Pharmacologic modulators of fracture healing: The role of cyclooxygenase inhibition

J.E. Puzas, R.J. O’Keefe, E.M. Schwarz, X. Zhang

Department of Orthopaedics, University of Rochester School of Medicine and Dentistry, Rochester, NY, USA

Keywords: Biomechanics, Muscle, Mechanical Influences, Collagenous Tissue, Sports Medicine, Plastic Surgery

Introduction

The physiological mechanisms of fracture healing have been the topic of active investigation for many years. At the present time ample data exist on the histological, mechanical and biochemical aspects of this process. Most investigators now believe that many of the steps in fracture healing are a recapitulation of what happens in endochondral bone formation. What lies ahead for a better understanding of this process is to uncover the regulatory molecules that control chondrocyte and bone cell proliferation and activity.

One area of fracture healing investigation that is just now coming to the forefront is the effect of pharmacologic agents. The recent widespread use of bisphosphonates, estrogen receptor modulators and teriparatide for the treatment of osteoporosis has raised questions about the effect of these agents on fracture healing. Yet another class of pharmacologic agents that requires investigation are COX-1 and COX-2 inhibitors. These agents are one of the most commonly used medications for minor musculoskeletal injuries and pain throughout the world, yet we have limited knowledge on how they affect the fracture healing process. The remainder of this discussion will explore fracture healing biology and the role played by prostaglandins and the cyclooxygenase enzymes.

Fracture healing

Unlike other tissues that heal through the generation of scar tissue, bone heals by regenerating new bone. This capacity may be due to similarities in the molecular programs of fetal skeletogenesis and adult fracture repair. The process of fracture healing can be subdivided into 3 phases: inflammatory, reparative, and remodeling. The first phase begins immediately following fracture and is characterized by the formation of a hematoma, migration of mesenchymal cells to the fracture site, and the release of cytokines and growth factors from leukocytes and fibroblasts. Following the initial inflammation, new bone is formed by intramembranous ossification as well as endochondral ossification; these processes are predominately mediated by osteoblasts. This phase is followed by an extended period of remodeling involving osteoclasts that resorb the new woven bone and osteoblasts that replace this matrix with lamellar bone. As with homeostatic remodeling, the important functional outcome of the remodeling phase of fracture healing is the restoration of mechanical strength and stability.

Defective fracture healing in OVX and steroid-induced osteoporosis

A recent study using the osteoporotic rat model was able to demonstrate the adverse effects of osteoporosis on the early phase of fracture healing. Callus formation and strength was monitored over a 3-week period by histological and biomechanical assessment. The investigators found a 40% reduction in fracture callus cross-sectional area and a 23% reduction in bone mineral density in the healing femur of the OVX rats observed on day 21 following fracture as compared with the control group. Biomechanical data from the healing femur of the OVX rats revealed a 5-fold decrease in the energy required to break the fracture callus, a 3-fold decrease in peak failure load, a 2-fold decrease in stiffness and a 3-fold decrease in stress as compared with the control group. Histomorphological analysis revealed a delay in fracture callus healing with poor development of mature bone in the OVX rats.

A study evaluating the effects of drug (corticosteroi) induced osteoporosis also documented its adverse effects on
Cyclooxygenase-1 and 2

Prostaglandins comprise a group of short fatty acid derivatives that are the most abundant eicosanoids in bone. Prostaglandin synthesis is controlled by three different enzymes: phospholipase A2s which release arachidonic acid (AA) from the cell membrane; the cyclooxygenases which catalyze the oxygenation and further reduction of AA to form PGH2; and isomerases which convert PGH2 to individual prostaglandins. Among these, the cyclooxygenases are the most important rate-limiting enzymes in the pathway. The identification of a second isoform of cyclooxygenase (COX-2) in the prostaglandin synthetic pathway has expanded our knowledge of the function of this group of fatty acid derivatives that serve as microenvironmental hormones.

Cyclooxygenase inhibitors have been widely used as anti-inflammatory and pain relief medications in clinical practice. These drugs, commonly known as NSAIDs (Non-steroidal anti-inflammatory drugs), are used to treat various inflammatory bone diseases, including rheumatoid arthritis and osteoarthritis. Traditional NSAIDs inhibit both COX-1 and COX-2. Selective inhibitors for COX-2 have been developed and are effective anti-inflammatory agents and avoid serious GI side effects. COX-2 inhibitors may also be useful in prevention of diseases such as colon cancer and Alzheimer’s disease. COX-2 isoforms have recently been identified in both bone cells and chondrocytes.

Cyclooxygenase knockout mice have been created. COX-1 null mice survive and have no gastric pathology, but show decreased platelet aggregation. COX-1-/- females have parturition difficulties resulting in few offspring when mating with male COX-1 -/- mice. In contrast, COX-2 -/- mice have shorter life spans of around 8-32 weeks. Although these mice show normal inflammatory responses to phorbol ester stimulation and are susceptible to peritonitis, absence of COX-2 protects knockout animals from LPS-induced hepatic necrosis, suggesting the role of COX-2 in tissue damage associated with inflammation. Developmental problems in the ovary and the kidney are observed, and cardiac fibrosis was found in some COX-2 -/- mice. The distinct phenotypic changes in these cyclooxygenase knockout mice further indicate different functions of COX-1 and COX-2 in pathological and physiological processes.

Cyclooxygenases and bone repair

NSAIDs are widely advocated for use as an analgesic in patients with fractures, including pediatric patients, elderly nursing home patients, those with stress fractures, and adult traumatic fractures resulting from participation in sports. Furthermore, NSAIDs are recommended for the relief of pain following spinal fusion. Because of the wide use of NSAIDs in patients undergoing reparative bone formation, the role of cyclooxygenase in this process is a critically important clinical issue.

Animal studies demonstrate inhibition of fracture healing with multiple NSAIDs, including ibuprofen, indomethacin, and ketorolac. A recent human study examined features associated with non-union of the femoral shaft and included 32 patients with non-union of a fracture of the diaphysis of the femur and 67 comparable patients whose fracture had united. Both groups were comparable with regard to gender, Injury Severity Score and soft tissue injury. There was no relationship between the rate of union and the type of implant, mode of locking, reaming, distraction or smoking. There was a marked association between non-union and the use of NSAIDs (p = 0.000001).

Animal studies also strongly suggest an inhibitory effect of NSAIDs on spine fusion. Several human studies have demonstrated a significant reduction in the rate of spinal fusion in patients taking NSAIDs. This information suggests that NSAIDs should perhaps be avoided in the post-operative period after spinal fusion but there is a lack of basic science information regarding reparative bone healing and NSAIDs. Finally, NSAIDs have been shown to be associated with a decreased incidence of heterotopic ossification following hip or pelvic surgery, and in other areas, including the forearm.

In contrast, the administration of PGE2 has increased the rate of fracture healing in several models. It was recently shown that ultrasound (sine wave of 1.5MHz repeating at 1kHz, 30mW/cm2, 20 minutes) increases both COX-2 and PGE2 expression in the mouse osteoblastic cell line, MC3T3-E1. The findings suggest that ultrasound may act, at least in part, through a COX-dependent pathway in accelerating fracture repair. Thus the metabolites of cyclooxygenase activity appear to be important in reparative process.

Prostaglandin production and COX-2 mRNA are increased in fracture callus during the first two weeks following injury, suggesting a role in the early phase of bone healing. Most remarkable was the reduction in osteoblasto-
genesis, a finding that was confirmed by two independent in vivo models of intramembranous bone formation as well as by in vitro studies that demonstrated a critical requirement for COX-2 in osteoblastogenesis. These findings demonstrate that the production of COX-2 metabolites during the inflammatory phase is required for efficient bone healing and that mesenchymal cell differentiation is a major target of cyclooxygenase activity.

Prior work has demonstrated that prostaglandins such as PGE2 induces bone nodule formation in bone marrow stromal cell cultures46,49 and in cultured rat calvaria osteoblasts50-53. Furthermore, systemic or local injection of PGE2 stimulates bone formation in vivo and appear to induce osteoblastogenesis from bone marrow precursors54,55. Ex vivo cultures of bone marrow stromal cells from rats injected with PGE2 for 2 weeks yield 4 times more mineralized bone nodules compared with cultures from vehicle-injected rats56. Finally, Scott et al. demonstrated that PGE2 increases bone nodule formation in low density cultures of rat bone marrow cells by recruiting osteoblast precursors from a population of non-adherent mesenchymal precursor cells present in the bone marrow56.

Based on these findings, we propose a mechanism of action for COX-2 in bone repair. Under basal conditions, COX-2 activity maintains a population of mesenchymal stem cells in a pre-osteoblast state responsive to additional osteoblastic signals. During injury, the elevated COX-2 expression increases the osteoblastic potential of mesenchymal stem cells and supports their differentiation to osteoblasts in response to osteogenic signals. COX-2 may exert its effect through regulation of transcription factors such as cbfa1 and osterix. Since cbfa1 is upstream of osterix, the COX-2 dependent regulation is likely at this earlier stage of differentiation, although the findings do not rule out a direct effect of cyclooxygenase metabolites on osterix expression. Furthermore, the effects could be mediated by induction of BMPs as previously reported57. This model postulates a unique mechanism of COX-2 in bone repair that is significantly different from that in the fetal skeletal development. This mechanism may be particularly important in injury or during inflammation where large amounts of PGE2 are produced by COX-2, as opposed to normal skeletogenesis where the role of COX-2 is limited.

References

Gastroenterol 2000; 95:1681-1690.


