Pamidronate therapy as prevention of bone loss following renal transplantation

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Background. Very rapid bone loss, osteopenia and skeletal morbidity after renal transplantation have been well documented and found to occur in a sex dependent fashion. Glucocorticoids, cyclosporine and pre-existing uremic osteodystrophy have been implicated in the pathogenesis of the skeletal lesions. Glucocorticoid induced osteopenia is also a serious clinical problem in patients with various nonrenal diseases and can be prevented, or at least attenuated, by pamidronate and other bisphosphonates.

Method. We prospectively studied 26 male patients undergoing renal transplantation, and randomized them to receive either placebo or intravenous pamidronate (0.5 mg/kg) at the time of transplantation and again one month later. All patients received immunosuppression comprising prednisolone, cyclosporine and azathioprine. The bone mineral density (BMD) of the second, third and fourth lumbar vertebrae and of the femoral neck was measured at the time of transplantation and at three months and 12 months after transplantation using dual energy X-ray absorptiometry (DXA).

Results. Twelve months after transplantation, the mean (± SEM) BMD of the lumbar vertebrae in patients who received placebo had decreased 6.4% (P < 0.05). In contrast, patients who received pamidronate experienced no significant reduction of BMD at the lumbar vertebrae. At the femoral neck, placebo-treated patients showed a reduction of BMD of 9% (P < 0.005), whereas there was no significant change in the pamidronate treated group. The two study groups had similar patient profiles, serum parathyroid hormone (PTH) and aluminium concentrations. After transplantation, comparable falls in the serum creatinine and PTH concentration were found in the two groups. Apart from transient hypocalcemia in two patients, no significant adverse effects of pamidronate were noted.

Conclusion. This study has shown that the early rapid bone loss that occurs in men during the first 12 months after renal transplantation can be prevented by two intravenous doses of pamidronate given at transplantation and one month later. The regimen was simple to administer, well tolerated and potentially applicable to other clinical groups of glucocorticoid treatment patients.

Renal transplantation is associated with marked skeletal morbidity. Osteopenia is frequent and the fracture risk is increased [1]. This appears likely to be the result both of unresolved abnormalities of bone and mineral metabolism related to previous uremia (residual hyperparathyroidism, low turnover or high turnover osteodystrophy) and also of new deleterious consequences of transplantation and its treatment. Dalen and Alvesstrand found accelerated bone loss following transplantation [2], and Julian et al [3] while confirming rapid early bone loss, also demonstrated that the histological changes seen in bone six months after transplantation were consistent with the toxic effects of glucocorticoids.

We have previously shown that the rapid bone loss during the first year after renal transplantation occurred in a sex dependent fashion: females lost bone mainly from the lumbar spine and males from the femoral neck [4]. These studies and others [2, 3, 5] have shown that bone loss during the first six months occurs at a very high rate, with subsequent slowing and in some cases stabilization of bone density [6]. Glucocorticoids and cyclosporine are thought to be implicated, although the role of the latter is controversial [7, 8]. Whereas cyclosporine was shown to increase bone remodeling in some animal models, and thereby lead to a high remodeling osteopenia with accelerated resorption [9], bone resorption in vitro is inhibited by cyclosporine [10]. Glucocorticoids inhibit intestinal calcium absorption [11], increase calciuria (with resulting subtle hyperparathyroidism) and reduce the secretion of sex hormones [12, 13]. Glucocorticoids also have direct and complex effects on bone metabolism [14] with clear-cut osteoblast toxicity and enhanced resorptive responsiveness to parathyroid hormone (PTH) [15, 16]. Thus, the renal transplant recipient faces a formidable array of risk factors for bone loss.

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Table 1. Clinical details

<table>
<thead>
<tr>
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<th>Control</th>
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</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Age (range)</td>
<td>53 (23–66)</td>
<td>50 (23–74)</td>
</tr>
<tr>
<td>Modality hemodialysis: CAPD</td>
<td>7:7</td>
<td>5:7</td>
</tr>
<tr>
<td>Time on dialysis years</td>
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<td>4.75</td>
</tr>
<tr>
<td>Number with diabetes mellitus</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Etiology of renal failure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic glomerulonephritis</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Polycystic kidneys</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Diabetic nephropathy</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Dysplastic kidneys</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Unknown/others</td>
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</tr>
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On the background, we have now studied the impact of anti-resorptive therapy given in the immediate post-transplant phase. In the knowledge that bisphosphonates have been found to be effective in the treatment of glucocorticoid induced osteopenia in patients without renal disease [17], we have now evaluated the ability of pamidronate to prevent the early bone loss following renal transplantation. Pamidronate has been used in patients with renal failure who have hypercalcemia secondary to malignancy [18, 19], and it has also been shown to be effective at preventing bone loss in patients receiving glucocorticoids for respiratory [20] and rheumatological [21] diseases. In the absence of data on the use of pamidronate in the immediate post-transplant period, we selected a dose in the lower range of that used to treat hypercalcemia due to malignancy. Our previous finding of marked sex differences in the pattern of bone loss in this setting [4] led us to study one sex only, in this case, men.

METHODS

Subjects

We studied 26 male patients admitted for cadaveric kidney transplantation. The study was approved by the local institutional review body and all patients provided written informed consent before the start of the study. The patients were randomized to receive either pamidronate (14 patients) or placebo (12 patients).

The details of the enrolled patients are summarized in Tables 1 and 2. The patients in the two groups were comparable with respect to age, duration of previous dialysis, previous transplant history and pretransplant biochemistry, including PTH, total alkaline phosphatase, serum aluminium, calcium and phosphorous.

Study protocol

Patients were randomized individually to one of two groups. One group received pamidronate (0.5 mg/kg in 500 mL 0.9% saline intravenously preoperatively and again 1 month postoperatively) and the other group received placebo (500 mL 0.9% saline) at the same time points. 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 rejection with either three 500 mg doses of methylprednisolone or a 10 day course of anti-thymocyte globulin (Me-rieux Transplant Ltd., Maidenhead, UK).

Dual energy X-ray absorptiometry (DXA) was performed in the first week after transplantation, at three months and at one year. Serum intact PTH concentrations were measured preoperatively and 12 months postoperatively. Serum aluminium concentrations were measured preoperatively. Routine blood chemistry including calcium and phosphate was monitored daily for two weeks, and thereafter at a frequency dictated by clinical events.

Laboratory measurements

Parathyroid hormone was measured by a two site radioimmunoassay for intact PTH (Diagnostic Product Corp., Los Angeles, CA, USA). The normal range for this assay is 10 to 65 pg/ml, with intra-assay and interassay coefficient of variations (CV) <7% over the range of 10 to 1000 pg/ml. Routine blood chemistry including calcium (adjusted for serum albumin [22]), phosphate and alkaline phosphatase was measured in serum using a DAX autoanalyzer (Bayer Diagnostics, Basingstoke, UK). Plasma cyclosporine levels were measured by a monoclonal cyclosporine specific radioimmunoassay (Incastar, Stillwater, MN, USA), with inter assay and intra-assay CVs of 7 to 10%. Serum aluminium was measured by flameless atomic absorption spectrometry [23].

Bone densitometry

Dual energy X-ray absorptiometry (DXA) was performed using a Lunar DPX scanner (Lunar Radiation Corp., Madison, WI, USA). We measured bone mineral density (BMD) at the second, third and fourth lumbar vertebrae in the anterior-posterior projection (L2-4), and at the femoral neck. The in-house precision of these measurements was <2% for all indices. BMD was expressed in g/cm², calibrated against calcium hydroxyapatite, and the reproducibility of repeated measurements using the machine was also <2%. The scans were performed by a single operator throughout the study. The results were compared with the UK reference database (age/sex-matched) to generate a T score.

Statistical analysis

Paired Student's t-tests (two tailed) were used to compare intragroup changes of the regional BMD. Regression analysis was used to relate serum PTH and aluminium concentrations to the changes in BMD. P values of
Table 2. Biochemistry

<table>
<thead>
<tr>
<th></th>
<th>Normal range</th>
<th>Pamidronate</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pretransplant</td>
<td>1 year</td>
<td>Pretransplant</td>
</tr>
<tr>
<td>Serum calcium mmol/L</td>
<td>2.10–2.60</td>
<td>2.41 (0.07)</td>
<td>2.41 (0.05)</td>
</tr>
<tr>
<td>Serum phosphate mmol/L</td>
<td>0.8–1.4</td>
<td>1.55 (0.12)</td>
<td>1.10 (0.06)</td>
</tr>
<tr>
<td>PTH pg/ml</td>
<td>10–65</td>
<td>305 (83)</td>
<td>108 (19)</td>
</tr>
<tr>
<td>Creatinine μmol/L</td>
<td>44–120</td>
<td>169 (13)</td>
<td>71 (7)</td>
</tr>
<tr>
<td>Alkaline phosphatase IU/L</td>
<td>30–130</td>
<td>121 (22)</td>
<td>86 (13)</td>
</tr>
<tr>
<td>Aluminium μg/L</td>
<td>&lt;50</td>
<td>27 (8)</td>
<td>19 (8)</td>
</tr>
</tbody>
</table>

Results are expressed as means (SEM).

Table 3. Immunosuppression protocol

<table>
<thead>
<tr>
<th></th>
<th>Cyclosporine</th>
<th>Azathioprine</th>
<th>Prednisolone</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>mg/kg/day</td>
<td>mg/kg/day</td>
<td>mg/kg/day</td>
</tr>
<tr>
<td>Day 1</td>
<td>10</td>
<td>2</td>
<td>80</td>
</tr>
<tr>
<td>Day 2–3</td>
<td>8</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Day 4–week 4</td>
<td>Titrated according to serum levels 200–250 μg/L</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Week 4–week 8</td>
<td>2</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Week 8–week 12</td>
<td>2</td>
<td>2</td>
<td>10</td>
</tr>
</tbody>
</table>

RESULTS

Twenty-six patients were enrolled and 25 patients (13 pamidronate and 12 control) were alive with a functioning graft at 12 months and able to complete the study. One patient who received pamidronate was undergoing his third transplant. He suffered intractable rejection and became dialysis dependent after 11 months. Analyses have been confined to the 25 patients completing the study protocol.

Serum biochemistry

At the time of transplantation, both groups of patients (placebo and treatment) had similar serum PTH concentrations (261 ± 77 pg/mL vs. 305 ± 83 pg/mL, P > 0.7), blood aluminium concentrations (19 ± 8 μg/L vs. 27 ± 8 μg/L, P > 0.5) and serum alkaline phosphatase concentrations (71 ± 1U/L vs. 121 ± 22 IU/L, P > 0.05; Table 2). At baseline, neither serum calcium nor serum phosphate differed between the two groups.

Sustained and significant falls in serum creatinine and phosphate concentrations were seen in both groups of patients. Final values of serum creatinine were 149 ± 13 μmol/L in control and 169 ± 16 μmol/L in pamidronate treated patients. Median PTH decreased to a similar extent in both groups, but nevertheless remained above normal in both groups (Table 2).

Two pamidronate treated patients experienced marked but asymptomatic reduction of serum calcium concentration. The nadirs were 1.62 mmol/L and 1.61 mmol/L on day 7. They were treated with alphacalcidol to a maximum dose of 3 μg daily with rapid tapering to zero during the first month, and both patients were given and tolerated the second dose of pamidronate without requirement for further support of serum calcium concentration. No other side effects were encountered.

Immunosuppression

There were no significant differences in the doses of prednisolone, azathioprine and cyclosporine prescribed, nor of the trough blood cyclosporine concentrations in the two groups of patients over the 12 months. In the control group, four patients received methylprednisolone for rejection and one required a second course (each of 3 bolus of 500 mg/day). Six patients treated with pamidronate received methylprednisolone, and three required a second course.

Bone mineral density

At the femoral neck, significant reduction of BMD was found in the control patients three months after transplantation (1.00 ± 0.06 vs. 0.95 ± 0.07 g/cm², mean ± SEM, P < 0.05). Between three months and one year, there were no significant changes in BMD, which remained significantly below baseline. At 12 months, 10 of the 12 patients still manifested a lower femoral neck BMD than at baseline, with a reduction of the mean BMD to 0.92 ± 0.06 g/cm² (P < 0.005 when compared with baseline). These reductions were equivalent to 5% at three months and 9% at 12 months. No such reduction of mean BMD at the femoral neck was seen in the pamidronate treated patients. The mean BMD at the femoral
neck was $0.94 \pm 0.06$ g/cm$^2$ at baseline versus $0.92 \pm 0.04$ g/cm$^2$ at three months and $0.94 \pm 0.05$ at 12 months (all NS; Fig. 1). Ten of the 13 patients who received pamidronate experienced a reduction of BMD at the femoral neck at three months, but progressive loss of BMD occurred in only four of the 13 patients.

Figure 1. Effect of renal transplantation on bone mineral density (BMD) at the femoral neck. Changes in BMD in (A) control patients and (B) pamidronate treated patients.

The T score at the femoral neck showed similar trends. At the time of transplantation the mean T score in the control group of patients was $-0.64 \pm 0.49$ and fell to $-1.32 \pm 0.47$ at 12 months. This included four patients who had a T score less than $-1.5$ at baseline, increasing to five at 12 months. In contrast, there was an improvement in the BMD at the femoral neck in two patients who received pamidronate such that their T score increased to over $-1.5$ during the 12 month period after transplantation. No patient in the pamidronate group experienced a fall of their T score to less than $-1.5$ (the mean T scores at baseline were $-1.13 \pm 0.44$ and $-1.10 \pm 0.45$ at 12 months; Tables 4 and 5).

At the lumbar spine, progressive reduction of BMD was found in the control patients reaching statistical significance by 12 months after transplantation ($1.26 \pm 0.06$ vs. $1.17 \pm 0.04$ g/cm$^2$, $P < 0.05$, equivalent to a loss of 6.4%). At this site, 9 of the 12 patients manifested BMD reduction compared with baseline. In contrast, patients treated with pamidronate experienced no reduction of lumbar spine BMD at 12 months, although there was a transient reduction of BMD at three months (Fig. 2). The mean BMD of the pamidronate treated group at 12 months was indistinguishable from that at baseline ($1.17 \pm 0.06$ vs. $1.14 \pm 0.05$ g/cm$^2$, $P = NS$) and eight of the 13 patients lost BMD over this period.
At the lumbar spine, two control patients with a T score above -1.5 at the time of transplantation experienced a fall in their T score to below this level at 12 months. At 12 months, four of the 12 patients had a T score below -1.5. In contrast, none of the pamidronate treated patients experienced a fall to below this threshold over the 12 month period, although the four patients who were below this level remained so.

Regression analysis done in both groups separately and combined showed that the bone loss at both sites after transplantation was not predicted by age or by the final level of renal function as judged by serum creatinine.
DISCUSSION

Adverse skeletal effects of glucocorticoids and measures to prevent them are matters of intense interest [20, 21, 24]. We and others have previously shown that the bone loss rate after renal transplantation is exceptionally high [2–5]. In the present study, we have found that the early rapid bone loss that occurs in men during the first 12 months after renal transplantation can be prevented by a simple regimen comprising two intravenous doses of pamidronate given at transplantation and again one month later. As judged by the results in the untreated patients, without such prophylaxis the skeleton is at risk of losing substantial amounts of bone during the first year: −0.08 ± 0.03 g/cm² (6.4%) at the lumbar spine and −0.09 ± 0.02 g/cm² (8.8%) at the femoral neck in this instance, and even more in some other studies [1–3].

Guidance on the management of corticosteroid induced osteoporosis has been provided by the National Osteoporosis Society and is endorsed by the British Society for Rheumatology and the Royal Society of Medicine. They have suggested that a T score less than −1.5 indicates a sufficiently high risk of osteoporotic fractures to justify consideration of treatment with a bisphosphonate. This is consistent with the European Regulatory Guidelines [25] recently proposed. Adachi et al have shown that 15% of patients who are treated with corticosteroids and have a T score less than −1.44 suffer new vertebral fractures [17]. In our study, no patients who received pamidronate at the time of transplantation lost BMD at the lumbar spine to below this critical T score. In contrast, two patients in the control group experienced a fall of BMD at the lumbar spine to less than a T score of −1.5. Similar results were found at the femoral neck; no patients in the pamidronate treated group experienced a fall in BMD such that their T score fell below −1.5, but one patient in the control group experienced a significant decline in BMD. Taken together, these results suggest that a bisphosphonate given at the time of kidney transplantation will act as an effective anti-resorptive agent and prevent significant bone loss.

The regimen of two intravenous doses of pamidronate was well tolerated. No discernible side effects were encountered apart from transient hypocalcemia in two subjects; febrile reactions sometimes associated with pamidronate were not seen and were almost certainly prevented by the concomitant steroid therapy. The intravenous regimen ensured high compliance and avoided the practical difficulties of oral bisphosphonates in these complex patients receiving multiple therapies.

The study did not demonstrate any significant detrimental effect on renal function. There were more episodes of cellular rejection in the treatment group, but the difference was small (5 vs. 9) and not significant. There are no data to suggest that bisphosphonates have immunomodulatory actions that could account for an increased frequency of rejection. The serum half-lives of the bisphosphonates are in the order of 0.5 to 2 hours, and although much of the administered dose remains in the skeleton for many years, it is in an inert location and unlikely to alter the frequency of rejection.

The very rapid early bone loss in these patients, largely abrogated by pamidronate in this study, is probably due to the combination of relatively high glucocorticoid and cyclosporine exposure and persisting hyperparathyroidism. If cyclosporine is, as suggested by some authors [9, 26], capable of accelerating bone resorption in clinical practice, the combination of reduced formation (glucocorticoid induced) and increased resorption (cyclosporine and in some patients persistent hyperparathyroidism) would have serious skeletal consequences. It is encouraging that, in the early months at least, this appears preventable by means of the simple anti-resorptive regimen described here.

Not addressed by this study is the question of longer term anti-resorptive or other therapies in renal transplant recipients. Although still not established with certainty, it is possible that cyclosporine-driven bone resorption remains inappropriately high, even when the glucocorticoid therapy has been reduced to very low doses or was withdrawn altogether [8]. Continuing hyperparathyroidism, present in many transplanted patients as confirmed in this study, is likely to exacerbate this further. Cyclic therapy with clodronate has been shown to induce a gain in BMD in patients with documented osteopenia after renal transplantation [27], and thus there may well be a role for longer term bisphosphonate therapy to counter cyclosporine driven resorption. Conversely, it should be remembered that progressive osteopenia may not occur in all patients beyond 24 months of transplantation. Data from Grotz et al suggest that bone density may even increase in patients transplanted for more than three years, although many of those studied may not have been treated with cyclosporine [6].

Calcitonin, vitamin D analogues and calcium supplements have also been proposed as effective therapeutic agents to reduce the bone loss associated with glucocorticoids [24, 28]. Oral calcitriol and calcium administered to patients in the first year of starting glucocorticoid was effective at preventing significant bone loss during the first year of treatment. However, this effect was not sustained and bone loss was detected after two years [24]. The efficacy of intranasal calcitonin is unproven. Sodium
fluoride is attractive in that it directly stimulates bone formation [29–31]. However, no convincing data exist regarding sodium fluoride treatment in renal transplant recipients.

In conclusion, this study has demonstrated the effectiveness of two intravenous doses of pamidronate in the prevention of femoral neck and lumbar spine bone loss in men during the first 12 months after renal transplantation. The regimen used was well tolerated, simple to administer and required minimal additional monitoring of the patients during the post-transplant period. Additional studies are needed in females in whom the need for effective countermeasures to post-transplant osteopenia may be even greater. The role of this, or similar, regimens in other groups of patients commencing glucocorticoid therapy remains to be defined, as does the necessary duration of treatment. The emerging understanding of the actions of glucocorticoids and cyclosporine on bone should help to direct further clinical studies in these high risk patients.

Reprint requests to Dr. Cunningham, Department of Renal Medicine and Transplantation, The Royal London Hospital, Whitechapel, London E1 1BB, England, United Kingdom.

REFERENCES