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Combined effects of zoledronate and mechanical stimulation on bone adaptation in an axially loaded mouse tibia

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ABSTRACT

Background: Local bisphosphonate delivery may be a solution to prevent periprosthetic bone loss and improve orthopedic implants fixation. In load-bearing implants, periprosthetic bone is exposed to high mechanical demands, which in normal conditions induce an adaptation of bone. In this specific mechanical situation, the modulation of the bone response by bisphosphonate remains uncertain.
Methods: We assessed the combined effects of zoledronate and mechanical loading on bone adaptation using an *in-vivo* axial compression model of the mouse tibia and injections of zoledronate. Bone structure was quantified with *in-vivo* μCT before and after the period of stimulation and the mechanical properties of the tibias were evaluated with 3 point-bending tests after sacrifice.
Findings: Axial loading induced a localized increase of cortical thickness and bone area. Zoledronate increased cortical thickness, bone perimeter, and bone area. At the most loaded site of the tibia, the combined effect of zoledronate and mechanical stimulation was significantly smaller than the sum of the individual effects measured at the same site in the control groups.
Interpretations: The results of this study suggested that a negative interaction between zoledronate and mechanical loading might exist at high level of strain.

1. Introduction

Periprosthetic bone loss is initiated at the very early stage after an implant is set and facilitates the occurrence of aseptic loosening. Different authors have suggested the solution of local bisphosphonate release from the orthopedic implant to prevent the bone resorption at this early post-operative stage (Horowitz and Gonzales, 1996; Peter et al., 2001). It has been shown that local bisphosphonate preserves periprosthetic bone stock, in rats and sheep (Peter et al., 2005; Stadelmann et al., 2008; Wermelin et al., 2007) and also increases the fixation strength, in rats (Peter et al., 2005, 2006).

In these studies, the implants were not specifically loaded, in contrast to the clinical situation of load-bearing implants, where the periprosthetic bone is exposed to high mechanical demands (Huiskes et al., 1987). In this specific mechanical situation, the modulation of the bone response by bisphosphonate remains uncertain.

An early study showed that the effect of a dichloromethylene bisphosphonate on the bone apposition rate was increased when combined with a mechanical stimulation (Shellhart et al., 1992). On the other hand, by using 3-amino-1hydroxypropylidene-1-bisphosphonate, Jagger et al. have shown that, in rats caudal vertebrae exposed to mechanical stimulus, the rate of bone apposition is not affected (Jagger et al., 1995). When fatigue loading is evaluated in rat bone, the use of alendronate did not protect the bone from fatigue in highly strained bone (Barrett et al., 2007). In another study, also in highly strained rat bone, alendronate was shown to suppress the apoptosis of osteocyte induced by the mechanical stimulation (Follet et al., 2007). The combination of mechanical loading and bisphosphonate, in particular zoledronate needs then to be further studied.

The aim of the present study was to assess the effect of zoledronate, the newer member of the third generation bisphosphonates (Green et al., 1996; Green, 1996; Pataki et al., 1997), on bone adaptation when a mechanical loading is applied. For this purpose, we used an *in-vivo* axial compression model of the mouse tibia (De Souza et al., 2005; Fritton et al., 2005, Stadelmann et al., 2009) and analyzed the effect of zoledronate on site-specific bone adaptation.

2. Methods

2.1. Animals

Eleven C57BL6 male mice, 17 ± 1 weeks old, were acclimated to our facility for three weeks. Mice were caged in groups of three or four. They were maintained under standard no barrier conditions and

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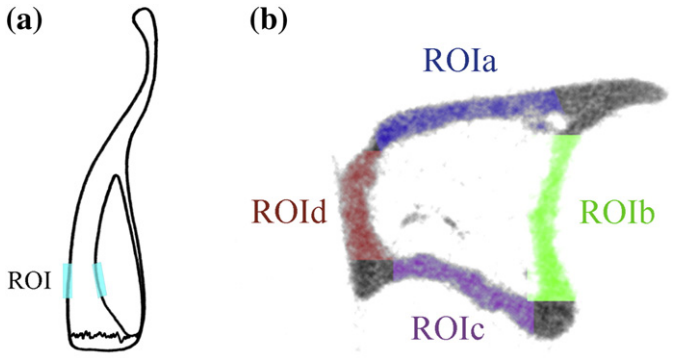

Fig. 1. Region of interest. Definition (a) of the global ROI and (b) of the 4 sub-ROI.

had access to mouse chow and water *ad libidum*. The local ethics committee on animal care approved all animal procedures (Protocol #2006.1).

The mice were separated randomly into two groups: on day 0, the five animals of the zoledronate group received a single subcutaneous injection (80 µl) of 1 µg/kg zoledronate (Novartis Pharmaceuticals AG, Switzerland) while the six animals of the control group received an equivalent injection of saline.

2.2. Anesthesia

General anesthesia was induced with a ketamine (80 mg/kg) and xylazine (5 mg/kg) cocktail administered intraperitoneally unless specified.

2.3. µCT

We assessed bone architecture using in vivo micro-computed tomography (µCT1076 in vivo, SkyScan, Belgium) 11 days after zoledronate injection. Animals were anesthetized. The lower limbs were fixed in a custom polystyrene support and aligned with the axis of rotation of the scanner. The tibias were then scanned with 9 µm isotropic voxel size, 50 kV beam, 0.8° step rotation. Reconstructions and analysis were performed with built-in routines of manufacturer's softwares NRecon and CTan, following the standard protocols. The reconstructed tibia contained about 1900 slices. Cortical thickness (Ct.Th), bone perimeter (B.Pm) and bone area (B.Ar) were evaluated at the proximal diaphysis (1/5 of the tibial length) (Fig. 1a). This region of interest (ROI) was then divided in four sub-regions of interest (ROIa-d), corresponding to the four facets, to assess orientation specific remodeling (Fig. 1b).

2.4. In vivo compression

A compression machine was developed to apply controlled compression cycles on the tibias (Stadelmann et al., 2009), based on a previously published work (De Souza et al., 2005; Fritton et al., 2005). On day 1, 3, 5, 8 and 10 the left tibia of all animals were mechanically stimulated with dynamic axial compression sequences. Custom molded pads were designed on the axes end to apply the compression on the leg.

Each animal was anesthetized and placed on a warm support with eye gel until completely unresponsive. The animal was then placed on the stimulation machine with the left leg between the moving pad on the knee and the fixed pad on the ankle (Fig. 2a).

To maintain the initial position of the leg, a pre-load of 0.5 N was applied before the dynamic compression. The compression waveform was composed of square-like cycles at 2 Hz frequency, and amplitude from a force of 0.5 N during 0.25 s followed by a force of 8 N during 0.25 s (Fig. 2b). The sequence of compression was applied for 1 min. Then the animal was placed at rest on the warm support. After 15 min, a second sequence of 1 min of dynamic compressions was applied. The animal was again placed on the warm support until it moved.

Because of the natural curvature of the tibia, this simple axial loading induced combined compressive and bending strains. Axial compression of 8 N induces maximum octaedral shear strain at the postero-tibial crest (1800 $\mu\varepsilon \pm 40\ \mu\varepsilon$) and the antero-distal tibia (1940 $\mu\varepsilon \pm 30\ \mu\varepsilon$) (Stadelmann et al., 2009).

2.5. Sacrifice and tibias extraction

On day 11, while still under anesthesia for the µCT, the animals were sacrificed with an overdose of ketamin. Both tibias were extracted surgically and placed in wet conditions at 4 °C.

2.6. Mechanical tests

Tibial mechanical properties were assessed by 3-point bending (Brodt et al., 1999; Jepsen et al., 2003), using the Instron Microtester 5848 (Instron, MA, USA), equipped with a 100 N gauge and custom bone supports. The fibula was removed with a surgical blade before the tests. The lower supports distance was set to 12 mm for all tibias, and the tibias always placed proximal end to the left and up, distal on the right. The crosshead speed was set to 0.02 mm/s and the force-displacement data sampling to 100 Hz.

The ultimate force, stiffness and post-yield energy to failure were calculated. The yield point was defined by using a 0.3 N offset from the stiffness line (Schriefer et al., 2005).

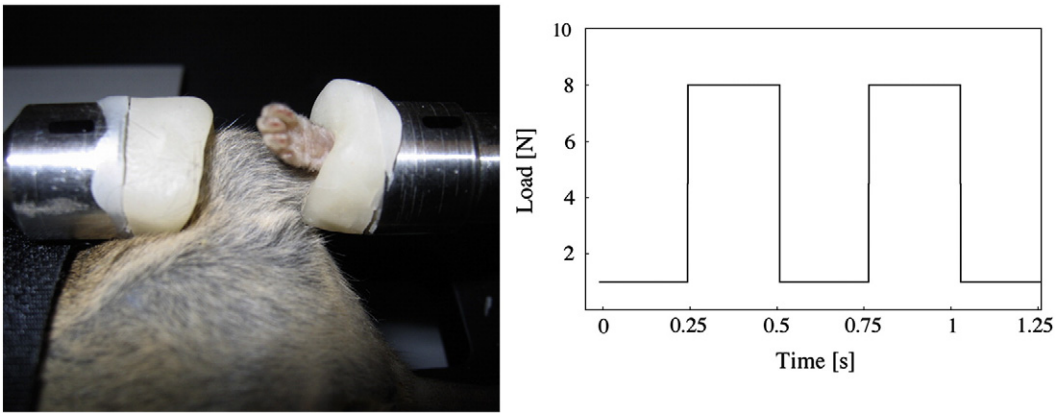

Fig. 2. (a) Animal's left tibia placed in the compression machine between the molded cups. (b) Compression waveform.