9% saline peroperatively and 1 month postoperatively. The control patients did not receive any prophylaxis against bone loss. Both groups received standard immunosuppression with cyclosporine, azathioprine, and prednisolone (Table 3). Episodes of renal dysfunction were managed conventionally by renal biopsy and treatment of confirmed rejection with three 500 mg doses of methylprednisolone. None of these patients required biologic agents for immunosuppression. DXA scans were performed in the first week of transplantation and at 4 years. Serum intact parathyroid hormone (PTH) concentrations were measured preoperatively and at 4 years. Routine blood chemistries, including calcium, phosphate, and total alkaline phosphatase concentration, were measured daily for 2 weeks and thereafter at frequencies dictated by clinical events.

Laboratory measurements

Routine blood chemistries, including calcium (adjusted for albumin), alkaline phosphatase, and creatinine, were measured using a DAX autoanalyzer (Bayer Diagnostics, Basingstoke, UK). Plasma cyclosporine concentrations were measured by a monoclonal cyclosporine-specific radioimmunoassay (Incstar, Stillwater, MN, USA). PTH was measured by two-site radioimmunoassays for intact PTH supplied by Diagnostic Product Corp., Los Angeles, CA, USA. At the time of transplantation, the Immulite assay was used, while repeat PTH estimations at 4 years were measured using the poly-poly Immulite 2000 that used the same polyclonal antibodies. The normal ranges for these assays were 10 to 65 pg/mL and 1.1 to 6.8 pmol/L, respectively. Intra-assay and interassay coefficient of variations were less than 7% in the range of our patients' results.

Bone densitometry

DXA was performed using a Lunar DPX scanner (Lunnar Radiation Corp., Madison, WI, USA). We measured BMD at the second, third, and fourth lumbar vertebrae in the anterior-posterior projection (L2-4) and at the femoral neck. In-house precision of these measurements was <2% for all indices. BMD was expressed in g/cm^2, calibrated against calcium hydroxapatite and reproducibility of repeated measurements using the machine was also <2%. The results were compared with the United Kingdom reference database (age/gender matched) to generate a T score.

Statistical analysis

Paired Student *t* tests (two-tailed) were used to compare intragroup changes of the regional BMD. Regression analysis was used to relate serum PTH concentrations to the changes in BMD. *P* values of less than 0.05 (two-tailed) were considered to indicate statistical significance.

RESULTS

Serum biochemistry

At the time of transplantation, both pamidronate and control patients had similar serum PTH concentrations (30.6 ± 9.4 pmol/L vs. 21.9 ± 7.9 pmol/L, respectively, P = NS), serum alkaline phosphatase, and calcium concentrations (Table 2). At 4 years, there was no statistically significant difference in mean serum creatinine concentrations between pamidronate and control patients (180 ± 18 μmol/L vs. 138 ± 9.5 μmol/L, respectively, P = NS). In addition, there were no significant differences in the mean serum calcium and alkaline phosphatase concentrations between the two groups. However, the mean 4-year serum PTH concentration in the pamidronate group of patients was significantly higher than in the control patients (15.8 ± 2.1 pmol/L vs. 9.8 ± 1.8 pmol/L, resepectively, $P < 0.05$ by Student *t* test).

Table 2. Biochemistry

Serum concentration	Pamidronate ($N = 9$)			Control ($N = 8$)		
	Pretransplant	1 year	4 years	Pretransplant	1 year	4 years
Creatinine *μmol/L*		160 (6)	180 (18)		148 (17.0)	138 (9.5)
iPTH *pmol/L*	30.6 (9.4)	11.5 (2.8)[a]	15.8 (2.13)[a]	21.8 (8.1)	9.2 (1.9)[a]	9.8 (1.8)[a,b]
Calcium *mmol/L*	2.42 (0.11)	2.38 (0.05)	2.38 (0.05)	2.41 (0.10)	2.41 (0.04)	2.46 (0.03)
Alkaline phosphatase *IU/L*	86.4 (12.1)	62.4 (9.9)	55.2 (7.0)	67.9 (10.1)	72.4 (8.5)	57.6 (6.1)

[a] $P < 0.05$ vs. pretransplant values; [b] $P < 0.05$ vs. 4-year pamidronate group

Table 3. Immunosuppression protocol

	Cyclosporine *mg/kg/day*	Azathioprin *mg/kg/day*	Prednisolone *mg/day*
Day 1	10	2	80
Day 2 and 3	8	2	20
Day 4 to week 4	Titrate dose to serum	2	20
Weeks 4–8	concentration of:	2	15
Weeks 8–12	200–250 μg/L	2	10
Week 12 to month 12	150–200 μg/L	2	10
After 1 year	100–150 μg/L	2	10

Rejection and immunosuppression

A total of 15 episodes of acute rejection were diagnosed and treated. Five patients in the pamidronate group received a total of eight courses of pulsed methylprednisolone, while six patients in the control group suffered a total of seven episodes of acute rejection. Glucocorticoids were not withdrawn in any patients in either group. The mean dose of prednisolone prescribed to the pamidronate group of patients was 8.9 ± 0.4 mg/day vs. 7.8 ± 0.8 mg/day (P = NS) for the control patients. Mean blood cyclosporine concentrations at 4 years were similar in the pamidronate and control patients (151 ± 12 μg/L vs. 132 ± 6.6 μg/L, P = NS).

Bone mineral density

At the femoral neck, significant reduction of BMD was found in the control patients at 1 and 4 years post transplantation (Fig. 1A). The mean BMD was 1.08 ± 0.07 g/cm^2 at the time of transplantation, falling to a mean BMD of 0.98 ± 0.06 g/cm^2 at 1 year ($P < 0.005$) and 0.94 ± 0.06 g/cm^2 at 4 years ($P < 0.01$). The mean percentage reduction of BMD over 1 and 4 years were 8.8 ± 2.2% and 12.3 ± 2.8%, respectively. In these control patients, five of eight patients experienced >10% reduction of BMD at the femoral neck. In contrast, the 4-year femoral neck BMD of patients who received pamidronate was not significantly different from baseline. BMD at baseline was 0.93 ± 0.05 g/cm^2, at 1 year it was 0.94 ± 0.04 g/cm^2, and at 4 years it was 0.88 ± 0.04 g/cm^2, P = NS (Fig. 1A). However, three patients experienced bone loss >10% (−10.9%, −13.5%, and –16.2%). Neither the initial nor 4-year PTH concentration correlated with the 4-year femoral neck BMD or the change in femoral neck BMD.

Control patients experienced a significant reduction of lumbar spine BMD at 1 year (baseline BMD 1.27 ± 0.07 g/cm^2 while 1-year BMD was 1.20 ± 0.05 g/cm^2, $P <$ 0.05). This was not seen in pamidronate-treated patients (baseline BMD 1.15 ± 0.07 g/cm^2 vs. 1.11 ± 0.05 g/cm^2 at one year, P = NS). At 4 years, neither the control nor the pamidronate group of patients showed statistically significant reductions in lumbar spine BMD from baseline (BMD at 4 years was 1.21 ± 0.08 g/cm^2 in the control and 1.10 ± 0.04 g/cm^2 in the treatment groups) (Fig. 1B). Neither the initial nor 4-year PTH concentration correlated with the 4-year lumbar spine BMD or the change in lumbar spine BMD.

DISCUSSION

Adverse skeletal outcomes after renal transplantation and measures to prevent them remain a matter of intense interest [10]. We have previously shown that two doses of pamidronate, given at the time of renal transplantation and 1 month later, can protect the skeleton from early bone loss [7]. Grotz et al [8] studied the effects of ibandronate given at three monthly intervals during the first year after renal transplantation and showed similar early protection against bone loss. They also showed a reduction of spinal deformation. However, issues regarding the appropriate duration of prophylaxis and identification of patients requiring prolonged therapy remain unresolved.

We have now demonstrated that early prophylaxis

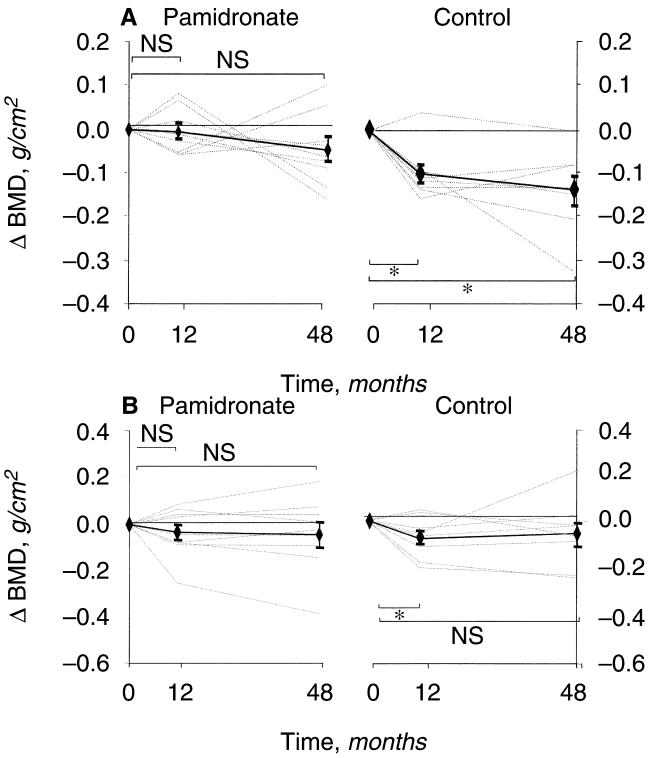

Fig. 1. Effect of renal transplantation on bone mineral density (BMD) at (*A*) femoral neck and (*B*) lumbar spine. Individual patient's ΔBMD are shown in dashed gray lines and mean (SEM) ΔBMD are shown in solid black lines. $*P < 0.005$.

with two doses of pamidronate not only prevented statistically significant loss of BMD at the femoral neck and the lumbar spine at 1 year after renal transplantation [7], but also provided sustained protection at the femoral neck up to 4 years after transplantation. By contrast, patients who did not receive pamidronate lost a statistically significant amount of bone at the femoral neck at 1 and 4 years (mean loss was 8.8 ± 2.2% and 12.3 ± 2.8% respectively). The BMD at the lumbar spine of control patients fell significantly 1 year after transplantation (mean loss was 5.2 ± 2.4%) but appeared to recover at 4 years such that there was no statistical difference in BMD compared with baseline. Although controlled, this study was small and of low power. However, it provides the first insight into the long-term action of bisphosphonates following renal transplantation.

In this study, the mean 4-year PTH concentration was significantly higher in the pamidronate group than the controls. Although PTH can be anabolic to bone under certain conditions (particularly if administered in a pulsatile manner) and a license for PTH as a treatment of osteoporosis has been sought in the United States, sustained hyperparathyroidism increases bone resorption and is an important cause of secondary osteoporosis. However, in this study, neither the pretransplant nor the 4-year PTH concentration correlated with bone loss or absolute BMD at either site measured. Although this may seem counter intuitive, a correlation between PTH and bone loss after transplantation has only been found in one study and was limited to women [3].

The pathogenesis of posttransplant osteoporosis is multifactorial. Bone histomorphometry at 6 months after renal transplantation was compatible with the toxic effect of glucocorticoids [1]. Moreover, calcineurin inhibitors and persisting hyperparathyroidism are known to increase bone resorption. However, Monier-Faugere et al [11] have recently shown that the majority of patients after renal transplantation have both generalized and focal osteomalacia on bone biopsy. Carlini et al [12] also showed similar bone pathologies, but encouragingly the abnormalities of bone formation rate and mineralization improved with time and approached normal after approximately 10 years. These findings have raised the possibility that prolonged treatment with bisphosphonates with extended biologic activity might further reduce bone turnover and potentially increase morbidity. It is, therefore, important to ascertain if bone loss continues at a high rate after the initial 12 months posttransplantation.

Results from this and our previous study [7] showed that the high rate of bone attrition at the femoral neck during the first few months slows thereafter (equivalent to 20% per annum at 3 months, 9% per annum at 12 months, and 3% per annum at 4 years). This contrasts with the bone loss that is experienced by women during menopause which is much slower at 1% to 2% per year. However, there was wide variation in bone loss whether or not patients received pamidronate. One third of the pamidronate-treated patients lost >10% BMD at the femoral neck over the 4-year study period. Thus, continued vigilance of patients' BMD is necessary to identify those who might benefit from continued treatment. Such a strategy minimizes the number of patients exposed long-term to the potential deleterious effects of bisphosphonates, a concern as osteomalacic lesions are present in many posttransplant patients [11]. It is encouraging to find that for the majority of patients, the two-dose pamidronate regimen was well tolerated and appears to provide considerable long-term skeletal protection.

A major limitation of this study is that it is powered to only examine intragroup changes in BMD within patients who did or did not receive pamidronate after transplantation but was not designed nor powered to examine the effects of bisphosphonates at reducing the bone fracture rates or bone histomorphometry. Larger studies powered to examine clinical end points directly are required to define further how we should monitor and treat patients after renal and other solid organ transplants.

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