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# Short-courses of dexamethasone abolish bisphosphonate-induced reductions in bone toughness

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ABSTRACT

Atypical femoral fractures, which display characteristics of brittle material failure, have been associated with potent remodeling suppression drugs. Given the millions of individuals treated with this class of drugs it is likely that other factors play a role in these fractures. Some evidence suggests that concomitant use of corticosteroids may contribute to the pathogenesis although data in this area is lacking. The goal of this study was to assess the combined role of bisphosphonates and dexamethasone on bone mechanical properties. Skeletally mature beagle dogs were either untreated controls, or treated with zoledronic acid (ZOL), dexamethasone (DEX), or ZOL + DEX. Zoledronic acid (0.06 mg/kg) was given monthly via IV infusion for 9 months. DEX (5 mg) was administered daily for one week during each of the last three months of the 9 month experiment. Ribs were harvested and assessed for bone geometry, mechanical properties, and remodeling rate (n = 3–6 specimens per group). DEX significantly suppressed intracortical remodeling compared to vehicle controls while both ZOL and the combination of DEX + ZOL nearly abolished intracortical remodeling. ZOL treatment resulted in significantly lower bone toughness, determined from 3-point bending tests, compared to all other treatment groups while the toughness in ZOL + DEX animals was identical to those of untreated controls. These findings suggest that short-courses of dexamethasone not only do not adversely affect toughness in the setting of bisphosphonates, but also actually reverse the adverse effects of its treatment. Understanding the mechanism for this tissue-level effect could lead to novel approaches for reducing the risk of atypical femoral fractures.



## Introduction

Atypical femoral fractures have been associated with potent remodeling suppression pharmaceutical drugs such as bisphosphonates [1]. Although a definitive causal link between the pharmaceutical agents and these fractures does not exist, several reports provide intriguing data regarding the proposed association [2–7]. The mechanism underlying these atypical femoral fractures remains unclear although if anti-remodeling agents do in fact play a role it is likely that tissue-level changes related to low bone remodeling contribute. What is clear about atypical femoral fractures is that they are catastrophic and debilitating [1].

Given the millions of individuals treated with anti-remodeling agents, the rarity of atypical femoral fractures suggests that they are multifactorial. The 2010 American Society for Bone and Mineral Research task force report on fractures identified glucocorticoid treatment as one potential co-factor [1]. Chronic high dose glucocorticoid treatment has well-established negative effects including increased osteoclast and decreased osteoblast activity, induction of osteocyte apoptosis, loss of bone mass, and reductions in mechanical properties [8]. Although bisphosphonates are approved for reducing the fracture risk associated with glucocorticoid-induced osteoporosis [9], they produce their beneficial effect in this setting by suppressing bone loss and thus maintaining bone mass. The effects of bisphosphonates on material properties, those independent of bone mass, in the setting of concomitant glucocorticoid treatment have not been extensively studied [10].

Pre-clinical data demonstrate that bisphosphonates reduce bone toughness, the ability of the bone material to absorb energy prior to fracture [11–16]. Reduced toughness is analogous to increased brittleness and thus these data are consistent with the fracture characteristics of atypical femoral fractures. Recently, our laboratory undertook a study focused on a condition known as osteonecrosis of the jaw in which we treated animals with a combination of bisphosphonates and glucocorticoids [17]. Using material saved from this experiment, the aim of this study was to test the hypothesis that the combination of zoledronic acid and dexamethasone would significantly reduce bone toughness more than either treatment alone.

## Materials and methods

### Animals

Twenty-four skeletally mature female beagles (~1–2 years old) were purchased from Marshall Farms USA (North Rose, NY) and housed throughout the experiment in environmentally controlled rooms at

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Indiana University School of Medicine's AAALAC accredited facility. All animal procedures were approved prior to the study by the IU School of Medicine Animal Care and Use Committee.

### Experimental design

Following two weeks of acclimatization, animals were assigned to the untreated control group (VEH; n = 6), or the zoledronic acid (ZOL; n = 6), dexamethasone (DEX; n = 6) or zoledronic acid plus dexamethasone (ZOL + DEX; n = 6) treatment groups. The primary goal of this study was to investigate the combined effects of ZOL and DEX on oral wound healing [17], thus ZOL was administered via IV infusion at a dose of 0.06 mg/kg (40 mL total volume over 15 min), which corresponds to the dose used in cancer patients, adjusted on a mg/kg basis [18]. ZOL was infused every 2 weeks, roughly twice as frequently as used clinically, in order to maximize drug exposure during the experimental period. Dexamethasone was given via daily oral dosing (5 mg) for the first seven days of the 7th, 8th, and 9th months of the experiment. This dosing was based on a modified version of a low-dose protocol used clinically in multiple myeloma patients [19].

All animals were administered calcein (5 mg/kg, intravenous) using a 2–12–2–5 schedule, meaning it was administered on two consecutive days, 12 days were allowed to pass, it was injected for another two consecutive days, and then 5 days passed prior to euthanasia. After 9 months animals were euthanized by intravenous administration of sodium pentobarbital and the 9th right and left ribs were dissected free and placed in 70% ethanol and frozen PBS soaked gauze, respectively. Bones from all animals were available for histology (n = 6/group) but bones for mechanical testing were only saved for a subset (n = 3) of both the control and dexamethasone groups. The rib was chosen because it has traditionally shown consistent alterations in mechanical properties associated with bisphosphonate-treatment [11,15].

### Peripheral quantitative computed tomography (pQCT)

Volumetric bone density and geometry were quantified using a Norland Stratec XCT Research SA + pQCT (Stratec Electronics, Birkenfeld, Germany). Specimens were cut to 40 mm in length and a single scan at the mid-point was conducted using a voxel size of 0.07 × 0.07 × 0.50 mm. Total bone mineral content (BMC, mg/mm), total volumetric bone mineral density (vBMD, mg/cm$^3$), bone area (BA, mm$^2$), periosteal circumference, cortical thickness, and cross-sectional moment of inertia (CSMI, mm$^4$) were obtained using standard scanner software and segmentation algorithms (Cortbd mode with a threshold of 710 mg/cm$^3$) were used to separate cortical bone from marrow. Bone diameter was measured using digital calipers. Diameter and CSMI values were calculated in the plane perpendicular to the axis of three-point bending.

### Biomechanical testing

Three-point bending was conducted in accordance with our previously described method [11]. Briefly, bones were thawed to room temperature in saline and then placed on a three-point bending fixture (bottom support span = 25 mm) with the convex rib surface facing up. Specimens were loaded to failure at a displacement rate of 20 mm/min, and data were collected at 10 Hz. Structural mechanical properties, ultimate load, stiffness, displacement (pre-yield, post-yield, and total), and energy absorption (pre-yield, post-yield, and total) were determined from the load–deformation curves using standard definitions [20]. Material-level properties, ultimate stress, modulus, and toughness (pre-yield, post-yield, and total) were estimated by normalizing the structural parameters using standard equations that include bone diameter and CSMI [20].

### Histology

Ribs were processed for assessment of fluorochrome labels using standard methods of undecalcified histology [21]. Two serial semi-thin sections (80–100 μm) were cut approximately 5 mm apart using a diamond wire saw. Fluorochrome labels were assessed using an analysis system (Bioquant OSTEO 7.20.10; Bioquant Image Analysis, Nashville, TN) attached to a microscope equipped with an ultraviolet light source. Dynamic histomorphometric measures of the intracortical bone envelope were made on one section per animal using methods previously published and in accordance with recommended standards [22,23].

### Statistics

Statistical tests were performed using SAS software (SAS Institute, Inc.). Histological parameters were compared among groups using one-way ANOVA with Fisher's protected least significant difference (pLSD) post-hoc tests. Because of unequal and small sample sizes among groups for geometry and mechanical testing parameters, data were assessed using Kruskal Wallis non-parametric tests followed by Mann–Whitney tests to determine group differences. For all tests, $p < 0.05$ was considered to be statistically significant.

## Results

Material-level estimates of toughness, the ability of the material to absorb energy, were significantly lower in ZOL-treated animals compared to vehicle controls (Fig. 1). These effects were driven by significant differences in post-yield toughness with no significant difference among groups in pre-yield toughness. The negative effects of ZOL on total and post-yield toughness were abolished in animals treated with both ZOL + DEX, which had levels of toughness significantly higher than ZOL and statistically similar to VEH animals (Fig. 1). There was no significant difference among groups for structural-level energy to fracture (Fig. 1) indicating that the alterations in geometry with ZOL were sufficient to maintain whole-bone energy absorption.

There was no significant treatment effect on whole bone stiffness or material-level modulus (Table 1). While there was no difference among groups in ultimate load, the material-level strength (ultimate stress) was significantly higher in both ZOL treated groups compared to DEX-treatment alone (Table 1). Pre-yield displacement was similar among groups while post-yield displacement was significantly lower in ZOL compared to both VEH and DEX; the combination of ZOL + DEX returned post-yield displacement to VEH-level values.

Mid-diaphysis cortical BMC, volumetric BMD cortical bone area, and cortical thickness were all significantly higher in both ZOL-treated groups compared to vehicle control (Table 2). There was no significant difference among groups for periosteal circumference or cross-sectional moment of inertia.

Intracortical labeled osteon number and bone formation rate were both significantly lower in DEX-treated animals compared to control animals (Table 3). The labeled osteon number in the ZOL and ZOL + DEX groups was 98% lower than the control group. The lack of double labeled osteons negated the calculation of bone formation rate in the ZOL and DEX + ZOL groups.

## Discussion

The current study provides evidence that bisphosphonate-induced reductions in toughness, which have been consistently shown in multiple studies [11–16], can be overcome with in vivo treatment. Using a short-course dexamethasone protocol, one that is consistent with what is used clinically in some situations [19], the reductions in toughness brought about by zoledronate treatment were completely abolished. The effect of dexamethasone in the absence of zoledronate treatment was unimpressive, although the low number of samples (n = 3) in this