Influence of Bone Morphogenetic Protein on Glucocorticoid-Inhibited Fracture Healing in a Closed Femoral Fracture Rat Model

Introduction:
While endogenous glucocorticoids are essential for the maintenance of normal bone turnover, pharmacologic doses ultimately result in osteopenia and may inhibit fracture healing. Patients with fractures receiving therapeutic glucocorticoids are at risk of delayed fracture union or nonunion. This investigation was undertaken to examine the utility of an induction agent of bone formation, Osteogenic Protein-1 (OP-1 / BMP-7) (Stryker Biotech, Hopkinton, MA) in the augmentation of fracture healing in an experimental animal model.

Methods:
One hundred and ninety-two male Sprague-Dawley rats received subcutaneous implants of either timed-release prednisolone (equivalent to 2.2 mg/kg/d) or placebo pellets. Two weeks following implantation, surgery was performed for femoral intramedullary pin placement and subsequent fracture using an established closed fracture model (Bonnarens and Einhorn, 1984). In a subset of rats, a limited approach to the femur was performed for application of 25 µg of recombinant human OP-1 mixed with 50 mg of its collagen carrier, or collagen carrier alone. The collagen carrier employed was Type I collagen processed from a bovine source (morselized, guinidine-extracted, lyophilized, and gas sterilized; Stryker Biotech). Femurs from six rats were harvested at 10, 28, and 42 days for radiographic analysis. Mineralized callus area was estimated from high resolution Faxitron radiographs which were digitized and measured on a semi-automated histomorphometry workstation (Bioquant Image Analysis Corp., Nashville, TN). Twenty-eight and 42 day femurs were stripped of all soft tissues and frozen in saline at -20°C for biomechanical analysis. Following thawing, the metaphyses were potted in aluminum frames with a low melting temperature medium while maintaining the callus areas moist. These constructs were then tested to failure in torsion in a materials test frame. Femurs from animals euthanized at 3, 10, 21, 28, and 42 days were fixed in 10% buffered formalin for histomorphometric analyses. Following 48 hours of fixation, bones were carefully stripped of soft tissues and demineralized in a 1N HCl+ EDTA solution (Fisher Scientific, Chicago, IL) for 48-72 hours. The intramedullary pin was removed, the femur was paraffin embedded and sectioned on a radial microtome at 5µm. Sections were stained with hematoxlin and eosin. Cartilaginous and non-cartilaginous callus, mineralized callus, and total callus areas were measured on the histomorphometry workstation (Bioquant Image Analysis Corp., Nashville, TN).

Results:
High resolution radiographs showed no significant difference in fracture callus at 10 days among animals treated with or without prednisolone alone. However, by 28 and 42 days, there was significantly less fracture callus seen radiographically in the corticosteroid treated animals (Figure 1). Femurs harvested at 28 days for biomechanical analysis showed significantly less torsional strength and were less stiff. Histomorphometric analyses demonstrated less total callus at 21 and 42 days, and less hard callus at 42 days in prednisolone treated rats. However, cartilaginous soft callus was greater in rats treated with prednisolone at 42 days. Differences between prednisolone treatment and control groups following application of OP-1 or collagen alone were not detected for radiographic, biomechanical, or histomorphometric analysis at any time (hard callus; Figure 2). However, comparison of application of OP-1 or collagen alone in prednisolone treatment groups revealed significantly greater fracture healing with OP-1, compared to collagen alone.

Conclusion:
This investigation has shown that while a corticosteroid significantly inhibits fracture healing by impeding the process of endochondral ossification, Osteogenic Protein-1 is highly effective in countering this inhibition. This osteoinduction cytokine may therefore serve as an important adjunct to fracture repair in patients receiving therapeutic glucocorticoids.

Reference:

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