Bone 92 (2016) 70–78

Contents lists available at ScienceDirect

Bone

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

Full Length Article

Effect of combined treatment with zoledronic acid and parathyroid hormone on mouse bone callus structure and composition

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ARTICLE INFO

Article history:
Received 24 March 2016
Revised 12 August 2016
Accepted 14 August 2016
Available online 16 August 2016

Keywords:
Bone healing
Bone quality
Zoledronic acid
Parathyroid hormone
Micro-computed tomography
Raman spectroscopy

ABSTRACT

In recent years, great interest in combined treatment of parathyroid hormone (PTH) with anti-resorptive therapy has emerged. PTH has been suggested to aid bridging of atrophic fractures and improve strength in closed fracture models. Bisphosphonate treatments typically result in a larger woven bone callus that is slower to remodel. The combination of both drugs has been demonstrated to be effective for the treatment of osteoporotic bone loss in many preclinical studies. However, the effect of combined treatment on fracture repair is still largely unexplored. In this study, we aimed to compare these drugs as single-agent and in combination in a murine closed fracture model. We wanted to assess potential differences in material properties, morphometry and in the development of the lacuno-canalicular network. A total of 40 female, 11-week-old wild type mice underwent a closed fracture on the midshaft of the tibia and were assigned to four groups (n = 8–10 per group). Beginning on postoperative day 8, animals received different subcutaneous injections. Group 1 received a single injection of saline solution and Group 2 of zoledronic acid (ZA). Group 3 received daily dosing of PTH. Group 4 received a dual treatment, starting with a single dose of ZA followed by daily injection of PTH. Three weeks after fracture, all animals were euthanized and tibiae were assessed using micro-computed tomography (micro-CT), high-resolution micro-CT (HR micro-CT), Raman spectroscopy, quantitative histomorphometry, and deconvolution microscopy (DV microscopy). Combined treatment showed a significant increase of 41% in bone volume fraction and a significant decrease of 61% in the standard deviation of the trabecular spacing compared to vehicle, both known to be strong predictors of callus strength. An analysis via HR micro-CT showed similar results on all groups for lacunar numerical density, whereas mean lacuna volume was found to be higher compared to vehicle in treated groups, but only PTH mono-treatment showed a significant increase compared to vehicle (+45%). Raman spectroscopy did not reveal detectable changes in material properties of the bone calluses. Sclerostin staining, tartrate resistant acid phosphatase (TRAP) staining and canalicular analysis with DV microscopy on a subset of samples did not display distinctive difference in any of the treatments.
We therefore consider PTH + ZA treatment beneficial for bone healing. No clear negative effect on bone quality was detected during this study.

1. Introduction

Both systemic anabolic and anti-catabolic agents are used clinically to modulate bone turnover, particularly in individuals with osteoporosis or metabolic bone disease. PTH (1–34) is a potent anabolic drug that is commonly used to increase bone formation in osteoporotic patients [1–4]. Bisphosphonates (BPs) are also routinely used to treat osteoporosis and potently suppress bone resorption, leading to a higher bone mass [5,6]. Zoledronic acid (ZA) is a third generation nitrogen-containing bisphosphonate, and it is one of the most potent BPs in current clinical use [7,8]. In the past years, we gained expertise in its administration and in the understanding of its effect as anti-catabolic agent in preclinical studies [9–11].

PTH treatment has been suggested as a method for reducing the local osteonecrotic effects that are a rare adverse event associated

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http://dx.doi.org/10.1016/j.bone.2016.08.012
8756-3282/

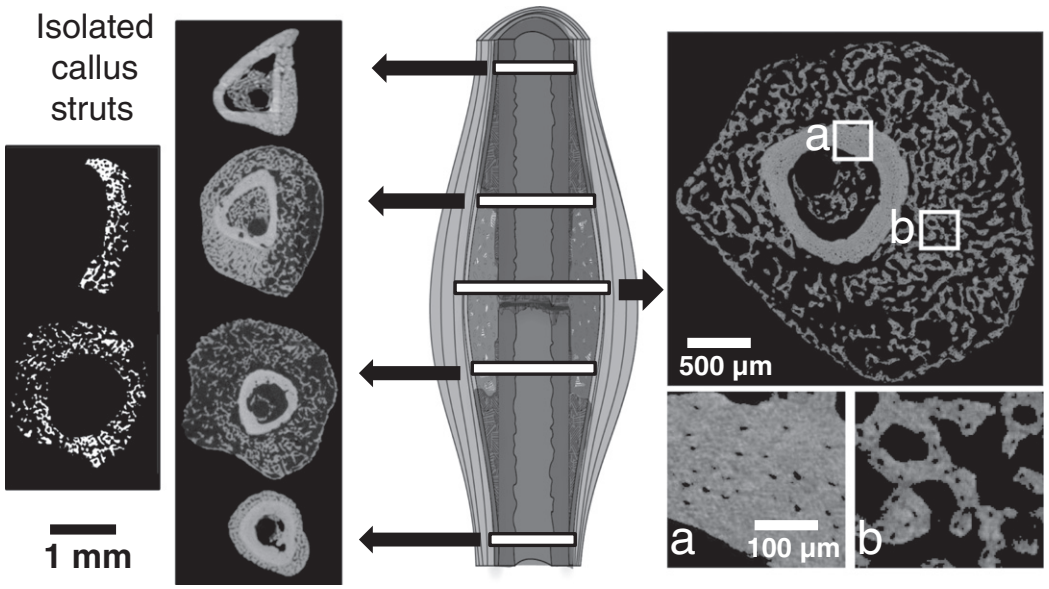

Fig. 1. (Left) A schematic representation of the isolation of the trabecular region of the callus. The regions with dense woven bone were removed since they are not suited for trabecular analysis. (Right) High-resolution acquisition for lacunar analysis in the old bone (a) and in the new bone (b).

with BP treatment [12]. PTH may also lead to improvements in bone quality in combination with BP by enabling the repair of microdamage, which if untreated can lead to fracture [13].

Studies reporting on the effect of combined PTH or teriparatide and BPs treatment show conflicting results for preventing bone loss in humans [14–25]. Some studies found PTH treatment to diminish morphological and/or microarchitectural measures when combined with BPs [14,20,21,25]. In other investigations, a significant increase in bone mineral density or an improvement of the microarchitecture was observed [18,19,24].

Combined treatment with PTH and BPs during fracture repair is still largely unexplored with a limited number of studies investigating the combination [26–29]. A study of Li et al. showed a significant increase of mechanical strength in osteoporotic rat femoral calluses with combined PTH + ZA treatment compared to vehicle and to mono-treatments [26]. However, no study to date has specifically addressed bone quality and material properties of the bone formed under these conditions. We hypothesized that combined treatment with PTH and ZA (PTH + ZA) would increase bone volume and improve bone callus quality with respect to macro-/microarchitecture and material composition by accelerating the healing process.

The study design was to examine the effects of PTH + ZA on the structure and composition of the fracture callus in a mouse tibial fracture model, compared to mono-treatments and no treatment three weeks post-operative (post-op). We therefore wanted to examine the impact of bone modulating drugs on the early stages of callus quality, osteocyte embedding and canalicular formation. We believe that a deeper knowledge on the effects of treatments at different hierarchical levels of the bone healing process will help find a better interpretation of past and future studies focusing on PTH-BP treatments [30]. Eleven-week-old mice which underwent closed tibial fracture were used in this study. Three weeks after inducing the fracture, animals were euthanized. Calluses and contralateral intact tibiae were then extracted for analysis.

Outcome measures included bone standard bone morphometrical measures, such as bone volume fraction (BV/TV) and bone mineral density (BMD) or tissue mineral density (TMD), in both the callus and the contralateral tibiae. To investigate differences in the microstructure of the callus struts, high-resolution micro-computed tomography (HR micro-CT) was adopted. Since in recent years it was suggested that changes in osteocyte lacunar numerical density and size might significantly alter bone stiffness [31], a lacunar analysis was also performed. Possible changes in compositional properties were evaluated with Raman spectroscopy, both in the calluses and in the contralateral tibiae. To gain insight into the underlying cellular expression a limited number of samples were checked for osteocytic expression of the SOST protein and for osteoblastic expression of the tartrate-resistant acid phosphate (TRAP). In addition, we also checked for differences in canalicular density in the latter samples.

By exploring the effects of interventions that reduce and augment remodeling, this study provides a detailed insight into how modulating these fundamental processes affects bone quality during repair.

2. Materials and methods

2.1. Animals, treatments and radiography

Female C57BL/6 mice were purchased from the Animal Resources Center (Perth, WA, Australia) and used at eleven weeks of age. All animals underwent a closed tibial fracture and were assigned to four groups (n = 10 per group). Fractures were induced in the midshaft by three point bending with an in-house-made surgical instrument. Groups 1 and 2 received a single subcutaneous (subQ) injection on post-op day 8 of saline solution (VEH) and ZA (0.1 mg/kg), respectively. Group 3 received daily subQ injections of PTH (1–34) (25 µg/kg) starting from post-op day 8. Group 4 received a dual treatment, starting with a subQ injection of ZA (0.1 mg/kg) on post-op day 8 and of daily subQ injections of PTH (25 µg/kg) starting on the same day. Three weeks after fracture, all animals were harvested and their tibiae extracted. All animal experiments were approved by the Westmead Hospital Animal Ethics Committee.

Fracture repair was monitored by radiography (Faxitron X-ray, Tucson, AZ, USA) one week, two weeks and three weeks after fracture. We then assessed union rate for the three time points for each group.

2.2. Micro-CT

Micro-CT analyses were performed according to standard procedures [32,33]. To assess bone volume fraction and bone mineral density of the calluses, eight to ten samples per group were scanned using a desktop micro-CT system (Skyscan 1174; Aartselaar, Belgium). We excluded two calluses we classified as proximal and the non-unions,