BIOMOLECULAR FACTORS IN OVARIECTOMIZED RAT FRACTURE HEALING

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Introduction

Females who have undergone either natural or surgical menopause, often later develop osteoporosis, a condition characterized by reduced bone mass and an increased risk of fractures. The major pathogenic factor is a deficiency in circulating estrogen levels\(^1\). Ovariectomized rat is a useful animal model as it displays similar bony changes to that in humans. Using this model, some have shown differences in the mechanical properties of the fracture callus during the course of healing when compared with normal rats\(^2\). The underlying molecular cascade in normal fracture healing has been described previously showing that bone morphogenic proteins (BMPs), transforming growth factor-beta (TGF-\(\beta\)) and their downstream signal transducers, Smads, are important regulators in normal rat fracture healing\(^3\). However, it is unknown whether these biomolecular factors are altered in an estrogen deficient environment. In women, serum insulin-like growth factor-I (IGF-I) concentrations decrease after menopause\(^4\). In vitro studies have shown that IGF-I stimulates collagen synthesis\(^5\) and decreases collagen degradation by inhibiting matrix metalloproteinases (MMPs)\(^6\).

We therefore hypothesized that during fracture healing in ovariectomized rats, the inferior mechanical properties are associated with alterations, as a result of estrogen deficiency, in the underlying molecular cascade.

Methods

Forty-eight 3-month old female Sprague-Dawley rats were used following approval from the Animal Care and Ethics Committee of UNSW. Half underwent bilateral ovariectomy (OVX) and the other half subjected to sham surgery (Sham). Ten weeks later, a closed fracture was produced in the right femur with a custom-made three-point bending device, after stabilizing the femur with an intramedullary K-wire. Animals were killed in groups of 6 at 1, 2, 4 and 6 weeks post-fracture and their femurs were harvested for dual-energy X-ray absorptiometry (DEXA), histological and immunohistochemical analysis. Bone mineral density (BMD) of the non-fractured femur and of the fracture callus, histo-morphology of the fracture callus and the expression and distribution of BMP-2, 7, TGF-\(\beta\), Smads-1-7, IGF-I, IGF-I receptor, MMP-1 (collagenase) and -3 (stromelysin-1), tissue inhibitor of MMPs (TIMPs)-1 and -2 within the fracture callus were examined. One-way ANOVA was used for statistical analysis.

Results

The BMD of the left femurs (non-fracture) at 1 week post-fracture (11 weeks post-ovariectomy) as measured by DEXA, was significantly lower in the OVX group compared with the Sham group (p<0.002). The differences in BMD within the fracture callus of the two groups at the designed time points however, did not reach statistical significance.

On haematoxylin and eosin (H&E) staining, morphological differences were seen during fracture healing of the Sham and OVX animals. More intramembranous ossification occurred adjacent to the cortical bone at the fracture site in the Sham group (Figure 1a) at 1 week post-fracture while the OVX sections showed either more fibrous tissue infiltration and cortical bone resorption (Figure 1b) or a high ratio of cartilage/bone (Figure 1c) in the fracture callus. Newly formed bone filled the gap and resolution of the fracture callus were noted in both groups from 4 week post-fracture. No delayed healing was noted in the OVX animals in this study.

The protein expressions of TGF-\(\beta\), BMP-2, -7, Smads-1-7 and IGF-I receptor, MMP-1 and TIMP-1 and -2 detected by immunohistochemistry were elevated with a similar pattern in the fracture callus in both Sham and OVX groups during fracture healing. IGF-I expression however was down regulated in the OVX groups throughout the healing stages (Figure 2). MMP-3 expression was up regulated in the OVX groups at early time points (1 and 2 weeks post-fracture, Figure 3).

Figure 2 Immunoperoxidase staining of IGF-I in 2 week (a and b) and 4 week (c and d) post-fracture sections. Stronger staining (brown) was seen in the sham sections (a and c) at both time points compared to the OVX sections (b and d). The staining was located in the osteoprogenitor cells and the osteoblast-like cells. (OM 20x)

Figure 3 Immunoperoxidase staining of MMP-3 in 1 week post-fracture sections. Positive signals were only detected in the OVX section (b) not in the Sham section (a). The staining was located in the osteoprogenitor cells, the osteoblast-like cells, the mesenchymal cells and some bone and cartilage matrix.

Discussions

Fracture healing is a complex biological cascade regulated by many systemic and local factors including BMPs, TGF-\(\beta\), Smads, IGF-I, MMPs and TIMPs. Postmenopausal women are more susceptible to fractures, especially of the hip, radius and lumbar vertebrae, and these fractures are unique from other fractures, in that they heal in an estrogen deficient environment. This study showed that during fracture healing in the ovariectomized rat, IGF-I expression in the fracture callus was down-regulated and MMP-3 expression up-regulated, when compared to Sham-operated rats. IGF-I is known to be important in the maintenance of bone mass and MMP-3 in degrading bone matrix. The alterations in these molecular factors may explain the inferior mechanical properties of OVX rat fractures observed in earlier studies.

The IGF-I receptor and TIMPs have the ability to directly regulate the levels of IGF-I and MMPs respectively, but their patterns of expression were similar in both groups suggesting that they are not the cause of the altered IGF-I and MMP-3 expression. We propose that the down-regulation of IGF-I may be a direct result of the change in estrogen levels and that the consequent lack of local IGF-I may in turn, cause the up-regulation of MMP-3.

Potential limitations in this study included the use of young animals, where their skeletal system has not yet fully matured, and also the time interval to fracturing following ovariectomy was only 10 weeks. The results do however provide a possible molecular basis for the impaired healing observed in the estrogen deficient state.

References