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Polyethylene particles inserted over calvarium induce cancellous bone loss in femur in female mice

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ABSTRACT

Focal bone resorption (osteolysis) induced by wear particles contributes to long-term orthopedic joint failure. However, the impact of focal osteolysis on remote skeletal sites has received less attention. The goal of this study was to determine the effects of polyethylene particles placed over calvaria on representative axial and appendicular skeletal sites in female mice. Because recent work has identified housing temperature as an important biological variable in mice, response to particle treatment was measured in animals housed at room (22 °C) and thermoneutral (32 °C) temperature. Osteolysis was evident in skeletal tissue adjacent to particle insertion. In addition, cancellous bone loss was observed in distal femur metaphysis. The bone loss was associated with lower osteoblast-lined perimeter and lower mineralizing perimeter in distal femur, lower osteocalcin gene expression in tibia, and lower serum osteocalcin, suggesting the response was due, at least in part, to reduced bone formation. Mild cold stress induced by sub-thermoneutral housing resulted in cancellous bone loss in distal femur and lumbar vertebra but did not influence skeletal response to particles. In summary, the results indicate that focal inflammation induced by polyethylene particles has the potential to result in systemic bone loss. This is significant because bone loss is a risk factor for fracture.

1. Introduction

> 600,000 orthopedic hip and knee replacements are performed annually in the United States (Kurtz et al., 2007). These procedures are used to treat bone fracture and a variety of degenerative joint disorders. Typically, quality of life rapidly improves following joint replacement. Unfortunately, approximately 17% of orthopedic hip replacements and 8% of knee replacements eventually fail and require surgical revision (Kurtz et al., 2005). The failure rate of joint prostheses has remained largely unchanged over the last 30 years (Kurtz et al., 2005). However, the number of joint replacements performed annually has increased dramatically (Kurtz et al., 2005) and, as a consequence, it is likely that the number of joint replacement revisions performed will also increase (Kurtz et al., 2007). Additionally, some studies find accelerated age-related bone loss in the contralateral limb following total joint replacement, suggesting arthroplasty can negatively influence the skeleton at distant sites (Gundry et al., 2017; Meek et al., 2011).

The precise mechanisms mediating implant failure are not entirely known but focal bone resorption (osteolysis) associated with particle-induced inflammation contributes to long-term orthopedic joint failure (Dattani, 2007; Gallo et al., 2002). Friction between the implant and the bone surface is responsible for generation of orthopedic wear particles (Dattani, 2007; Gallo et al., 2002). Wear particles from all commonly used orthopedic material (e.g., polyethylene, metal, and ceramic) can stimulate osteolysis (Dattani, 2007; von Knoch et al., 2004a). Of these, ultra-high molecular weight polyethylene is of particular importance as it has been and continues to be widely used in joint replacements. The process of ultra-high molecular weight polyethylene breakdown in hip replacement is reported to begin at the articular surface of the cup (Witkiewicz et al., 1993). Interaction between the polyethylene particles and the host cells leads to oxidative changes of the polyethylene and to size reduction of the particles. Wear particles are commonly found in and around tissues resected during joint revision and in distal lymph nodes (Witkiewicz et al., 1993; Kobayashi et al., 1997), the latter indicating that there is transport of the particles from site of generation to remote sites.

The effects of wear particles on bone are commonly investigated using small rodent models (von Knoch et al., 2004b; Wedemeyer et al.,

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2007; Nich et al., 2010; Kauther et al., 2010; Jin et al., 2011; Takahashi et al., 2011; von Knoch et al., 2005a; Darowish et al., 2009; Childs et al., 2001; von Knoch et al., 2005b; Ren et al., 2006). In mice, placement of particles over the calvarium induces osteolysis that is readily detectable using histology (von Knoch et al., 2004a; von Knoch et al., 2004b; Wedemeyer et al., 2007; Nich et al., 2010; Jin et al., 2011; Takahashi et al., 2011; von Knoch et al., 2005a; Darowish et al., 2009; Childs et al., 2001; von Knoch et al., 2005b; Ren et al., 2006; Yang et al., 2004; K. Ren et al., 2011) or micro-computed tomography (μCT) (Wedemeyer et al., 2007; Nich et al., 2010; Kauther et al., 2010; Darowish et al., 2009; Ren et al., 2006; K. Ren et al., 2011; Burton et al., 2013; Green et al., 2013). Animal studies to date have appropriately focused on the role of focal bone loss contributing to implant loosening. However, focal inflammation can also lead to systemic bone loss (Desimone et al., 1993) but few studies have evaluated the impact of polyethylene particle-induced local inflammation on bone mass, microarchitecture, or turnover at remote skeletal sites.

Housing temperature has emerged as an important biological variable that may influence experimental outcomes in mouse studies (Eng et al., 2015; Kokolus et al., 2013; Rosania, 2014; Stemmer et al., 2015; Ganeshan and Chawla, 2017). Mice are generally housed at 18–23 °C, a temperature range that is well below the thermoneutral zone (temperature range where basal rate of heat production is in equilibrium with heat loss) for this species (~32 °C). Importantly, housing temperature affects age-related bone loss (Iwaniec et al., 2016). Specifically, mild cold stress induced by room temperature housing leads to premature cancellous bone loss in mice. Although the precise mechanism is not fully established, the bone loss is associated with increased sympathetic outflow accompanying adaptive thermogenesis. Additionally, housing temperature influences the immune system (Kokolus et al., 2013), an important mediator of particle-induced osteolysis. Therefore, the purpose of this study was to (1) evaluate whether focal osteolysis induced by placement of polyethylene particles over the calvarium alters bone metabolism at remote skeletal sites and (2) determine whether the response is influenced by housing temperature.

2. Methods

2.1. Experimental protocol

Four-week-old female C57BL/6 (B6) mice were purchased from Jackson Laboratory (Bar Harbor, MN). The mice were housed individually in climate-controlled rooms on a 12 h light dark cycle. All mice were fed standard rodent chow (Teklad 8604, Harlen Laboratories, Indianapolis, IN). The animals were maintained in accordance with the National Institutes of Health Guide for the Care and the Use of Laboratory Animals. The Oregon State University Institutional Animal Care and Use Committee approved all protocols.

2.1.1. Experiment 1: effects of particle-induced calvarial osteolysis and housing temperature on bone in femur and lumbar vertebra

Mice (n = 43) were maintained at room temperature (22 °C) from 4 to 10 weeks of age. At 10 weeks of age, the mice were randomized by weight into one of four treatment groups: (1) 22 °C control (sham surgery, n = 11), (2) 22 °C particles (n = 11), (3) 32 °C control (sham surgery, n = 10), or (4) 32 °C particles (n = 11). Particle treatment was initiated 1 week following randomization to allow groups 3 and 4 to adapt to thermoneutral housing. The animals were sacrificed 2 weeks following particle implantation (at 13 weeks of age). The 2-week duration was selected based in part on precious work by von Knoch et al. (2005a) showing a robust bone response to particles two weeks following implantation. For tissue collection, mice were anesthetized (2% isoflurane delivered in oxygen), weighed, and killed by decapitation. Calvariae, femora, and the 5th lumbar vertebrae were excised and placed in 10% formalin for 24 h and then transferred to 70% ethanol for storage prior to evaluation.

2.1.2. Experiment 2: effects of particle-induced calvarial osteolysis on bone in femur, gene expression in tibia, and biochemical markers of bone turnover in serum

4-week-old mice (n = 19) were placed in a 32 °C room upon arrival at Oregon State University. At 6 weeks of age, the mice were randomized by weight into one of two treatment groups: (1) control (n = 10) or (2) particles (n = 9). Particles were implanted over calvaria and the animals sacrificed 2 weeks later (at 8 weeks of age). The fluorochrome calcein (15 mg/kg; Sigma Chemical, St Louis, MO) was administered by subcutaneous injection to label mineralizing bone at 4 days and 1 day prior to sacrifice. As in Experiment 1, calvariae and femora were excised for evaluation of bone. In addition, trunk blood was collected for evaluation of serum osteocalcin, a global marker of bone turnover, and tibiae were flash frozen in liquid nitrogen for evaluation of gene expression. Serum and tibia were stored at −80 °C until analysis.

2.2. Particle implantation

A polyethylene particle stock solution was prepared to deliver 2.5 mg of particles in 15 μl of solution. Polyethylene particles (S-395 *N1*, Shamrock Technologies Inc., Newark NJ), mean diameter 5 μm, were washed 6 times in 70% ethanol. 2 ml of wet particles were suspended in 95% ethanol. For particle placement, mice were anesthetized (2% isoflurane delivered in oxygen), and particles implanted using a model described by von Knoch et al. (2004b). A one cm skin incision was made over the calvarium. The skin was retracted and 15 μl of particle solution delivering 2.5 mg of particles were applied by pipette on top of the exposed calvarial surface between bregma and lambda. The incision was closed with 7 mm surgical staples (Reflex 7 Wound Closure System). Sham-operated controls underwent the same procedure excluding particles.

2.3. Micro-computed tomography

Calvaria, femora, and 5th lumbar vertebra were imaged using microcomputed tomography (μCT; μCT40 scanner, Scanco Medical AG, Bassersdorf, Switzerland) at 55 kVp x-ray voltage, 145 μA intensity, and 200 ms integration time using cubic voxels, 12 μm on a side. Filtering parameters sigma and support were set to 0.8 and 1, respectively. All samples were scanned immersed in 70% ethanol. All μCT data are reported using standard 3 dimensional nomenclature (Bouxsein et al., 2010).

Bone segmentation in femur and lumbar vertebra was conducted at a threshold of 245 (scale, 0–1000) determined empirically. Femora (cortical + cancellous bone) were evaluated for total femur bone volume (mm^3). Femur length was measured as the distance between the proximal end of the femoral head and distal end of the femoral condyles. Cortical bone architecture was evaluated in a 0.24 mm (20 slices) region of the diaphysis that started 60% distal from the top of the femoral head. Cross-sectional volume (mm^3, cortical bone volume + marrow volume), cortical bone volume (mm^3), marrow volume (mm^3), cortical thickness (μm), and polar moment of inertia (mm^4, an index of bone strength in torsion) were measured. Cancellous bone architecture was evaluated in the femoral metaphysis and in 5th lumbar vertebra. For the femoral metaphysis, 42 slices (0.50 mm) of bone were measured in a region that began 45 slices (0.54 mm) proximal to the growth plate. The entire cancellous compartment was evaluated in the vertebral body. Direct cancellous bone measurements included cancellous bone volume fraction (%, ratio of the segmented bone volume to the total volume of the region of interest), connectivity density (mm^{-3}, measure of the degree of connectivity of trabeculae), trabecular thickness (μm), trabecular number (mm^{-1}, number of trabeculae intercepted per unit length), and trabecular spacing (μm).