Transpl Int (2002) 15: 290–295
DOI 10.1007/s00147-002-0402-4

ORIGINAL ARTICLE

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Effects of a single infusion of pamidronate prior to liver transplantation: a bone histomorphometric study

Received: 5 December 2000
Revised: 19 June 2001
Accepted: 4 February 2002
Published online: 16 April 2002

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Abstract Osteoporosis is a common and serious complication of solid organ transplantation. Effective therapeutic regimens have not been established but evidence that increased bone turnover is responsible for bone loss early after transplantation provides a rationale for the use of anti-resorptive agents in the peri-operative period. We have examined the effects of a single pre-operative infusion of pamidronate, 60 mg, on bone remodelling and turnover in a prospective study of 12 patients, four male and eight female aged 19–61 years, with chronic liver disease, who formed a subgroup of a larger randomised controlled single-blind study. Iliac-crest biopsies were obtained before and 3 months after liver transplantation and histomorphometry performed using image analysis. In untreated patients ($n=5$) a significant increase in bone formation rate at tissue level was demonstrated at 3 months in comparison to pre-operative values (0.035 ± 0.013 vs. 0.161 ± 0.12 $\mu m^2/\mu m$/day; mean ± SD, $P=0.003$). In patients treated with pamidronate ($n=7$) no significant increase in bone formation rate was demonstrated at 3 months, although there was a trend towards an increase in indices of bone turnover. In this group there was also a significant reduction in erosion cavity length (210.4 ± 63.8 vs. 179.8 ± 67.5 μm; $P=0.03$) and non-significant reductions in other indices of erosion cavity size. These results indicate that pre-operative administration of pamidronate in patients with chronic liver disease prevents, at least in part, the increase in bone turnover which occurs in untreated patients after transplantation.

Keywords Liver transplantation · Osteoporosis · Pamidronate · Bone turnover

Introduction

Solid organ transplantation is associated with bone loss and increased fracture risk, resulting in significant morbidity [1, 2, 3, 4, 5, 6, 7, 8, 9]. Bone loss is most rapid during the first 3 to 6 months post-operatively, subsequent increases in bone-mineral density being reported in some studies. The pathogenesis of post-transplantation osteoporosis has not been clearly established but is likely to be multifactorial, glucocorticoid therapy, administration of other immunosuppressive agents, vitamin D deficiency, hypogonadism and pre-existing bone disease all being potential contributory factors [10, 11].

Effective treatment regimens for patients undergoing solid organ transplantation have not been definitively established. The early onset of bone loss post-operatively, and the demonstration, using bone histomorphometry, that there was a significant increase in bone turnover 3 months after liver transplantation [12] provide a rationale for the use of anti-resorptive therapy in the peri-operative period. The effects of the

bisphosphonate, pamidronate, on post-transplantation osteoporosis have been reported in several studies; these have generally demonstrated beneficial effects, although the design of the majority of studies was suboptimal. We have recently conducted a large randomised controlled trial of the effects of a single intravenous infusion of pamidronate prior to liver transplantation [13]. In this prospective study we report the effects of pamidronate on bone turnover and remodelling in a subgroup of patients recruited into the trial.

Patients and methods

Patients

Twelve patients with chronic liver disease (four male and eight female) aged 19–61 years (mean 47.3 years) taking part in a randomised controlled single-blind trial of pamidronate in the prevention of post-transplantation osteoporosis, consented to undergo bone biopsy before and 3 months after liver transplantation The cause of liver disease in these patients was primary biliary cirrhosis ($n=6$), alcoholic cirrhosis ($n=2$), sclerosing cholangitis ($n=1$), hepatitis C infection and hepatoma ($n=1$), hepatitis B cirrhosis ($n=1$) and Wilson's disease ($n=1$). All patients were undergoing their first liver transplantation. Five of the eight women were post-menopausal. None of the patients was receiving treatment with bisphosphonates, hormone replacement therapy or vitamin D.

Patients randomised to pamidronate therapy received a single dose of 60 mg by intravenous infusion before transplantation. No placebo preparation was given to the control group.

In five patients (two pamidronate treated, three control), immunosuppression consisted of peri-operative intravenous methylprednisolone, 10 mg/kg, followed by oral prednisolone 1 mg/kg/day reducing to a maximum of 30 mg/day at 1 month and 5–10 mg/day at 3 months. Five patients (three pamidronate treated, two control) received peri-operative methylprednisolone 10 mg/kg followed by oral prednisolone 20 mg daily, reducing to zero by 3 months. Two patients in the pamidronate-treatment group received an immunosuppressive regimen of Campath 1-H peri-operatively in addition to cyclosporin. No glucocorticoids were given to these patients except for the treatment of acute rejection in one case. Trough serum cyclosporin levels of 150–200 ng/ml were maintained immediately post-operatively, reducing to 125–175 ng/ml at 3 months, and azathioprine was administered at a dose of 1 mg/kg/day. Acute rejection, which occurred once in six patients, was treated with 3 g intravenous methylprednisolone over 3 days.

Trans-iliac biopsies were obtained pre-operatively and 3 months after transplantation from contralateral sides of the iliac crest, under local anaesthetic using a modified Bordier trephine with an internal diameter of 8 mm. Informed written consent was obtained from all patients, and the study was approved by the local ethics committee. All patients were given double tetracycline labelling prior to each biopsy [14]. All biopsies were coded and the histomorphometric analysis was performed "blind" by the same observer (S.V.).

Bone histomorphometry

Bone biopsies were embedded in LR white medium resin (London Resin, London, UK). Eight-micrometre undecalcified sections were stained using von Kossa and toluidine blue stains. Histomorphometric measurements were made using a 'Digicad' digitising tablet and cursor with an LED point light source (Kontron, München, Germany) and an Olympus BHS-BH2 binocular transmitted light microscope with a BH2-DA drawing attachment (Olympus Optical UK, London, UK.). All histomorphometric measurements are described according to ASBMR nomenclature [15].

Primary measurements

Bone area/tissue area (B.Ar/T.Ar), osteoid perimeter/bone perimeter (O.Pm/B.Pm) and osteoid seam width (O.Wi) were measured on von Kossa stained sections on a minimum of 25 fields from three to six sections. Osteoid width was measured at four approximately equidistant points or eight points on seams longer than 600 μm. A minimum of 20 seams per biopsy was measured on the same sections used for O.Pm. All seams with a width greater than or equal to 3 μm were measured.

Tetracycline labelling was viewed by fluorescence microscopy on a minimum of six 15-μm unstained sections at ×156 magnification. Mineralising perimeter (Md.Pm.) was calculated as follows:

$$\text{Md.Pm/B.Pm}(\%) = \text{dL.Pm} + (0.5 \times \text{sL.Pm})/\text{B.Pm}$$

where dL.Pm is the double-labelled perimeter and sL.Pm is the single-labelled perimeter.

The mean distance between double labels was measured directly at ×312 magnification. Measurements were made at approximately four equidistant points along each double label. A minimum of 20 labels was measured for each biopsy on a minimum of six sections.

Mineral apposition rate (MAR) was calculated as:

$$\text{MAR}(\mu\text{m/day}) = \text{L.Wi/LP}$$

where L.Wi is the inter-label width and LP is the labelling period (12 days).

Derived indices

The tissue-based bone formation rate (BFR/B.Pm) was calculated as follows:

$$\text{BFR/B.Pm}(\mu\text{m2}/\mu\text{m/day}) = \text{MAR} \times (\text{M.Pm/B.Pm}).$$

Measurement of resorption cavity characteristics

The method described by Garrahan et al. [16] was adapted for use with the digitising tablet and light cursor for measurement. Cavities were identified on toluidine blue-stained sections viewed under polarised light at ×156 magnification and measured at ×156, ×375 or ×750 magnification depending on the size of the cavity. Criteria for the identification of resorption cavities included interruption of lamellae at an angle to the bone surface, absence of osteoid seam and depth greater than 3 μm. A minimum of 20 cavities from four to six sections per biopsy was measured. The following indices were obtained for each resorption cavity: mean eroded depth (E.De, μm), maximum eroded depth (E.De Max, μm), eroded area (E.Ar, μm^2) and mean cavity length (E.Le, μm).

Structural analysis

Strut analysis [17] and assessment of trabecular bone pattern factor [18] and marrow star volume [19] were performed as described previously [20].

Statistical analysis

Log transformation of all non-normally distributed data was carried out. Paired data were compared by a two-tailed paired t-test. Data are expressed as mean ± SD.

Results

Demographic details of the 12 patients are provided in Table 1. The mean age was similar to that in the larger cohort participating in the randomised controlled trial (53 and 49 years in the treated and untreated groups, respectively), although in the larger study the male-to-female ratio was lower (1:1) and fewer patients had cholestatic liver disease (31%). Seven were randomised to pamidronate and five to the no-treatment group. Paired biopsies from all 12 patients were adequate for standard histomorphometric analysis. Structural analysis, which requires the presence of both intact cortices, was possible in only eight pairs of biopsies (pamidronate treated $n=5$, untreated $n=3$). The median interval between pamidronate administration and transplantation in the seven treated patients was 31 days (range 4–150).

Indices of bone remodelling before and after transplantation in patients treated with pamidronate and the untreated patients are shown in Table 2. In untreated patients a significant increase in bone turnover was demonstrated post-operatively, as reported previously in a separate cohort [12]. There was also a significant decrease in cancellous bone area ($P=0.013$). In the seven patients treated pre-operatively with pamidronate, no significant changes in bone turnover were seen 3 months post-operatively, although there was a trend towards increased bone turnover (bone formation rate 0.025 ± 0.037 vs. 0.062 ± 0.071 $\mu m^2/\mu m/day$).

Resorption cavity characteristics in both groups are shown in Table 3. In untreated patients, indices of resorption cavity size were similar at pre-transplant and post-transplant time points. In patients treated with pamidronate there was a trend towards reduction in resorption cavity size, with a significant reduction in mean cavity length. No significant changes were seen after liver transplantation in any of the structural indices assessed in either the treated or untreated group.

Discussion

Our results confirm the increase in bone turnover previously reported in the first 3 months after liver transplantation in untreated patients [12]; even in the small number of these patients in the present study, statistically significant increases in indices of bone turnover were seen. In contrast, no significant increase in bone turnover was observed in patients who had received pamidronate, although for all indices related to tissue-level turnover there was a trend towards an increase, suggesting that administration of pamidronate reduced but did not reverse the effects of transplantation on bone turnover. No significant changes in erosion cavity size were seen in untreated patients 3 months post-operatively, whereas there was a significant reduction in cavity length and a reduction in cavity area (which did not achieve statistical significance) in pamidronate-treated patients. Wall width was not measured in this study, since, as a result of the time course of bone remodelling in humans, changes would not be detectable over the study period of 3 months.

Bisphosphonates are widely used in the prevention and treatment of post-menopausal and glucocorticoid osteoporosis, reducing osteoclast numbers and activity and hence decreasing bone turnover. The precise mechanisms by which these effects are achieved have not been established and to some extent differ between bisphosphonates; however, inhibition of osteoclastogenesis and

Table 1. Demographic details of the patients

Characteristic	Pamidronate treated ($n=7$)	Untreated ($n=5$)
Age (years)	53.7	48.5
Male/female	1:6	3:2
Acute rejection (n)	3	3
Cholestatic liver disease (n)	5	2

Table 2. Bone remodelling before and after liver transplantation in patients treated with pamidronate and in untreated patients. Results are shown as mean ± SD

Variable	Pre-transplant + Pamidronate $n=7$	Post-transplant + Pamidronate $n=7$	Pre-transplant Untreated $n=5$	Post-transplant Untreated $n=5$
B.Ar/T.Ar (%)	17.9 ± 4.3	14.7 ± 5.9	21.7 ± 5.7	16.5 ± 5.1[a]
O.Pm/B.Pm (%)	$8.1\bullet8.9$	12.3 ± 10.9	6.4 ± 4.5	24.5 ± 15.8[b]
Md.Pm/B/Pm (%)	4.1 ± 5.7	10.7 ± 12.7	6.2 ± 2.7	25.0 ± 19.6[c]
O.Wi (μm)	7.0 ± 1.2	6.2 ± 1.5	8.4 ± 1.6	10.4 ± 2.1
BFR/B.Pm ($\mu m^2/\mu m/day$)	0.025 ± 0.037	0.062 ± 0.071	0.035 ± 0.013	0.161 ± 0.12[d]
MAR(μm/day)	0.54 ± 0.26	0.57 ± 0.07	0.58 ± 0.11	0.67 ± 0.11

[a] $P=0.02$
[b] $P=0.01$
[c] $P=0.002$
[d] $P=0.003$

increased osteoclast apoptosis [21, 22] both contribute and inhibitory effects on osteoblast and osteocyte apoptosis have also been reported [23]. Histomorphometric studies of the effects of bisphosphonates in humans are sparse. In patients with glucocorticoid-induced or post-menopausal osteoporosis, treatment with alendronate resulted in a significant reduction in bone turnover, with no detectable effect on erosion cavity size [24, 25]. In another study of the effects of oral pamidronate in post-menopausal women with osteoporosis, there was a trend towards a reduction in bone turnover, although this did not achieve statistical significance and similar changes were seen in the placebo group [26]. No evidence of impaired mineralisation, a potential concern with some bisphosphonates, was observed in any of these three studies, although histological evidence of osteomalacia has been described in patients with Paget's disease treated with high doses of intravenous pamidronate [27] and after administration of etidronate [28]. In the present study, no adverse changes in mineralisation were seen in either group after transplantation.

Effective preventive regimens for post-transplantation osteoporosis have not been established. Several intervention studies have been reported in patients undergoing liver transplantation but these have mostly been small and non-randomised. Combined treatment with calcium, alphacalcidol and cyclic etidronate did not prevent bone loss after liver transplantation in a non-randomised study reported by Riemens et al. [29]; moreover, 24% of treated patients suffered new vertebral fractures during the 1st year after transplantation. However, in another study both calcitonin and cyclic etidronate were reported to result in significant increases in vertebral bone-mineral density after 1 year's treatment in patients undergoing liver transplantation [30]. In a large, non-randomised study Neuhaus et al. [31] recently reported beneficial effects on bone-mineral density of various combinations of calcitriol, calcium and sodium fluoride, treatment being commenced 6 months post-transplantation and continued for 2 years.

In a non-randomised study in which historical controls were used, Reeves et al. [32] reported that 38% of untreated patients developed vertebral fracture post-transplantation compared with none of the 13 patients treated with intravenous pamidronate, 60 mg every 3 months before and for 9 months after transplantation. In the large randomised controlled trial of intravenous pamidronate in which the patients in the present study were participating, no significant treatment benefits of pamidronate were detected [13], significant bone loss being observed during the 1st year in both groups in the proximal femur, whereas none was seen in either group in the lumbar spine; moreover, the fracture incidence was very low in both groups, indicating that the more recent use of lower doses of glucocorticoids and selection of patients for transplantation earlier in the natural history of their disease may have resulted in a much lower incidence of post-transplantation bone disease.

Our study has several limitations. The number of patients studied was small, although the use of paired biopsies, which avoids the problem of the large intra-individual variations in bone remodelling and turnover, increases the power of the study to demonstrate significant effects. Secondly, since only one dose of pamidronate was administered prior to transplantation it could be argued that its effects might no longer be evident at the 3 months' time point. However, the histomorphometric data would be consistent with pamidronate-induced effects at 3 months, not only because of the lack of a significant increase in bone turnover in treated patients but also because of the reduction in erosion cavity size seen in pamidronate-treated, but not untreated patients. Finally, for logistic reasons it was not possible to standardise the timing of pamidronate infusion prior to biopsy, although four patients received it within a month of their operation, and all but one within 2 months pre-operatively. Furthermore, we previously reported that the early pamidronate-induced increase in serum parathyroid hormone concentration was related to the timing of liver transplant rather than to the infusion per se [33].

In summary, these histomorphometric data confirm the increase in bone turnover which occurs early after liver transplantation in the absence of treatment and indicate that pamidronate may partially mitigate these changes. The small magnitude of pamidronate-induced effects on bone turnover after transplantation is consistent with the lack of any significant effect, in our study, on bone-mineral density and may be related, in part, to

Table 3. Indices of resorption cavity size before and after liver transplantation in patients treated with pamidronate and in untreated patients. Results are shown as mean ± SD

Variable	Pre-transplant + Pamidronate $n=7$	Post-transplant + Pamidronate $n=7$	Pre-transplant Untreated $n=5$	Post-transplant Untreated $n=5$
E.De (μm)	25.2 ± 7.9	23.2 ± 8.1	21.5 ± 4.1	21.4 ± 1.7
E.De.Max (μm)	35.6 ± 11.5	33.0 ± 11.5	27.1 ± 3.7	30.3 ± 3.9
E.Ar (μm^2)	3,852 ± 1,859	3,014 ± 1,585[a]	2,296 ± 492	2,291 ± 580
E. Le (μm)	210.4 ± 63.8	179.8 ± 67.5[b]	172.5 ± 15.1	173.4 ± 29.0

[a] $P=0.06$
[b] $P=0.03$

the low bone turnover state which is characteristic of hepatic osteoporosis [34, 35] and is present prior to transplantation in the majority of these patients [12]. Our own experience suggests that the incidence of osteoporosis after liver transplantation is decreasing and fragility fractures in these patients are now uncommon; nevertheless, post-transplantation osteoporosis remains a serious clinical problem in other cohorts of patients undergoing solid organ transplantation [11], and further studies are required to establish effective prophylactic regimens.

Acknowledgements J.E.C. and S.V. are supported by the Wellcome Trust.

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