Bone 44 (2009) 629–633

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Bone structure and remodelling in stroke patients: Early effects of zoledronate ☆

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ARTICLE INFO

Article history:
Received 7 August 2008
Revised 27 October 2008
Accepted 10 November 2008
Available online 11 December 2008

Edited by: R. Recker

Keywords:
Histomorphometry
Stroke
Zoledronate
Bone formation
Bone resorption

ABSTRACT

Introduction: We have reported that after an acute stroke, intravenous zoledronate prevented bone loss in the hemiplegic hip. Participants from the trial also volunteered for trans-iliac bone biopsy, to assess the early effects of stroke and zoledronate on iliac bone remodelling.
Methods: Patients with acute stroke were randomly assigned to a single intravenous dose of zoledronate 4 mg or placebo within 5 weeks of stroke. Biopsies from 14 patients (3 female, 11 male, mean age 71 ± 11) were suitable for analysis. These were taken at mean 10 weeks (±2) post-stroke, and included 5 patients who had received zoledronate. Histomorphometry was performed on undecalcified sections using light and fluorescence microscopy. Static and dynamic indices of remodelling were compared to a local reference range from healthy controls. Osteoclasts and their precursors were identified on frozen sections using tartrate resistant acid phosphatase (TRAP) staining. Dual-energy x-ray absorptiometry (DXA) of the proximal femora was performed at baseline and 6 months later.
Results: The eroded surface in cancellous bone (ES/BS) was significantly higher in stroke patients than controls (5.7% vs. ref 1.6%, $p<0.0001$). Although ES/BS did not differ between zoledronate and placebo-treated groups, there were significantly fewer osteoclasts and their precursors in zoledronate-treated individuals ($p=0.023$). Bone formation indices (osteoid surface, OS/BS and mineralising surface, MS/BS) were significantly lower in stroke patients than controls and although OS/BS was higher in the zoledronate group than the placebo group ($p=0.033$), MS/BS was not different ($p=0.924$). There were no differences between hemiplegic and unaffected sides for any histomorphometric parameter despite asymmetric reductions in hip bone mineral density ($p=0.013$).
Conclusion: Stroke patients had higher resorption indices and lower bone forming surfaces than controls, consistent with uncoupling of bone remodelling. These findings are preliminary and a larger study is required to evaluate the contributions of gender, age and hemiplegic status to the remodelling imbalance. Zoledronate therapy was associated with a reduction in osteoclastic cell numbers consistent with its known mode of action in bone.

Introduction

We previously demonstrated that a single intravenous infusion of zoledronate given within 5 weeks of acute stroke protected against the deleterious effects of hemiplegia on hip bone mineral density [1]. After a stroke, there is a reduction in bone mineral density in the hemiplegic hip as assessed by DXA. Despite the magnitude and rapidity of these effects, the underlying mechanisms at the bone tissue level in stroke patients are unexplained. Biochemical bone marker studies have suggested an increase in bone resorption and reduced bone formation in the first week after stroke compared to controls [2], which persisted throughout the first year [3,4]. Although bone density reduction following stroke is site specific (with the greatest losses at sites such as the affected hip [5]), more generalised bone loss as a result of bed-rest and alterations in calciotropic hormone regulation is also described [6]. Year-long treatment with daily oral risedronate after acute stroke was associated with suppression of bone resorption markers, but also an unexpected increase in bone formation markers [4]. Bone formation might be suppressed after stroke due to a combination of reduced PTH and reduced active vitamin D [7], the result of raised ionised calcium inhibiting the parathyroid gland [8]. The aims of this study were three-fold: to assess indices of cancellous, endocortical and cortical bone turnover in the iliac bone 10 weeks

☆ Funding sources: This work was supported by a project grant from the National Osteoporosis Society (UK). KESP acknowledges funding from the arthritis research campaign (Clinician Scientist, Fellowship) and Medical Research Council (Training Fellowship). ID and CR acknowledge funding from the Cambridge NIHR Biomedical Research Centre. The funding bodies had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; nor in the decision to submit the paper for publication.

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8756-3282
doi:10.1016/j.bone.2008.11.017

following stroke; to evaluate the early effects of zoledronate on bone remodelling; and to compare histomorphometric parameters in patients with stroke to a UK healthy reference range. Secondary aims were to investigate the relative contributions of the side of biopsy (hemiplegic or contra-lateral), baseline 25 hydroxyvitamin D (25OHD), stroke severity and functional status to changes in bone remodelling in stroke patients.

Methods

Approval for the study was obtained from the Cambridge Local Research Ethics Committee (Cambs LREC C, 2001). All the study participants were admitted to an acute stroke unit with first ever stroke and were taking part in the randomised placebo controlled trial of 4 mg intravenous zoledronate vs. placebo to prevent bone loss in hemiplegia. As part of that study protocol, all patients who were randomised to either zoledronate or placebo were invited to volunteer for a single trans-iliac bone biopsy. Patients received calcium (1 g) and vitamin D (800 IU) daily during the trial. Full inclusion and exclusion criteria for this trial have been published [1]. Additional exclusion criteria for the biopsy study were disorders of blood coagulation, warfarin therapy, respiratory disease and obesity. Patients had been independent and ambulant before admission with a stroke and all had hemiplegia affecting the lower limb. They were unable to walk 1 week following stroke. All patients underwent measurement of bone mineral density of both hips by DXA at study entry, repeated at 6 months. Two scales were used to evaluate patients' activity and motor power at study entry and immediately prior to biopsy. These were the Barthel Index (/100) [9] and the long term score (/48) of the Scandinavian Stroke Scale [10].

Fifteen of the 31 trial subjects volunteered for a single trans-iliac bone biopsy that was timed to occur approximately 10 weeks after the stroke (mean 10.4+/−1.9 SD). In one subject, the biopsy specimen was incomplete leaving 14 samples for analysis. The reasons for not having a biopsy were as follows: 9 patients declined, 5 were taking oral warfarin therapy, and 2 had discontinued the study. The trans-iliac bone biopsies were taken between January 2002 and October 2003 from 4 women (1 unsuitable for analysis) and 11 men, aged between 56 and 89 (mean 77.1 +/− 11) years. In order to explore the side-specific effects of hemiplegia on bone turnover, it was decided at the start of the study to sample alternately from the affected and unaffected side, so 7 patients had a biopsy from the hemiplegic side and 7 from the contra-lateral side. Upon unblinding, five patients had received zoledronate and 9 had received placebo infusions. Biopsies were obtained using a 7.5 mm internal diameter modified Bordier trephine using local anaesthetic infiltration and intravenous sedation. In 11 patients it was possible to double demeclocycline labelling before the biopsy (300 mg demeclocycline twice daily for 2 days, then a ten day gap, followed by 300 mg twice daily for 2 days, followed by a biopsy 4 days after the last dose) [11]. Biopsies were cut in half longitudinally, coded by the laboratory technician and all histomorphometric measurements on blinded biopsies were made by the same observer (KESP) with the exception of ES/BS (done by SV).

One half of the biopsy was embedded in methylmethacrylate for histomorphometry (British Drug House Chemicals Ltd, Poole, Dorset), the other was briefly immersed in polyvinylalcohol (PVA) and chilled in hexane for 10 min at −70 °C before mounting in PVA on a brass chuck for TRAP staining and immunohistochemistry on cryosections [12]. Undecalcified cryosections (8 μm) were cut onto sectioning tape using a Bright cryostat microtome (Huntingdon, UK) and reacted for tartrate resistant acid phosphatase activity [13]. After air drying, cryotape sections were placed in a freshly prepared buffered solution of 18.75 mg of naphthol AS B1 phosphate, 93.75 mg of sodium tartrate and 37 ml of 0.1 M tri sodium citrate (buffered to pH 4.5 with 2.1% citric acid). They were then washed rapidly in a solution of 0.25 g of sodium fluoride in 250 ml distilled water before placing in a reagent consisting of 18.75 mg of fast garnet in 35 ml of buffer (250 ml distilled water and 3.4 g sodium acetate buffered to pH 6.2 with acetic acid). Tape sections were then washed in distilled water twice and cover slipped with an aqueous mounting media.

After methylmethacrylate embedding, 8 μm undecalcified sections were cut with a Jung Polycut microtome (Leica, Milton Keynes, UK) and stained with Von Kossa/Van Gieson stain for osteoid measurement, trabecular surface and structural parameters and toluidine blue stain for eroded surface and wall thickness (using polarised light) before mounting on slides with DPX (Fisher Chemicals). Additional 15 μm sections were examined for fluorescent labels using ultraviolet light microscopy and a 365-nm filter. For all cancellous indices at least two sections from levels separated by more than 150 μm (a reduced thickness due to the limitations of using a half core biopsy) were analysed from each biopsy to avoid replicate sampling of a single surface event. Our detailed histomorphometric methods have been published previously [14,15] but additions are described here. For the present study, cancellous bone was defined as the area bounded by, parallel to and separated from the endocortical surface by 250 μm. The remaining surface including the entire endosteum was analysed separately. Osteoid thickness (O.Th) was measured at ×200 magnification, bone surface (BS) at ×80, wall thickness (W.Th) using polarised light at ×125 and eroded surface (ES) at ×200. For O.Th, a minimum number of 20 osteoid seams was analysed from each biopsy. All osteoid widths greater than 3 μm were included in the analysis [16]. For W.Th a minimum of 25 bone remodelling units was measured for each biopsy. Mineralising surface (MS/BS%) was defined as the surface extent of labelled (double-labelled + half single-labelled surface, dL + 1/2 sL) surface to cancellous bone surface. Mineral apposition rate (distance between labels) in placebo patients included values of 0.3 μm/d for 2/7 biopsies that had single cortical labels only [17]. Bone formation rate (BFR/BS $um^3/um^2/d$) was defined as the amount of new bone mineralised per day per unit of cancellous bone surface. Based on the geometric probability density function, measures of mean apparent widths were transformed into 3D mean apparent thicknesses by multiplying them by π/4 [18]. All histomorphometric measurements are described using nomenclature approved by the American Society for Bone and Mineral Research (ASBMR [19]), with the exception of 'cortical osteoid area', Ct. OAr/BAr.

Endocortical histomorphometry

The endocortical surfaces were defined for the purposes of this study as both inner bone surfaces and cancellous surfaces up to 250 μm into the medullary cavity measuring parallel to the plane of the inner surfaces. Osteoid surface (Ec. OS/BS) was measured on these surfaces in the same manner as the cancellous OS/BS.

Cortical histomorphometry

Mean cortical thickness (Ct. Th) was measured automatically after drawing periosteal and endocortical surfaces from three sections and using correction for section obliquity. Cortical osteoid area (Ct. OAr/BAr) and cortical porosity (%) were measured as follows. The entire Von Kossa stained cortex was captured at 100× magnification (2 cortices per biopsy) using automated montaging software and a digital camera. Canals were defined on the cortex image using a digital drawing pen and osteoid was filled in. The osteoid area was measured directly using ImageJ software (ImageJ 1.32e, NIH, USA available to download at http://rsb.info.nih.gov/ij/) and osteoid area as a percentage of cortical bone area was calculated. Assumptions of isotropy used to convert 2D to 3D values in cancellous bone are not met in cortical bone [19,20], so mean cortical areas in 2D cannot be extrapolated to mean volumes in 3D. Therefore, Ct. OAr/BAr is reported, acknowledging that the correct ASBMR nomenclature is to use either 2D or 3D referents only in a single publication.