Can an anti-fracture agent heal fractures?

Thomas A. Einhorn, MD

Professor and Chairman Department of Orthopaedic Surgery
Boston University School of Medicine

Address for correspondence:
Thomas A. Einhorn, MD
Boston University School of Medicine
720 Harrison Avenue, Suite 808
Boston, MA 02118
Telephone: 617-638-8435
Fax: 617-638-8493
Email: thomas.einhorn@bmc.org

Summary

Anti-fracture agents typically prevent fractures by augmenting bone mass and enhancing skeletal integrity. These agents exert their effects by means of anti-catabolic or anabolic actions. Because fracture healing involves bone formation as well as bone resorption, it is reasonable to hypothesize that agents that affect these activities may also modulate skeletal repair. Bisphosphonates, agents that inhibit bone resorption, may enhance the healing of fractures or permit patients with fractures to bear weight earlier by delaying the conversion of calcified cartilage to woven bone, or woven bone to lamellar bone. In doing so, they increase the size of the fracture callus and small increases in the radius of fracture callus can have dramatic positive effects on fracture callus stiffness and strength. Another possibility is that certain hypertrophic nonunions fail to unite because of excessive remodeling of the callus. Use of a bisphosphonate may modulate this catabolic activity, uncouple it from the associated bone formation and promote healing. Inhibitors of RANKL have undergone far less investigation but may also act on osteoclast precursors to down-regulate bone resorption. Parathyroid hormone may enhance fracture repair by promoting chondrogenesis early in the healing process and osteogenesis at a later time. The former effect improves callus geometry while the latter effect improves bone quality as well as quantity. Several anecdotal reports and one randomized, controlled trial have suggested that parathyroid may enhance skeletal repair in specific clinical settings.
Although these reports are based on solid scientific data, there are limited clinical data at this time. The use of anti-fracture agents for the enhancement of fracture healing will ultimately depend upon high quality evidence from well-designed, well-controlled clinical trials.

KEY WORDS: bisphosphonates, teriparatide, parathyroid hormone, denosumab, fracture healing.

Introduction

Over the past decade, there has been increasing interest in developing new technologies for the enhancement of skeletal repair. Although the use of locally implanted or injected growth factors has received most of the attention, the ability to enhance bone repair by systemic means is an attractive idea. A growing body of evidence supports the notion that pharmaceutical agents active in bone, and known to improve bone mass, may be candidate agents for the systemic enhancement of fracture repair. This article will provide a minireview of the evidence to support this concept.

Can Bisphosphonates Enhance Fracture Healing?

Bisphosphonates are the most widely used class of compounds for the treatment of bone diseases characterized by enhanced osteoclastic activity, such as osteoporosis, Paget Disease, metastatic bone disease, osteogenesis imperfecta, and fibrous dysplasia (1-4). The mechanism of action by which these compounds exert their activity depends on the chemical structure. Nitrogen-containing bisphosphonates such as alendronate, pamidronate, risedronate, ibandronate, and zoledronate, exert their effects by inhibiting components of the intracellular mevalonate pathway resulting in impaired membrane localization of small guanosine triphosphatases, an important signaling molecule involved with maintaining osteoclast cell morphology, integrin signaling, membrane protein trafficking and cell survival (5-7) Non-nitrogen-containing bisphosphonates, such as etidronate and clodronate, preferentially bind to the mineral phase of bone, are released during osteoclastic bone resorption, and accumulate in these cells eventually inducing apoptosis (5) (Table 1).

Because bisphosphonates consistently inhibit osteoclastic bone resorption, and because the remodeling phase of fracture healing involves osteoclast-directed bone remodeling, concern has been expressed regarding the potential role of bisphosphonates in the bone healing process (8). Goodship et al. (9) studied the effects of pamidronate on the healing of a 3-millimeter osteotomy gap in the sheep tibial diaphysis after stabilization with a unilateral external fixator. Greater callus formation with an associated increase in bone mineral content was observed in the treatment group. There were no differences in fracture or torsional stiffness between the groups although torsional stiffness was also greater in the group treated with pamidronate. While callus remodeling was reduced, the authors concluded that reduced callus remodeling leads to an increased amount of bridging callus and an improvement in the early regain of strength during the healing process. Peter et al. (10) noted a similar increase in callus size, without an accompanying improvement in ultimate load-to-failure and flexural rigidity when alendronate was used in dogs that had undergone a mid-diaphyseal radius fracture. Li et al. (11) studied the long term effects of bisphosphonate on fracture healing in rats treated with two doses of incadronate (10 and 100 mg/kg). Animals were treated three times per week for two weeks and killed 25 and 49 weeks after fracture. Radiographs showed that the largest cross-sectional area at the fracture site was observed in the groups treated with bisphosphonate while histological analysis demonstrated delayed lamellar bone formation with reduced mineral apposition and bone formation rates. The net effect was a greater stiffness and ultimate load to failure measured in the animals treated with bisphosphonate. These findings suggest that long-term continuous treatment with incadronate delayed the remodeling phase of fracture healing but did not impair the recovery of mechanical integrity at the fracture site.

Table 1 - Commercially Available Bisphosphonates.

Generic Name	Route of Administration	Nitrogen or Non-Nitrogen Containing Compounds	Approved Uses
Clodronate	Oral, intravenous	Non-nitrogen	----------------------
Etidronate	Oral	Non-nitrogen	Osteoporosis
Tiludronate	Oral	Non-nitrogen	Paget disease
Alendronate	Oral	Nitrogen	Paget disease, osteoporosis
Pamidronate	Intravenous	Nitrogen	Paget disease, hypercalcemia of malignancy, metastatic osteolysis
Risedronate	Oral	Nitrogen	Page disease, osteoporosis
Zoledronate	Intravenous	Nitrogen	Hypercalcemia of malignancy, metatstic osteolysis, osteoporosis
Ibandronate	Oral, intravenous	Nitrogen	Osteoporosis

Recently, Amanat et al. (12) investigated the effects of systemic versus local dosing of bisphosphonate on fracture repair. Recognizing that prior reports have shown that continuous administration of bisphosphonate increases callus size by inhibiting bone remodeling, these investigators used an open rat femoral fracture model and administered either a single systemic bolus dose or a local application of pamidronate. By six weeks after fracture, the results demonstrated significant increases in bone mineral content and volume in the animals treated with pamidronate compared to saline-treated controls. In addition, the single bolus dose group showed increased callus strength by 50%. Although local application also increased callus size and bone mineral content, it did not increase the strength of the callus. These findings suggest that the normal process of bone remodeling during fracture healing is a mechanism to control callus size and thereby strength. A single systemic dose of bisphosphonate may lead to an increase in callus size and early gain of strength while delaying bone remodeling (Figure 1). This is in counterdistinction to that observed with local application of bisphosphonate which may have a more inhibitory effect on the fracture healing process. Thus, a single bolus administration of a potent, long-acting bisphosphonate may be a valuable adjunctive therapy for the treatment of fractures. One of the unsolved problems in clinical fracture care is the treatment of fracture nonunion. Although the pathophysiology of this condition is not fully understood, the concept that bone remodeling may be impaired has been considered. Indeed, if fracture callus fails to unite because of a hyperresorptive state, it is reasonable to believe that bisphosphonates may positively influence this process. Kakar et al. (13) describe the case of a 9-year old girl who sustained an open tibia/fibular fracture after being hit by a motor vehicle. She was treated by irrigation and debridement of the fracture followed by application of an external fixator. After 7 months, the fibula healed but the tibia showed delayed union despite frame removal and full weight bearing in a cast. Administration of two doses of 0.025 mg/kg zolodronic acid intravenously six weeks apart was followed by a rapid increase in bridging callus at the tibial fracture site. Remodeling was seen in the second year. This case suggests that sufficient anabolic activity was present to heal the fracture but that union was impaired by excess catabolic activity. The use of zoledronate to modulate bone catabolism may have led to healing and avoided the need for further operative procedures.

Can Parathyroid Hormone Enhance Fracture Healing?

Parathyroid hormone (PTH) is an 84 amino acid polypeptide whose role in mineral homeostasis is to increase serum calcium levels by enhancing gastrointestinal calcium absorption, increase renal calcium and phosphate reabsorption, liberate calcium from the
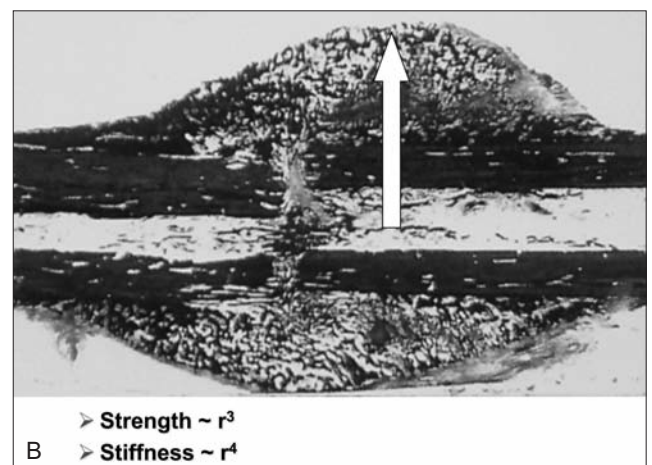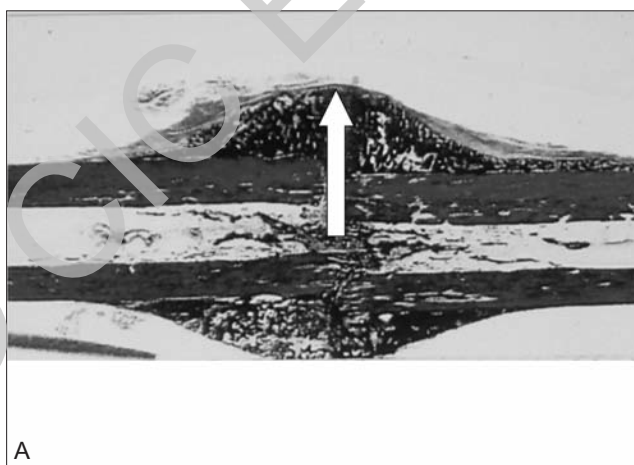
- Strength ~ r^3
- Stiffness ~ r^4

Figure 1A, B - **A.** Sagittal section of a rat fracture callus six weeks after injury. The arrow shows the radius of the callus (hematoxylin and eosin, x25). **B.** Sagittal section of rat fracture callus six weeks after injury in an animal that had been treated with alendronate. Note increased length of the radius. Because strength is related to the third power of the radius and stiffness to the fourth power of the radius, this construct has increased mechanical properties at this time point in the healing process (hematoxylin and eosin, x25).

skeleton in response to systemic demands, and participate in the regulation of vitamin D metabolism.14 Several reports have shown that, whereas continuous exposure to parathyroid hormone leads to an increase in osteoclastic activity, intermittent exposure stimulates osteoblasts and results in an increase in bone formation (15, 16). The first 34 amino acids of this peptide represents its active fragment and PTH (1-34) has very similar effects.

Studies in models of fracture healing have demonstrated that PTH (1-34) consistently enhances the stiffness and strength of experimental fractures (17-20). Andreassen et al. reported on the effects of intermittent PTH (1-34) on callus formation and mechanical strength in tibial fractures in healthy sexually mature adult rats after 40 days of healing. Compared with controls, fracture callus volume and mechanical properties were significantly enhanced (17). In an investigation by the same investigators in which aged rats were used, similar findings were observed (18). More recently, Alkhiary et al. (19) investigated the effects of recombinant human PTH (1-34) [teriparatide] on fracture healing in 270 rats that underwent standard closed femoral fractures and received doses of teriparatide that are similar to those shown to be effective in the treatment of osteoporosis in postmenopausal women. Using biomechanical tests, histomorphometry and quantitated computed tomography, these investigators demonstrated that daily systemic administration of both a 5 and a 30 mg/kg per day dose enhanced fracture healing by increasing bone mineral density, bone mineral content, and total osseous tissue volume. Nakajima et al. (20) reported similar findings using a dose of PTH (1-34) that was within this range (10 mgs/kg per day).

While these studies clearly document the potential enhancement of bone repair with PTH (1-34) in rodent models of fracture healing, the mechanism through which PTH exerts its effect has not been fully elucidated. A recent investigation by Kakar et al. (21) showed that PTH (1-34) primarily enhanced the earlier stages of endochondral bone repair by increasing chondrocyte recruitment and rate of differentiation (Figure 2). In coordination with these cellular events, increased canonical Wnt-signaling was observed in PTH-treated animals at multiple time points across the time-course of fracture repair. Quantitative microcomputed tomography analysis showed that PTH (1-34) treatment induced a larger callus cross sectional area, length and total volume compared with controls. Molecular analysis of the expression of extracellular matrix genes associated with chondrogenesis and osteogenesis showed that PTH (1-34)-treated fractures displayed a 3-fold greater increase in chondrogenesis relative to osteogenesis over the time course of the repair process. In addition, chondrocyte hypertrophy occurred earlier in the PTH-treated callus tissue. Analysis of the expression of potential mediators of the actions of PTH showed that PTH (1-34) treatment significantly induced the expression of Wnts 4, 5a, 5b, and 10b and increased levels of unphosphorylated, nuclear localized beta-catenein protein, a central feature of canonical Wnt signaling (21). Recent work from our laboratory (unpublished) shows that the expression of matrix metalloproteinase occurs earlier in fractures treated with PTH (1-34) suggesting an enhancement of cartilage turnover and an acceleration of the transition from calcified cartilage to bone.

Based on these studies, several clinical investigators have used teriparatide in the treatment of patients with fractures and reported individual cases or small case series (22-25). To date, only one clinical trial has been conducted investigating the use of teriparatide in patients with fractures. Post-menopausal women who had sustained a dorsally angulated distal radial fracture in need of closed reduction but not surgery were randomly signed to eight weeks of once-daily injections of placebo, PTH (1-34; teriparatide) 20 mcg, or 40 mcg within ten days of fracture. The results showed that the estimated median time from fracture to first radiographic evidence of complete cortical bridging in three of four cortices was 9.1, 7.4, and 8.8 weeks for placebo, teriparatide 20 mcg, and teriparatide 40 mcg, respectively (p=.015). There was no statistically signifi-
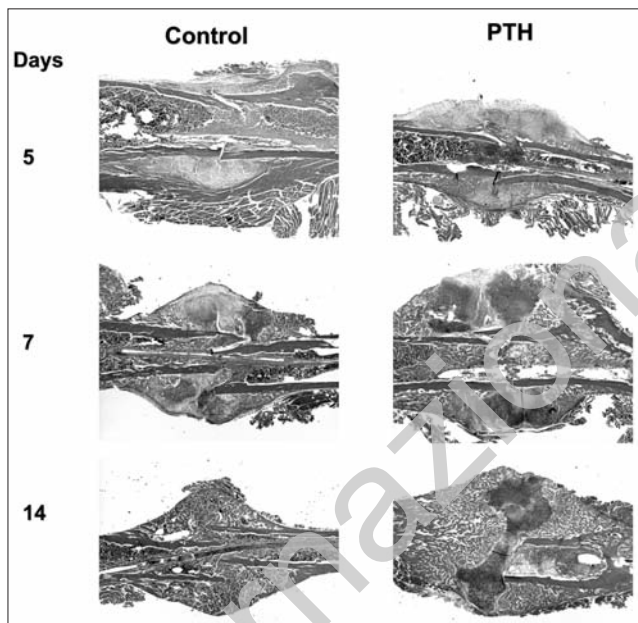

Figure 2 - Sagittal sections of rat femoral fracture calluses showing an increase in cartilage content in the animals treated with PTH (Safranin-o and toludine-blue, x2).

cant difference between the higher and the lower doses of teriparatide nor between the higher dose and the placebo group. The time to healing was shorter in the teriparatide 20 mcg group than in placebo (p=0.006). The investigators concluded that the shortened time to healing may suggest that fracture repair can be accelerated by teriparatide but that these results require further investigation (26).

Can an Inhibitor of Receptor Activator of NF kappa B Ligand (RANKL) Enhance Fracture Healing?

RANK is a receptor on osteoclast precursors which when bound by RANKL promotes the differentiation of these cells in the osteoclast lineage. Primarily produced by osteoblasts, RANKL is a membrane-bound ligand that exists in a soluble form and can be detected in the serum. Inhibition of RANKL is an evolving strategy for the control of conditions in which increased bone resorption is part of the pathogenesis of disease. Recently, a fully human monoclonal antibody to RANKL, denosumab, has been developed and tested in phase 3 clinical trials for its use in the treatment of postmenopausal osteoporosis (27). However, despite its efficacy against osteoperosis, because the resorption of calcified cartilage and bone occur during the normal process of fracture healing, the impact of osteoclast inhibition on fracture healing was compared between alendronate and denosumab, two osteoclast inhibitors with different mechanisms of action (28).

Male human RANKL knock-in mice that express a chimeric (human/murine) form of RANKL received unilateral transverse femur fractures. Mice were treated biweekly, and for 21 or 42 days, with either alendronate or denosumab. Treatment efficacy was assessed by measuring serum levels of tartrate resistant alkaline phosphatase (TRAP) 5b. The results showed almost a complete elimination of TRAP 5b in the denosumab-treated animals but only a 25% reduction in serum levels in the alendronate-treated mice. Immunohistochemical analyses showed similar findings. Mechanical testing showed that fractured femurs from both the alendronate and denosumab groups had significantly increased stiffness and strength at day 42 after fracture compared to controls. Microcomputed tomography analysis showed that fracture calluses from

denosumab-treated mice had significantly greater bone volume and bone mineral content in comparison to alendronate-treated bones at both 21 and 42 days after fracture. Although both alendronate and denosumab delayed the early removal of cartilage and the remodeling of the fracture callus, this did not diminish the mechanical integrity of the healing fractures. In contrast, strength and stiffness were enhanced in these treatment groups when compared to control bones (28). Hence, these two pharmaceutical agents, with different mechanisms of action, enhance fracture healing by inhibiting the resorption of calcified cartilage and woven bone.

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