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Publication details:
Orthopaedic Research Society, 50th Annual Meeting
pp. 478-478
0149-6433 (ISSN)

Event details:
50th Annual meeting of the Orthopaedic Research Society
San Francisco, CA, USA

Publication Date:
2004

DOI:
https://doi.org/10.26190/unsworks/758

THE EFFECTS OF LONG TERM OVARIECTOMY ON FRACTURE HEALING IN RATS

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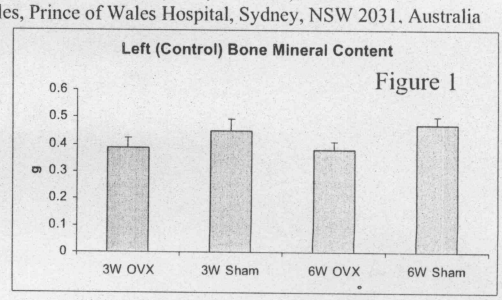
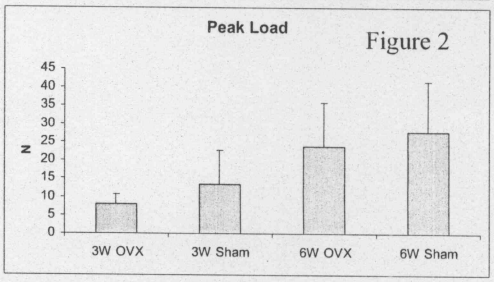
INTRODUCTION

Estrogen deficiency is a major pathogenic factor in the development of postmenopausal osteoporosis, a condition characterised by a reduction in bone mass and disrupted bone architecture, resulting in greater bone fragility and an increased risk of fractures [1]. In the United States alone, osteoporosis affects up to 15% of postmenopausal Caucasian women [2], with a large proportion of these requiring orthopaedic treatment for fragility fractures, mainly of the spine, hip and distal radius. Despite its prevalence and societal impact, relatively little research has been directed at the study of osteoporotic fractures.

Fracture healing is a complex biologic cascade [3] regulated by growth factors, cytokines and hormones and may in part be affected by mechanical and biological factors. The mechanical and histologic properties of fracture healing following short-term estrogen deficiency have demonstrated a general impairment in fracture healing [3,4,5,6,7]. Meyer et al., also reported that age and ovariectomy significantly impair the process of fracture healing in female rats [6]. While most studies employ a 12-week period of estrogen deficiency prior to fracture healing, the effect of long-term estrogen deficiency has yet to be reported. This study reports the tensile and histologic properties of healing fractures following 6 months of estrogen deficiency and age matched sham controls.





METHODS

Seventy female Sprague-Dawley rats (10 weeks old) were used following ethical approval (AEC 01/105). The ovaries were removed in 35 animals (OVX) and a sham operation (Sham) performed in 35 animals. The animals were housed for 6 months on standard rodent diet following the ovariectomy or sham procedure when the right femur was fractured by way of an open osteotomy using a gigli saw and fixed with an intramedullary K-wire [7]. The left limbs were used as contralateral controls. Animals were sacrificed at 1, 3 and 6 weeks and radiographed in the anteroposterior and lateral planes using a Faxitron and high resolution mammography film. Five from each group per time point were randomly selected for histology and ten were used for mechanical testing. Bone mineral content (BMC) and density (BMD) of the entire right femur, left femur and the fracture callus were obtained using a Norland pDEXA Sabre scanner (Norland Medical Systems, Inc, NY) and small animal software. Biomechanical testing of the femurs was conducted using an MTS 858 Bionix testing machine (MTS, MN). K-wires were passed through the proximal and distal femur and used to secure the bones during tensile testing to failure at 2mm/minute. The peak load and stiffness were obtained from the load versus displacement graphs. The femurs were then fixed for histology in 10% phosphate buffered formalin for a minimum of 48hrs and decalcified in 10% formic acid – formalin solution. Tissues were paraffin embedded and sectioned for routine Harris haematoxylin and eosin (H&E) staining. Three independent blinded observers qualitatively assessed histology. A two-way analysis of variance (ANOVA) was used to evaluate the differences between the groups at each time point.

RESULTS

All animals recovered from surgery without any adverse complications or infections. A statistical increase in body weight following the 6-month estrogen deficiency was noted between the Sham and OVX groups ($p<0.05$) (380.0 g +/- 44.4 versus 435.1 g +/- 55.5 respectively). DEXA analysis revealed a significant reduction in BMC and BMD ($p<0.05$) in the left, non-fractured femurs in the sham and OVX animals. No difference in BMC or BMD was found in the fracture callus in the Sham or OVX animals at 3 or 6 weeks following fracture.

Tensile data increased with time in the sham and OVX fractures ($p<0.05$). No difference in tensile peak load (Figure 1) or stiffness was found between the sham and OVX group at 3 or 6 weeks following 6-month estrogen deficiency.

Histology at 1 week revealed an infiltration of pro-inflammatory cells and haematoma present at the fracture site with no new bone in the OVX group; however some new bone was noted in the sham group. By 3 weeks, more newly formed bone was seen in the fracture callus in the sham group together with some cartilage and denser soft tissue thereby narrowing the fracture gap. In the OVX group, the fracture gap remained quite broad with some new bone formation exists. At 6 weeks, some soft tissue was still present in the fracture callus composed of cartilage and soft tissue. The fracture gap in the sham group was partially filled with cartilage and bone.

DISCUSSION

The capacity for fracture repair has been reported to decrease with age while the risk of fracture increases greatly with age. The biology and biomechanics of fracture repair following estrogen deficiency remains unclear. The current study used an open osteotomy model fixed with a k-wire to examine fracture healing in animals that were estrogen deficient for 6 months. In contrast to other studies on fracture healing where a time period of 3 month or less of estrogen deficiency is allowed prior to fracturing, we did not find any mechanical or bone mineral density differences in the fractured limbs following 6 months of estrogen deficiency. This may reflect the severity of this open fracture model compared to the closed fracture model reported in other studies [4] as well as a general reduction in healing in aged bone. Damage to the periosteum in the open model may have been too severe to enable this tissue to participate in the repair process. The contralateral limbs confirmed a significant bone loss at 6 months. Other compensatory mechanisms may also be involved that may play a role in fracture healing following long-term estrogen deficiency in contrast to the changes in bone healing observed following short-term estrogen deficiency.

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50th Annual Meeting of the Orthopaedic Research Society
Poster No: 0478