Bone healing: little secrets

Thomas A. Einhorn

Department of Orthopaedic Surgery
Boston University School of Medicine, Boston, MA

Address for correspondence:
Thomas A. Einhorn, MD
Professor and Chairman
Department of Orthopaedic Surgery
Boston University School of Medicine
720 Harrison Avenue, Suite 808
Boston, MA 02118
Ph.: 617-638-8435
Fax: 617-638-8493
E-mail: thomas.einhorn@bmc.org

Summary

The development of new strategies to enhance the healing of fractures continues to evolve with the introduction of both locally and systemically delivered compounds. The recent refinement in the use of autologous bone marrow as a bone graft material has brought the field of stem cell biology into orthopaedic practice. New recombinant peptides such as platelet-derived growth factor and teriparatide show promise as local and systemic enhancers respectively. Finally, recent evidence that mutations in elements of the Wnt signaling pathway lead to gain-of-function mutations in bone formation in rare clinical settings has provided the basis for the targeting of this pathway for the development of therapeutic agents for bone repair. These recent developments are presented as the "little secrets" of present day bone healing.

KEY WORDS: Platelet Derived Growth Factor; pathyroid hormone; bone marrow; Wnt signalling molecules.

Introduction

Fracture healing is considered to be a biologically optimized process leading to the repair and regeneration of bone in most cases. However, despite the expectation of successful healing, approximately 5-10% of the eight million fractures sustained annually in the United States alone have difficultly achieving union (1). To address this, clinicians, biologists, and bioengineers are developing new technologies to enhance fracture healing. Some of these technologies have already reached the clinic and within the past decade bone morphogenetic proteins have made a significant impact on enhancing bone repair. Other technologies including the development of novel synthetic peptides, recombinant molecules, and biophysical stimuli are currently in development. In addition, a new concept, the introduction of systemic agents to enhance skeletal repair is just now receiving attention.

Among the many possibilities for current and future therapies in fracture healing, a few standout as showing great potential and form the basis for what some might call "little secrets" of bone healing. These include the local technologies of autologous bone marrow stem cell injection and the use of recombinant human platelet derived growth factor; and systemic technologies including recombinant human parathyroid hormone, and modulators of the Wnt signaling pathway.

Percutaneous autologous bone marrow grafting

It has long been known that bone marrow is a reservoir of progenitor cells for both the hematopoietic and stromal stem cell systems. Over the years, surgeons and scientists have explored the use of bone marrow stem cells to enhance skeletal repair (2-10). Although the results have been inconsistent, recent work has suggested that methods to enrich stem cell populations using centrifugation, filtration and/or selective retention techniques, may improve the yield of progenitor cells (11,12). Moreover, while it has long been thought that mesenschymal stem cells would be the preferred cellular graft because of their ability to potentially differentiate into chondroblasts and osteoblasts, a reexamination of the hematopoietic niche suggests that a combination of mesenschymal and hematopoietic cells may be more desirable (8,9). As skeletal healing involves the participation of chondroblasts, osteoblasts, and also a healthy vascular supply, the presence of endothelial cells and the ability of hematopoietic stem cells to support the activities of stromal cell progenitors may provide an optimal grafting environment. Hernigou et al. (11) recently reported successful results in sixty patients with non-infected nonunions who underwent bone marrow aspiration from both iliac crests and injection of bone marrow aspirate concentrate. Union was obtained in 53 out of 60 patients and positive correlations were demonstrated between the volume of mineralized callus at four months and the number and concentration of colony forming units (CFUs) in culture. Indeed, the seven patients who did not unite had significantly lower numbers in concentrations of CFUs. The investigators confirmed that technical aspects of this procedure involve aspiration of a sufficient volume of bone marrow (at least 120 millimeters) and a harvesting technique that involves deep insertion of a beveled needle (approximately 6 to 8 centimeters in length and 1.5 millimeters in diameter) into spongy bone between the tables of the ilium. Marrow is then aspirated into 10 millimeter plastic syringes. At a given depth, the aspirating needle is turned 45 degrees to reorient the bevel during successive aspirations so that the largest possible space is aspirated. After one full turn, the needle is removed by one centimeter and the process is repeated. Marrow is aspirated in small fractions (up to 5 millimeters) to reduce the degree of dilution with peripheral blood. Three to five perforations may need to be made into the iliac crest in order to aspirate sufficient volume using this technique (13).
Centrifugation to obtain a concentrated buffy coat, which contains progenitor cells and other mononuclear cells is achieved with the appropriate device. Once the concentrated stem cells are obtained, direct injection into a nonunion site can lead to the healing of fractures in carefully selected, non-infected cases (Figure 1).

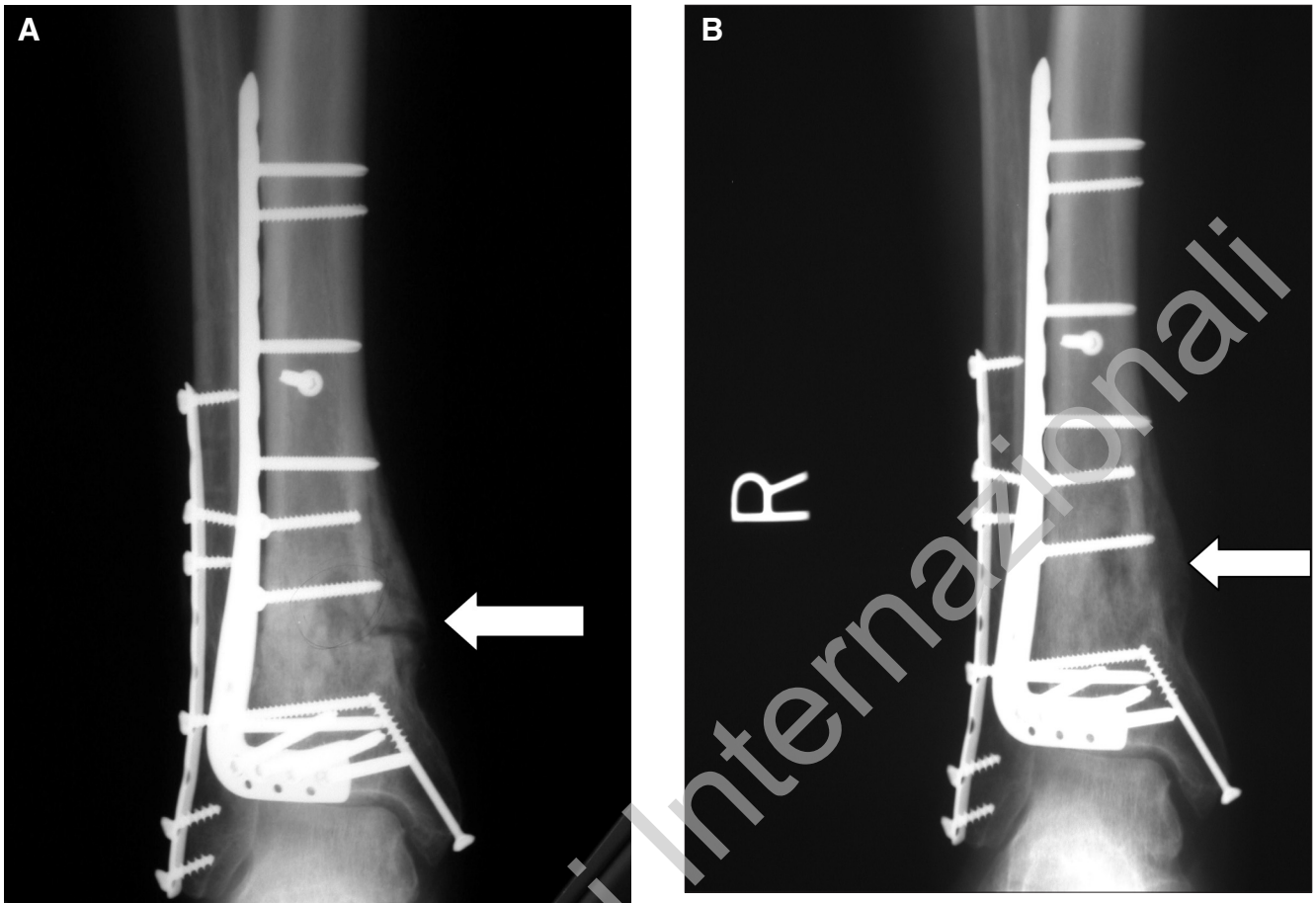

Figure 1 - **A.** Anteriorposterior radiograph of a 49 year old man with a nine months old tibial nonunion. The patient had undergone several attempts at operative fixation and grafting which included autologous iliac crest bone graft and recombinant human OP-1 (bone morphogenetic protein-7). An incomplete union is noted with radiolucency clearly visible approximately 4 centimeters proximal to the medial malleolus. **B.** Anteriorposterior radiograph four months after bone marrow aspirate concentrate injection. Note complete healing of the nonunion site.

Recombinant human platelet derived growth factor

During the normal process of fracture healing, a blood clot is formed at the fracture site and platelets produce, among other factors, platelet derived growth factor. The PDGF family consists of at least four subtypes and three of these (PDGF-AA, PDGF-AB, and PDGF-BB) have been identified in fractures. PDGF attracts neurotrophils, macrophages, osteogenic, chondrogenic, and myogenic progenitor cells and can increase the expression of vascular endothelial growth factor (VEGF), which regulates neoangiogenesis and is essential for skeletal repair. Indeed, conditions with an increased risk for impaired tissue healing have shown insufficient production of PDGF (14). Although previous reports have yielded inconclusive results regarding the use of PDGF in fracture healing (15,16), a recent clinical investigation in ankle fusions has reported encouraging findings. A North American Pivotal Study recently submitted its results to the United States Food and Drug Administration for consideration of premarket approval of recombinant human PDGF in the treatment of foot and ankle fusions (17). The investigators noted that out of 16 secondary endpoints measured at 52 weeks after operation, 15 were statistically significant for non-inferiority when compared to the use of autologous bone graft. Recombinant human platelet derived growth factor compared favorably to autograft with healing rates of 87.8% and 88.3% and a therapeutic failure rate of 7.3% and 8% respectively. The patients who were treated with recombinant human PDGF had fewer complications and infections when compared to those treated with autograft.

Parathyroid hormone

To date, strategies to enhance the healing of fractures have involved local applications of materials and substances. Although continued development of local strategies holds promise for the future, a systemic stimulation of healing could potentially prevent the need for operative intervention and enhance repair by providing a general up-regulated osteogenic response to the skeleton. One agent that may be effective in achieving this effect is parathyroid hormone (PTH). PTH (1-34; teriparatide) is currently available in many countries for the treatment of osteoporosis. It has been shown to increase bone mass and reduce the risk of fracture. Preclinical studies have also shown that intermittent systemic injection of PTH increases callus volume and bone strength in models of fracture healing (18-20). Although it is unclear if the doses currently tolerated in patients for the treat-

ment of osteoporosis will be sufficiently effective to enhance fracture healing, and while the mechanisms of action are not fully understood, it is believed that PTH mediates its effects on endochondral ossification by binding to its receptor on osteoprogenitor cells enabling interaction with PTHrp and Indian hedgehog which mediate chondrocyte development and differentiation (18). In addition, there are data to support a direct effect of PTH on intramembranous bone healing possibly by means of an interaction between PTH and the Wnt-signaling pathway (21). A clinical trial on the use of systemic teriparatide in the healing of closed distal radial fractures treated by non-operative means has suggested a beneficial effect at a clinically tolerable dose (20 µgs) (22). Post-menopausal women who had sustained a dorsally angulated distal radius fracture in need of closed reduction, but not surgery, were randomly assigned to eight weeks of once-daily injections of placebo (n=34), teriparatide 20 µgs (n=34) or 40 µgs (n=34) within ten days of fracture. 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 respectively for placebo, teriparatide 20 µgs, and teriparatide 40 µgs (p=0.015). Moreover, the time to healing was shorter in the teriparatide 20 µg group than in the placebo group (p=0.006). These results suggest that fracture repair may be accelerated by teriparatide and indicate the need for further study on the effects of this hormone on fracture healing (22).

Modifiers of the WNT signaling pathway

In recent years, a family of proteins known as Wnts has been shown to regulate embryological tissue development and the postnatal regeneration of skeletal tissue. Whereas bone morphogenetic proteins interact with BMP receptors, Wnt binds to a specific receptor called Frizzled, as well as to two co-receptors known as low-density lipoprotein-related protein 5 and LRP 6. These interactions lead to the stabilization of β-catenin, which translocates to the nucleus and regulates gene expression (23). Clinical cases with gain-of-function mutations in the Wnt co-receptor LRP-5 have been associated with increased bone mass (24, 25). This effect is thought to be mediated by an increase in intracellular β-catenin, which is favorable for osteoblast differentiation and bone healing. Two Wnt inhibitory targets; Dickkopf-1 and sclerostin, both negatively regulate bone mass and regeneration by modulation of the LRP-5 receptor and possibly by affecting the BMP-SMAD pathway (26,27). It has also been shown that inhibition of the complex protein GSK3, which regulates β-catenin degradation in the proteosome, also has effects on bone (28). These proteins have been suggested as targets for future enhancement of bone mass and fracture healing. Although no clinical data are currently available, Minear et al. (29) have recently demonstrated that Wnt signaling and perhaps application of Wnt protein could be used to stimulate fracture healing. Using a mouse strain, Axin-$2^{LacZ/LacZ}$ in which the cellular response to Wnt is increased, these investigators demonstrated accelerated fracture healing as a result of more robust cellular proliferation. In a more recent experiment, young male rats had a screw inserted into their proximal tibiae and were divided into groups that were administered sclerostin antibody or control substance twice a week, for two or four weeks. Sclerostin antibody significantly increased the pull-out strength by almost 50% compared with controls. Microcomputed tomography showed that the antibody led to a 30% increase in bone volume fraction in the region surrounding the screw. Thus, modulation of the Wnt signaling pathway may not only enhance fracture healing; it may also improve the fixation of fracture fixation implants (30). These findings are exciting not only because of the measured effects in these animal models, but because they involve the manipulation of a biological system that has already demonstrated how alterations in its signaling can lead to substantially altered human phenotypes.

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