J Musculoskel Neuron Interact 2003; 3(4):308-312

Perspective Article

Hylonome

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[1,2]. 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[3,4]. Following the initial inflammation, new bone is formed by intramembranous ossification as well as endochondral ossification; these processes are predominately mediated by osteoblasts[3,4]. 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[5]. As with homeostatic remodeling, the important functional outcome of the remodeling phase of fracture healing is the restoration of mechanical strength and stability[3,4].

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[6]. 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 (corticosteroid) induced osteoporosis also documented its adverse affects on

The authors have no conflict of interest.

Corresponding author: J. Edward Puzas, Ph.D., Donald & Mary Clark Professor of Orthopaedics, Department of Orthopaedics, Box 665, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
E-mail: edward_puzas@URMC.Rochester.edu

Accepted 1 August 2003

fracture healing in a rabbit ulnar osteotomy model[7]. In this study critical sized (1 mm) defects were created bilaterally in 18 adult female New Zealand White rabbits. Starting 2 months before operative intervention and continuing for 6 weeks during healing of the osteotomies, a subcutaneous dose of either sterile saline or prednisone (0.15 mg/kg) was administered daily. Serial radiographs of the forelimb were taken immediately post-operatively and weekly beginning the second week post-operatively. After sacrifice at 6 weeks, only 3 of 20 limbs from animals treated with prednisone achieved radiographic union while 13 of 16 control limbs achieved union. The radiographic densities of bone in the defect as well as callus size were greater in the control limbs than in the limbs from prednisone-treated animals. DEXA confirmed that the bone mineral content was lower in the ulnae of prednisone-treated rabbits both within the defect and in adjacent ulnar bone. Finally, mechanical testing data indicated that osteotomies from rabbits chronically treated with prednisone were weaker than in controls.

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[8-11].

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[12,13]. Traditional NSAIDs inhibit both COX-1 and COX-2[14,15]. Selective inhibitors for COX-2 have been developed and are effective anti-inflammatory agents and avoid serious GI side effects[16,17]. COX-2 inhibitors may also be useful in prevention of diseases such as colon cancer and Alzheimer's disease[18,19]. COX-2 isoforms have recently been identified in both bone cells and chondrocytes[20,21].

Cyclooxygenase knockout mice have been created[22,23]. 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[24]. 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[25,26], elderly nursing home patients[27,28], those with stress fractures[29], and adult traumatic fractures resulting from participation in sports[30]. Furthermore, NSAIDs are recommended for the relief of pain following spinal fusion[31]. 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[32,33]. 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 ($p = 0.000001$)[34].

Animal studies also strongly suggest an inhibitory effect of NSAIDs on spine fusion[35,36]. Several human studies have demonstrated a significant reduction in the rate of spinal fusion in patients taking NSAIDs[37,38]. 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[39,40], and in other areas, including the forearm[41].

In contrast, the administration of PGE2 has increased the rate of fracture healing in several models[42-44]. 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[45]. 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[46,47], 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 cultures[48,49] and in cultured rat calvaria osteoblasts[50-53]. Furthermore, systemic or local injection of PGE2 stimulate bone formation *in vivo* and appear to induce osteoblastogenesis from bone marrow precursors[54,55]. *Ex vivo* cultures of bone marrow stromal cells from rats injected with PGE2 for 2 weeks yield 4 times more mineralized bone nodule compared with cultures from vehicle–injected rats[55]. Finally, Scutt 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 marrow[56].

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 reported[57]. 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 skeletalogenesis where the role of COX-2 is limited.

References

1. Ferguson C, Alpern E, Miclau T, Helms JA. Does adult fracture repair recapitulate embryonic skeletal formation? Mech Dev 1999; 87:57-66.
2. Le AX, Miclau T, Hu D, Helms JA. Molecular aspects of healing in stabilized and non-stabilized fractures. J Orthop Res 2001; 19:78-84.
3. Einhorn TA. The cell and molecular biology of fracture healing. Clin Orthop 1998; (Suppl.355):S7-S21.
4. Buckwalter JA, Einhorn TA, Bolander ME, Crue RL. Healing of the musculoskeletal tissues. In: Rockwood CA Jr, Green DP (eds) Fractures in Adults. Lippincott-Raven, Philadelphia; 2001:261.
5. Cruess RL, Dumont J. Fracture healing. Can J Surg 1975; 18:403-413.
6. Namkung-Matthai H, Appleyard R, Jansen J, Lin JH, Maastricht S, Swain M, Mason RS, Murrell GA, Diwan AD, Diamond T. Osteoporosis influences the early period of fracture healing in a rat osteoporotic model. Bone 2001; 28:80-86.
7. Waters RV, Gamradt SC, Asnis P, Vickery BH, Avnur Z, Hill E, Bostrom M. Systemic corticosteroids inhibit bone healing in a rabbit ulnar osteotomy model. Acta Orthop Scand 2000; 71:316-321.
8. Kujubu DA, Fletcher BS, Varnum BC, Lim RW, Herschman HR. TIS10, a phorbol ester tumor promoter-inducible mRNA from Swiss 3T3 cells, encodes a novel prostaglandin synthase/cyclooxygenase homologue. J Bio Chem 1991; 266:12866-12872.
9. O'Banion MK, Sadowski HB, Winn V, Young DA. A serum- and glucocorticoid-regulated 4-kilobase mRNA encodes a cyclooxygenase-related protein. J Biol Chem 1991; 266:23261-23267.
10. O'Banion MK, Winn VD, Young DA. cDNA cloning and functional activity of a glucocorticoid-regulated inflammatory cyclooxygenase. Proc Natl Acad Sci USA 1992; 89:4888-4892.
11. Sirois J, Simmons DL, Richards JS. Hormonal regulation of messenger ribonucleic acid encoding a novel isoform of prostaglandin endoperoxide H synthase in rat prevulatory follicles. Induction *in vivo* and *in vitro*. J Bio Chem 1992; 267:11586-11592.
12. Lane NE. Pain management in osteoarthritis: the role of COX-2 inhibitors, J Rheumatol 1997; 24:20-24.
13. Simon LS, Lanza FL, Lipsky PE, Hubbard RC, Talwalker S, Schwartz BD, Isakson PC, Geis GS. Preliminary study of the safety and efficacy of SC-58635, a novel cyclooxygenase 2 inhibitor: efficacy and safety in two placebo-controlled trials in osteoarthritis and rheumatoid arthritis, and studies of gastrointestinal and platelet effects. Arthritis Rheum 1998; 41:1591-1602.
14. Giercksky KE, Huseby G, Rugstad HE. Epidemiology of NSAID-related gastrointestinal side effects. Scand J Gastroenterol 1989; 163:3-8.
15. Hirschowitz BI. Non-steroidal anti-inflammatory drugs and the gastrointestinal tract, Gastroenterologist. 1994; 2:207-223.
16. Masferrer JL, Zweifel BS, Manning PT, Hauser SD, Leahy KM, Smith WG, Isakson PC, Seibert K. Selective inhibition of inducible cyclooxygenase 2 *in vivo* is anti-inflammatory and non-ulcerogenic. Proc Natl Acad Sci USA 1994; 91:3228-3232.
17. Goldstein JL, Silverstein FE, Agrawal NM, Hubbard RC, Kaiser J, Maurath CJ, Verburg KM, Geis GS. Reduced risk of upper gastrointestinal ulcer complications with celecoxib, a novel COX-2 inhibitor. Am J

Gastroenterol 2000; 95:1681-1690.
18. O'Banion MK. COX-2 and Alzheimer's disease: potential roles in inflammation and neurodegeneration. Expert Opin Investig Drugs 1999; 8:1521-1536.
19. Dubois RN. Cyclooxygenase–a target for colon cancer prevention. Aliment Pharmacol Ther 2000; 1(Suppl.4):64-67.
20. Geng Y, Blanco FJ, Cornelisson M, Lotz M. Regulation of cyclooxygenase-2 expression in normal human articular chondrocytes. J Immunol 1995; 155:796-801.
21. Crofford LJ. COX-1 and COX-2 tissue expression: implications and predictions,. J Rheumatol 1997; 24:15-19.
22. Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD. Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. Cell 1995; 83:483-492.
23. Morham SG, Langenbach R, Loftin CD, Tiano HF, Vouloumanos N, Jennette JC, Mahler JF, Kluckman KD, Ledford A, Lee CA. Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. Cell 1995; 83:473-482.
24. Dinchuk JE, Car B, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM. Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. Nature 1995; 378:406-409.
25. Facchini R, Selva G, Peretti G. Tolerability of nimesulide and ketoprofen in paediatric patients with traumatic or surgical fractures. Drugs 1993; 46:238-241.
26. Pierce MC, Fuchs S. Evaluation of ketorolac in children with forearm fractures. Acad Emergency Med 1997; 4:22-26.
27. Egbert AM. Post-operative pain management in the frail elderly. Clin Geriatric Med 1996; 12:583-599.
28. Cramer GW, Galer BS, Mendelson MA, Thompson GD. A drug use evaluation of selected opioid and nonopioid analgesics in the nursing facility setting. J Amer Geriatr Soc 2000; 48:398-404.
29. Fredericson M, Bergman AG, Hoffman KL, Dillingham MS. Tibial stress reaction in runners. Correlation of clinical symptoms and scintigraphy with a new magnetic resonance imaging grading system. Amer J Sports Med 1995; 23:472-481.
30. Massart P, Bezes H. Comparison of the efficacy and tolerance of isoxicam and piroxicam following surgery for skiing accidents. Br J Clin Pharmacol 1986; 22:161S-165S.
31. Reuben SS, Connelly NR, Steinberg R. Ketorolac as an adjunct to patient-controlled morphine in postoperative spine surgery patients. Reg Anesth 1997; 22:343-346.
32. Altman RD, Latta LL, Keer R, Renfree K, Hornicek FJ, Banovac K. Effect of non-steroidal anti-inflammatory drugs on fracture healing: a laboratory study in rats. J Orthop Trauma 1995; 9:392-400.
33. Ho ML, Chang JK, Wang GJ. Effects of ketorolac on bone repair: A radiographic study in modeled demineralized bone matrix grafted rabbits. Pharmacology 1998; 57:148-159.
34. Giannoudis PV, MacDonald DA, Matthews SJ, Smith RM, Furlong AJ, De Boer P. Non-union of the femoral diaphysis. The influence of reaming and non-steroidal anti-inflammatory drugs. J Bone Joint Surg 2000; 82B:655-658.
35. Dimar JRN II, Ante WA, Zhang YP, Glassman SD. The effects of non-steroidal anti-inflammatory drugs on posterior spinal fusions in the rat. Spine 1996; 21:1870-1876.
36. Martin GJJ, Boden SD, Titus L. Recombinant human bone morphogenetic protein-2 overcomes the inhibitory effect of ketorolac, a nonsteroidal anti-inflammatory drug (NSAID), on posterolateral lumbar intertransverse process spine fusion. Spine 1999; 24:2188-2193.
37. Deguchi M, Rapoff AJ, Zdeblick TA. Posterolateral fusion for isthmic spondylolisthesis in adults: analysis of fusion rate and clinical results. J Spinal Disord 1998; 11:459-464.
38. Glassman SD, Rose SM, Dimar JR, Puno RM, Campbell MJ, Johnson JR. The effect of post-operative non-steroidal anti-inflammatory drug administration on spinal fusion. Spine 1998; 23:834-838.
39. Kjaersgaard-Andersen P, Hougaard K, Linde F, Christiansen SE, Jensen J. Heterotopic bone formation after total hip arthroplasty in patients with primary or secondary coxarthrosis. Orthopedics 1990; 13:1211-1217.
40. Moore KD, Goss K, Anglen JO. Indomethacin versus radiation therapy for prophylaxis against heterotopic ossification in acetabular fractures: a randomised, prospective study. J Bone Joint Surg Br 1998; 80B:259-263.
41. Beingessner DM, Patterson SD, King GJ. Early excision of heterotopic bone in the forearm. J Hand Surg 2000 ; 25:483-488.
42. Norrdin RW, Shih MS. Systemic effects of prostaglandin E2 on vertebral trabecular remodeling in beagles used in a healing study. Calcif Tissue Int 1988; 42:363-368.
43. Keller J, Klamer A, Bak B, Suder P. Effect of local prostaglandin E2 on fracture callus in rabbits. Acta Orthop Scand 1993; 64:59-63.
44. Suponitzky I, Weinreb M. Differential effects of systemic prostaglandin E2 on bone mass in rat long bones and calvariae. J Endocrinol 1998; 156:51-57.
45. Kokubu T, Matsui N, Fujioka H, Tsunoda M, Mizuno K. Low intensity pulsed ultrasound exposure increases prostaglandin E2 production via the induction of cyclooxygenase-2 mRNA in mouse osteoblasts. Biochem Biophys Res Com 1999; 256:284-287.
46. Dekel S, Lenthall G, Francis MJ. Release of prostaglandins from bone and muscle after tibial fracture. An experimental study in rabbits. J Bone Joint Surg Br 1981; 63-B:185-189.

47. Gerstenfeld L, Thiede M, Serbert K, Mielke C, Tewari S, Svagar B, Cullinane D, Einhorn T. Differential inhibition of fracture healing by non-specific and cyclooxygenase-2 (COX-2) specific non-steroidal anti-inflammatory drugs. Transactions ORS 2002; paper no: 0239.
48. Weinreb M, Suponitzky I, Keila S. Systemic administration of an anabolic dose of PGE2 in young rats increases the osteogenic capacity of bone marrow. Bone 1997; 20:521-526.
49. Weinreb M, Grosskopf A, Shir N. The anabolic effect of PGE2 in rat bone marrow cultures is mediated via the EP4 receptor subtype. Am J Physiol 1999; 276:E376-383.
50. Kaneki H, Takasugi I, Fujieda M, Kiriu M, Mizuochi S, Ide H. Prostaglandin E2 stimulates the formation of mineralized bone nodules by a cAMP-independent mechanism in the culture of adult rat calvarial osteoblasts. J Cell Biochem 1999; 73:36-48.
51. Tang LY, Kimmel DB, Jee WS, Yee JA. Functional characterization of prostaglandin E2 inducible osteogenic colony forming units in cultures of cells isolated from the neonatal rat calvarium. J Cell Physiol 1996; 166:76-83.
52. Nagata T, K. Kaho K, Nishikawa S, Shinohara H, Wakano Y, Ishida H. Effect of prostaglandin E2 on mineralization of bone nodules formed by fetal rat calvarial cells. Calcif Tissue Int 1994; 55:451-457.
53. Flanagan AM, Chambers TJ. Stimulation of bone nodule formation *in vitro* by prostaglandins E1 and E2. Endocrinol 1992; 130:443-448.
54. Suponitzky I, Weinreb M. Differential effects of systemic prostaglandin E2 on bone mass in rat long bones and calvariae. J Endocrinol 1998; 156:51-57.
55. Keila S, Kelner A, Weinreb M. Systemic prostaglandin E2 increases cancellous bone formation and mass in aging rats and stimulates their bone marrow osteogenic capacity *in vivo* and *in vitro*. J Endocrinol 2001; 168:131-139.
56. Scutt A, Zeschnigk M, Bertram P. PGE2 induces the transition from non-adherent to adherent bone marrow mesenchymal precursor cells via a cAMP/EP2-mediated mechanism. Prostaglandins 1995; 49:383-395.
57. Paralkar VM, Grasser WA, Mansolf AL, Baumann AP, Owen TA, Smock SL, Martinovic S, Borovecki F, Vukicevic S, Ke HZ, Thompson DD. Regulation of BMP-7 expression by retinoic acid and prostaglandin E(2). J Cell Physiol 2002; 190:207-217.