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Original Article

Mechanisms of Rapid Bone Loss Following Cardiac Transplantation

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Abstract. Rapid bone loss after orthoptic cardiac transplantation (OHTX) is a major problem; however, the mechanisms are poorly understood. To investigate these mechanisms we measured biochemical and hormonal indices of bone turnover serially in 25 patients (21 men, 4 women) after OHTX. Serum osteocalcin was reduced immediately post-OHTX (2.2 ± 0.5 ng/ml) but rose significantly by 6 and 12 months (14.1 ± 2.5 and 15.7 ± 2.2 respectively). Bone resorption indices (urinary hydroxyproline/creatinine and calcium/creatinine ratios) were increased immediately post-OHTX but fell by 6 months. Serum testosterone was reduced in males but recovered towards normal values by 6–12 months. Regression analysis showed lumbar bone loss was predicted independently by the change in both serum osteocalcin and testosterone. The data suggest that bone loss post-OHTX is due to a combination of accelerated turnover and hypogonadism.

Keywords: Corticosteroids; Cyclosporine; Hydroxyproline; Osteocalcin; Testosterone; Transplantation

Introduction

Osteoporosis is a major complication of organ transplantation [1–5]. Lumbar and femoral neck bone density have been reported to be reduced by 8%–13% compared with age-matched controls after bone marrow transplantation [1]. After renal transplantation vertebral bone density falls rapidly within 6 months and histomorphometry shows decreased bone formation, consistent with a corticosteroid effect [2]. Vertebral fractures are also frequent after orthoptic cardiac transplantation (OHTX), occurring in up to 35% of patients [4]; however, serum osteocalcin, a marker of bone formation, is increased [4,6] suggesting that the pathogenesis of bone loss after OHTX is not purely due to corticosteroid-induced bone loss. Cyclosporine has been shown to induce high-turnover osteopenia in the rat [7] and so may also contribute to bone loss post-OHTX.

We have previously observed in a preliminary report that the most rapid bone loss occurs from the lumbar spine post-OHTX [8]. In this report we aimed to investigate mechanisms of bone loss after OHTX by relating changes in biochemical and hormonal indices of bone turnover to bone loss and immunosuppressive therapy.

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Patients and Methods

Between 1984 and 1992, 318 cardiac transplants have been performed at St. Vincent's Hospital [9]. During 1991, 25 consecutive patients (21 men and 4 women; mean age (± SD) 47.6 (± 12.8) years) had assessments of biochemical and hormonal markers of bone turnover and bone density immediately post-transplantation and at 6 and 12 months. Data at 12 months were unavailable in 1 patient who died and excluded in 5 patients who started calcitriol after the 6-month measurement as prophylaxis against further bone loss. One female was postmenopausal but not receiving oestrogen replacement. Of the 3 premenopausal females, 2 were amenor-

rhoeic and 1 was taking the oral contraceptive pill. All patients received immunosuppression with cyclosporine, azathioprine, prednisolone and anti-thymocyte globulin as previously described [8]. Oral prednisolone was administered at 0.5 mg/kg per day in divided doses until day 14, then reduced to 0.18 mg/kg per day for 3–6 months and thereafter reduced to 0.13 mg/kg per day maintenance where possible. No patient was withdrawn totally from steroids during the 12 months of follow-up. The mean (± SD) combined cumulative dose of prednisolone was 9.2 (± 5.0) and 2.8 (± 1.0)g between 0–6 and 6–12 months post-OHTX respectively. The mean cumulative dose of cyclosporine for the corresponding periods was 63.2 (± 20.0) and 61.0 (± 29.5) g respectively.

Serum and a second voided urine specimen (2-h test) were collected after an overnight fast in the first week after OHTX and at 6 and 12 months. Serum osteocalcin was determined by an in-house radioimmunoassay using a heterologous rabbit anti-sheep osteocalcin antiserum as previously described [10]. Parathyroid hormone (PTH) was measured using an immunoradiometric assay of the intact PTH molecule (Nichols Institute Diagnostics, San Juan Capistrano, CA). Urinary calcium was measured by titration using a Corning Calcium Analyser 940 (Halsted, Essex, UK) and hydroxyproline quantified using a AA-1 autoanalyser (Technicon, Tarrytown, NY). Both were expressed as a ratio relative to the urinary creatinine (Astra autoanalyser, Beckman Instruments, Brea, CA). Serum testosterone was measured in benzene-extracted serum with charcoal separation. Luteinizing hormone (LH) was measured by radioimmunoassay using a second antibody to separate bound and free, labelled hormone. Serum dehydroepiandosterone sulphate (DHEAS) was measured in unextracted serum with charcoal separation. Sex hormone binding globulin (SHBG) was measured by an immunoradiometric assay. Serum chemistry analyses were determined by automated methods by the Department of Chemical Pathology, St. Vincent's Hospital.

Bone mineral density (BMD, g/cm^2) was measured by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX-L densitometer in each patient on the same day as bone turnover indices were collected. Measurements were made of lumbar spine BMD (L2–4) and total body calcium. The co-efficient of variation of DXA is 1.5% for measurements at the lumbar spine [12] and for total body calcium at our institution. The radiation dose with the DXA method is <0.1 μGy [13].

Changes in biochemical markers were evaluated by an analysis of variance. The relationship between biochemical markers and bone density trends in lumbar spine BMD and total body calcium, corticosteroid and cyclosporine therapy was evaluated by the use of regression analysis. Subregion analysis of BMD trends were not analyzed in regard to biochemical markers since the majority of loss occurred in the lumbar spine [8].

Results

The changes in hormonal and biochemical parameters of bone turnover are shown in Table 1. Serum calcium did not change significantly during the 12 months of follow-up. Serum creatinine increased marginally over 12 months ($p<0.005$ for baseline v 12 months). Intact PTH did not change significantly over the 12 months.

Bone formation, as measured by serum osteocalcin, was suppressed immediately post-OHTX but had increased sixfold by 6 months with a further small rise by 12 months. Change in osteocalcin correlated significantly with daily cyclosporine dose ($r = 0.48$, $p<0.0001$ for $t_{12}-t_0$) and daily prednisolone dose ($r = 0.42$, $p<0.0001$ for $t_{12}-t_0$). Bone resorption, as measured by the urinary hydroxyproline/creatinine ratio (OHP/Cr) was increased immediately post-OHTX but subse-

Table 1. Biochemical and hormonal indices

	Baseline	6 months	12 months	Reference interval
Serum calcium (mmol/l)	2.31 (0.06)	2.38 (0.03)	2.39 (0.02)	2.10–2.60
Serum creatinine (mmol/l)	0.11 (0.01)	0.12 (0.01)	0.12 (0.01)*	0.04–0.12
Intact PTH (ng/l)	4.3 (0.5)	4.1 (0.5)	3.9 (0.4)	1–7
Osteocalcin (μg/l)	2.2 (0.5)	14.1 (2.5)**	15.7 (2.2)**	3–18
Urinary OHP/Cr	0.034 (0.004)	0.020 (0.002)**	0.015 (0.001)**	<0.016
Urinary Ca/Cr	0.38 (0.06)	0.35 (0.09)	0.22 (0.05)	<0.46
Testosterone (nmol/l)	8.6 (0.9)	15.5 (1.3)**	16.9 (1.8)**	12–36[a]
LH (IU/l)	7.8 (3.3)	5.4 (0.9)	12.6 (6.0)	3–20[a]
DHEAS (μmol/l)	1.3 (0.1)	1.3 (0.2)	1.4 (0.2)	5.3–9.0[a]
SHBG (nmol/l)	24.0 (2.0)	29.0 (3.0)	28.0 (3.0)	10–50[a]

Values are the mean (± SE) at baseline and 6 and 12 months. For abbreviations see text.
*$p<0.01$ and **$p<0.001$ versus baseline.
[a]Males only.

quently fell significantly by 6 months with a further non-significant decline between 6 and 12 months. The urinary calcium/creatinine ratio (Ca/Cr) followed a similar pattern. Serum testosterone in males was reduced immediately post-OHTX but rose significantly by 6 months with a further small rise between 6 and 12 months. The change in testosterone correlated significantly with daily cyclosporine dose ($r = 0.50, p<0.0001$ for $t_{12}-t_0$ and daily prednisolone dose ($r = 0.28, p<0.02$ for $t_{12}-t_0$). Serum DHEAS concentrations were uniformly reduced below the normal range at all three time points.

Lumbar spine bone loss was rapid (mean −6.7%, 95% CI −4.8 to −8.6% by 6 months; mean −8.8%, 95% CI −6.7 to −10.9% by 12 months). Backwards stepwise regression revealed lumbar spine loss was predicted by the change ($t_{12,6}-t_0$) in osteocalcin and testosterone (standardized coefficients −0.40 ($p<0.04$) and 0.29 ($p<0.01$) respectively for change at 12 months). Lumbar spine bone loss was correlated inversely with the 6-month value for serum osteocalcin ($r = -0.46$) but not testosterone. Correcting osteocalcin for renal function did not affect these relationships. Total body calcium also fell significantly but to a lesser extent (mean −2.4%, 95% CI −1.3 to −3.4% by 6 months; mean −2.8%, 95% CI −1.4 to −4.3% by 12 months) but the loss was not predicted by an biochemical markers.

Discussion

Osteoporotic fractures in patients undergoing OHTX have been attributed at least in part to the use of corticosteroid therapy. Corticosteroids cause bone loss by a variety of mechanisms. They increase bone resorption, probably largely due to decreased calcium absorption and increased urinary calcium loss causing secondary hyperparathyroidism; however, we observed no elevation of serum PTH in the present study. There was, however, a trend for as light reduction in PTH over 12 months.

Corticosteroids also inhibit osteoblasts and bone formation directly. Although we found serum osteocalcin was reduced immediately post-OHTX, an inverse relationship was observed between serum osteocalcin and the lumbar bone trend such that those patients with higher osteocalcin at 6 and 12 months lost more bone. The increase in serum osteocalcin suggests relatively active bone remodelling and is consistent with our earlier study of corticosteroid bone loss [14]. Although osteocalcin is excreted by the kidney, the rise in serum osteocalcin cannot be explained by impairment of renal function due to cyclosporine, which was only of mild degree, since serum osteocalcin does not rise until renal impairment is severe [15]. Other studies have also indicated that serum osteocalcin correlates inversely with bone density [16,17] and our data indicate that increased serum osteocalcin levels do not reflect protection from corticosteroid bone loss.

Corticosteroids also impair LH response to LH releasing hormone and adrenal sex steroid secretion is decreased by suppression of ACTH secretion. In the present study although testosterone levels were suppressed immediately post-OHTX, LH levels were mid range and did not change significantly. Importantly serum testosterone levels recovered over 6–12 months in most patients and the relationship between serum testosterone and lumbar bone loss was such that those whose testosterone rose most lost least bone, suggesting hypogonadism is an additional factor in bone loss after OHTX. DHEAS concentrations were low in the present study, probably due to suppression of ACTH by the corticosteroid therapy, although reduced DHEAS levels can occur as an adrenocortical adaptive mechanism in patients with severe chronic disease [18]. Sex hormones are largely bound to the glycoprotein SHBG, but as there was no change in SHBG levels this cannot explain the reduction in testosterone or DHEAS.

In an earlier cross-sectional study of OHTX patients, Shane et al. [4] observed elevated serum osteocalcin levels and considered the biochemical pattern was not typical of corticosteroid-induced bone loss. The authors speculated that cyclosporine may diminish the inhibitory effects of corticosteroids on bone formation and lead to a state of accelerated bone formation. Our baseline biochemical data were obtained immediately post-transplantation, so may reflect the effects of underlying disease or the surgery as well as drug effects. However, regardless of the causes for the biochemical and hormonal picture immediately post-OHTX, our data demonstrate that by 6–12 months bone formation matches resorption in a 'high-turnover' state. Importantly the degree of lumbar bone loss during that period is related both to the level of bone turnover and to the degree of hypogonadism. These data suggest that biochemical and hormonal markers may be able to identify those patients losing spinal bone most rapidly and raise the possibility of allowing therapeutic intervention in selected patients.

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