Recent Developments in the Biology of Fracture Repair

Abstract
Fracture repair is dependent on local and systemic molecular and cellular processes. During fracture repair, mesenchymal stem cells are systemically recruited to the fracture site, and cytokines are released from the fracture site into the vascular system. In a significant minority of fractures, healing delays result from adverse clinical factors that interfere with these processes. Extrinsic factors, such as aging and smoking, adversely affect the molecular and cellular processes occurring locally in the fracture site.

Fracture fixation affects healing through local changes in the biologic signaling within the fracture callus. Current biologic treatment of fractures includes the local application of osteoinductive bone morphogenetic proteins (ie, BMP-2, BMP-7) and cell-based therapies. Although clinical results with bone morphogenetic proteins have been satisfactory, they have not been as impressive as those reported in animal studies. Further understanding of the biology of fracture repair may lead to improved treatment modalities.

Fracture healing is a highly efficient repair process resulting in newly formed bone, similar in quality to the original tissue. However, in a small but significant number of instances, adverse conditions impair this process, causing significant morbidity. Because fractures are common in the general population, delayed healing and nonunion are significant health care issues. Thus, there remains a need to develop treatment methods that enhance fracture healing and improve outcomes.

Biologic methods of bone regeneration will continue to have an increasing role in the treatment of fractures. To further develop these methods, knowledge of the pathologic changes in the fracture repair process that lead to delayed union or nonunion is important.

Fracture healing may be seen as both a local and a systemic process (Figures 1 and 2). There are local molecular and cellular signaling pathways, and evidence is emerging of the systemic recruitment of mesenchymal stem cells (MSCs) to the fracture site. Extrinsic factors, such as drugs and aging, influence local processes to alter the outcome of fractures, while the biomechanics of fracture fixation affect the biology of fracture healing.

Pathophysiology of Delayed Fracture Healing and Nonunion
Bone regeneration depends on three essential elements: progenitor cells, growth factors (osteoinduction), and the appropriate milieu (osteoaduction). Delayed fracture repair and
nonunion can result from a lack of osteoprogenitor cells, insufficient osteoinductive growth factors, or a defective milieu, or, more commonly, a combination of these factors. Disease or other adverse factors may delay fracture healing by affecting one or more of these elements.

For example, a critical-sized bone defect (ie, one that does not heal spontaneously because of its size) would result in the absence of human MSCs (hMSCs) within the defect, a lack of osteoinductive signals, and a milieu that is nonconducive to healing. Excess motion at the fracture site impairs both the molecular and cellular processes within the defect. Hypoxia affects osteogenic differentiation of hMSCs by reducing the number of viable cells and altering the molecular signals produced. Blood supply is important to fracture repair as blood provides nutrients and oxygen for cell survival, and blood vessels are the route for inflammatory and osteoprogenitor cells that are recruited to the fracture site. Systemic factors may affect fracture repair by reducing the number of osteoprogenitor cells recruited to the fracture site and/or by affecting the local osteoinductive signals.

Nonunions are commonly classified on radiographs as hypertrophic or atrophic. Hypotrophic nonunion is generally thought to be the result of mechanical instability and is treated by restoring stability, usually by skeletal fixation. Atrophic nonunion is thought to result from biologic causes, principally poor vascularization, and is treated by restoration of the osteogenic potential, with resection of fibrous tissue and bone grafting or some other method of osteoinduction. Both groups of nonunion contain fibrous tissue, fibrocartilage, and adipose tissue, which are not normally present in healing fractures. The hypertrophic group also has hyaline cartilage and bone in varying proportions.

**Local Molecular Signaling**

**Osteoinductive Molecules**

Fracture repair is regulated by several growth factors with varying osteogenic potential, such as transforming growth factor-β, platelet-
derived growth factor, insulin-like growth factor-1, and bone morphogenetic protein (BMP). Of these, BMP appears to be among the most consequential. BMP was discovered and named by Urist3 in 1965; he also first described the phenomenon of osteoinduction. Urist observed new bone formation occurring locally in rodents after they were given intramuscular implantation of bone cylinders decalcified with hydrochloric acid. This phenomenon was attributed to the presence of a protein, BMP, in bone matrix. Since that discovery, at least 16 different human BMPs have been identified. These proteins affect cells and tissues involved in the repair process in a number of ways, including the recruitment of MSCs from surrounding tissues to the fracture site, followed by their proliferation and differentiation into chondrocytes and osteoblasts, invasion of blood vessels, and, ultimately, bone formation. All of these effects are mediated by the binding of BMPs to specific transmembrane receptors on hMSCs, active osteoblasts, and mature chondrocytes as well as to the subsequent activation of various intracellular messenger systems.4

**Therapeutic Application of BMPs**

Extensive animal data have demonstrated the potential of BMPs to induce healing of critical-sized (ie, large) bone defects.5-7 In animal models, BMPs alone (with their carrier matrix) have been shown to induce rapid bone bridging of a defect. The quality of the repair tissue was equivalent to or better than that obtained with autologous bone grafting, the standard treatment for bone defects and nonunions in clinical practice.5-7 At present, only BMP-2 (Infuse; Medtronic Sofamor Danek, Memphis, TN) and BMP-7 (OP-1 Implant; Stryker Biotech, Hopkinton, MA) have been approved by the US Food and Drug Administration for clinical use. Several clinical studies have already demonstrated the positive effect of the application of BMPs on the outcome of fractures and nonunions. Because a review of all available evidence is beyond the scope of this article, we will focus on the treatment of segmental bone defects in patients because this allows a direct comparison with the studies of BMPs in animals.

In most clinical studies of the treatment of segmental bone defects, BMPs have been used in conjunction with allograft or autograft bone. Jones et al8 demonstrated that a combination of BMP-2 and allograft bone was equivalent to autologous bone for the treatment of segmental bone defects. In that study, patients with a tibial diaphyseal fracture and a residual cortical defect were randomly assigned to receive either autogenous bone graft or allograft with an onlay application of recombinant human BMP-2 (rhBMP-2). Radiographic and functional outcomes were similar in both groups. To date, the only published clinical study on the treatment of segmental defects with BMPs alone [with a nonosteocductive carrier matrix only] in humans showed healing of critical-sized fibular defects in patients undergoing opening wedge high tibial osteotomy with fibulectomy.9 RhBMP-7 bound to collagen type 1 sponge induced bony union in five of six patients with a critical-sized fibular defect, whereas there was no healing in any of the six patients treated with the type 1 collagen carrier only. Despite these favorable results, no large studies have been done on the use of BMPs alone in humans.

The pace of healing of segmental defects treated with BMPs differs significantly among species. Segmental defects in large animals treated with BMPs alone healed in <3 months,5,6 whereas in humans, critical tibial defects treated with allograft bone and BMP-2 required ≥6 months to achieve bony union.5 The cause for this difference remains unclear. Clinically, poor fracture healing in humans may be associated with adverse factors not present in animal studies. For example, soft-tissue coverage of the fracture may not be adequate. The initial dose of BMPs given to human subjects [7 mg of BMP-7 and 2 g of collagen carrier, or 12 mg of rhBMP-2 and collagen sponge] was much higher than in the animal studies. Because the release of BMP inhibitors depends on the extracellular level of BMPs, it is postulated that this higher concentration of BMPs leads to the expression of several BMP antagonists, which further limits their efficacy and reduces the rate of bone healing. It is also speculated that BMP receptors in animals and humans are different in their degree of responsiveness to the BMP molecules.

**Role of BMPs and Their Inhibitors in Fracture Healing**

The activity of BMPs can be limited by several antagonists, which bind to them and interfere with their ability to induce receptor activation. One of the most characterized BMP inhibitors is noggin, a protein that binds to both BMP-2 and BMP-7 and antagonizes their actions by preventing binding with their membrane receptors.10 Its expression by osteoblasts is induced by BMP-2,10 implying that BMP-2 and noggin are involved in a negative feedback loop during bone formation. This may provide a physiologic mechanism that prevents overexposure of osteoblasts to BMP signaling.

The balance between BMPs and their inhibitors is likely to be a critical determinant of fracture healing, with a decreased expression of BMPs and/or a relative increase of BMP antagonists adversely affecting healing. In a rat model of fracture nonunion, a downregulation of the gene expression of BMPs was demonstrated.11 In an animal model of atrophic nonunion, reversal of this decreased ex-
pression of osteoinductive factors, induced by an early local injection of rhBMP-7, prevented the development of nonunion. The expression of chordin, a BMP antagonist with a mode of action similar to that of noggin, was upregulated in an animal model of fracture nonunion, suggesting that downregulation of chordin in a fracture nonunion has the potential of improving bone healing.

In normally healing fractures, the balance between BMPs and their inhibitors can also be manipulated to hasten repair. This would involve the addition of the osteoinductive factors (eg, BMP-2), inhibition of the activity of BMP inhibitors, or a combination of both methods. So far, biologic methods of enhancing bone regeneration have centered on the promotion of osteoinduction via the delivery of BMPs. However, it was recently demonstrated that noggin or chordin suppression can accelerate osteogenesis in vitro and that noggin knockdown increased the rate of intramembranous ossification in an animal model. We believe that these findings will be extended to fracture healing and that blockade of the activity of BMP inhibitors may provide a novel strategy for expediting fracture repair.

**Local Cellular Signaling**

Complete fracture healing requires that a sufficient number of hMSCs differentiate into chondrocytes and osteoblasts, as well as other cells of the mesenchymal lineage, such as adipocytes, and stromal and endothelial cells. In addition to differentiation, the trophic, or nutritional, activity of MSCs in the repair of other tissues is now well established. This refers to the capacity of MSCs to secrete growth factors, which stimulate blood vessel formation and the proliferation of other local MSCs. It is postulated that MSCs exert a trophic activity in the early stages of fracture repair, although this has not yet been specifically demonstrated in fractures. MSCs are thought to be recruited locally from the cortex, bone marrow, periosteum, and external soft tissues (Figure 2). The relative contribution of MSCs from each tissue is uncertain but is thought to depend on the local parameters present at the injured tissue, such as growth factors, oxygen gradient, and mechanical stability. The clinical relevance of muscle as a source of progenitor cells during fracture repair has been the subject of several recent studies.

The presence in muscle of a population of adult stem cells that can differentiate into cells of different lineages has been suspected for some time based on two observations. First, muscle has the potential to turn into bone, as occurs during heterotopic ossification. Second, the original description of osteoinduction by Urist has been attributed to the effects of BMPs on progenitor cells within muscle tissue. However, the isolation of the relevant MSC population from this tissue is relatively recent. Clinically, the importance of muscle as a source of osteoprogenitor cells is underlined by the poor outcome of fractures in which muscle has been devitalized, although this poor outcome is often attributed to the coexisting damage to the periosteal blood supply. Muscle resection significantly reduces callus formation and the biomechanical properties of the healed bone, while a muscle crush does not significantly affect bone healing. Conversely, heterotopic ossification in acetabular fractures has been reduced as surgeons have become more aggressive in debriding injured and necrotic muscle from the surgical field.

**Systemic Recruitment of Cells and Molecular Signaling**

Traditionally, it was thought that the cells involved in fracture repair were recruited only locally. However, a systemic mobilization and recruitment of osteoblastic precursors to the fracture site from the peripheral circulation have now been demonstrated in several recent studies. In a rabbit ulnar osteotomy model, it was demonstrated that some osteoblasts involved in fracture healing were systemically mobilized and recruited to the fracture from remote bone marrow sites. Shen et al demonstrated in a murine model that, following systemic injection of MSCs, osteoprogenitor cells localized to the fracture callus.

These studies have implications for the development of future cell-based therapies for fracture healing. Cell-based therapies are needed when insufficient cells are present within a fracture callus [eg, segmental defect]. In such a situation, even when all of the osteoprogenitor cells at the site of fracture are working to the maximum, there will be no bony union, nor will any osteoinductive agents be effective because maximal osteogenesis per cell is already occurring. In a level III study [case-control], Hernigou et al demonstrated the clinical effectiveness of local percutaneous injection of bone marrow aspirate in treating tibial nonunions. We believe that the studies mentioned here suggest that it might be possible to develop cell-based therapies in which cells are systemically administered and localized to the site of injury.

Evidence is emerging that distant skeletal sites can be affected in response to a local bone injury. An increased osteogenic response has been detected in sites distant from the fracture in animal models. This may result from the release of growth factors (eg, transforming growth factor-β, insulin-like growth factor-1) from the fracture site into the systemic circulation, as shown in a clinical study. It is not known whether the level of these factors in serum reflects the repair activity of the fracture.
**Systemic Factors and Local Fracture Healing**

It is widely accepted that extrinsic factors have an influence on the outcome of fracture healing. However, it is often difficult to isolate the role of a particular systemic factor in clinical situations. For example, impaired fracture healing in the elderly may be related to age, osteoporosis, drugs, malnutrition, and/or anemia. Evidence gained from animal models as well as recently uncovered cellular and molecular processes have led to better understanding of the role of systemic factors.

**Nonsteroidal Anti-inflammatory Drugs**

During fracture repair, the enzyme cyclooxygenase-2 (COX-2) is activated to produce prostaglandins, which are needed during inflammation and are critical for starting the osteogenic response. Nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit COX-2, dramatically reducing prostaglandin production, and therefore have the potential to negatively affect fracture repair. Although results in various animal studies have been conflicting, it is generally accepted that NSAIDs impair fracture healing in animal models in which a high dose has been administered. This inhibitory effect is most potent during the early phase of healing, thereby underlining the importance of the initial inflammatory reaction.

Although it is not known in which clinical situations NSAIDs in high local concentrations will affect early-phase fracture healing, administration of NSAIDs has been associated with delayed union and pseudarthrosis. The inhibitory effect of COX-2 blockade in vitro has been shown to be reversed by the administration of BMP-2.

**Age**

Aging is an independent factor that negatively affects fracture repair. Delayed fracture healing in the elderly may be caused by differences in molecular signaling locally within the fracture callus as well as to systemic factors. Meyer et al reported a decreased expression of BMP-2 and Indian hedgehog (a factor related to endochondral callus formation) in fracture calluses of older rats. Noggin expression was not changed with age. However, the decrease in expression of BMP-2 implies that the BMP inhibitor predominated over BMP-2 in older rats.

Hormonal differences with aging may also be a factor. Sera obtained from aged donors are less potent inducers of osteoblast differentiation of hMSC than are sera obtained from young donors. These effects were specific for osteoblast differentiation because no donor age differences in the ability to support differentiation of other cell types were observed. Short-term bone marrow cultures established from young and old donors contain similar numbers of hMSCs and exhibit similar proliferation rates. In addition, the capacity of hMSC to differentiate into osteoblasts and adipocytes was maintained irrespective of donor age. These studies suggest that there are no intrinsic defects in hMSCs with aging and that extrinsic factors present in the aging environment of hMSCs may be responsible for the impaired osteoblast functions seen with aging.

These observations need further investigation. If poor fracture repair in the elderly is related to impaired osteoinductive signals rather than to differences in the cellular component of the healing fractures, then the elderly patient with trauma is more likely to benefit from an osteoinductive agent, such as BMP-2, than from cell-based therapies.

**Smoking**

Smoking has an adverse effect on fracture healing. In one study, the time to union for tibial fractures among smokers was significantly longer than in nonsmokers. This effect may be mediated by either nicotine or some other, yet undefined components in cigarette smoke, or both.

In animal models, nicotine has been shown to delay cellular differentiation into chondrocytes and to slow the physiologic transition from cartilaginous callus to bone. It is also highly likely that the compromise of microcirculation secondary to nicotine causes a delay in fracture repair. It remains to be seen whether the deleterious effects of smoking are reversible with smoking cessation and whether BMPs can improve healing in smokers. These questions should be investigated using animal models of fracture healing and smoking.

**Influence of Fracture Fixation**

The local mechanical forces on a fracture resulting in movement at the fracture site are critical factors in the success of fracture repair. Excess motion can delay healing, casting and fracture fixation aim to provide a mechanical environment in which strains are decreased to avoid delayed union or nonunion. However, some movement, referred to as micromotion, is beneficial to fracture healing. Different fractures and different areas of the skeleton respond differently to mechanical forces and resultant strains. Yet precisely how these changes in local mechanical loading result in a cartilaginous callus or intramembranous ossification remains speculative. The influence of the mechanical environment on the fracture repair can be viewed at three levels: tissue, cellular, and molecular.

Tissue differentiation requires mechanical stability. In an animal model, Claes et al demonstrated a strong association between fracture stability and the spatial distribution of newly formed blood vessels and specific tissue formation. It was also independently demonstrated in an...
Summary

Significant advances have been made in the understanding of the biology of fracture repair. In particular, it is now understood that fracture repair is not only a local phenomenon but is itself under the influence of extrinsic factors. Adverse local and systemic clinical factors can affect the molecular and cellular processes involved and can lead to delayed fracture repair and non-union. The management of these healing problems remains challenging, despite the introduction of therapeutic BMPs and other biologic methods of bone regeneration. Further understanding of the pathophysiology of fracture repair is needed to develop improved treatment strategies targeted to the molecular and cellular processes affected in specific clinical conditions.

References

Evidence-based Medicine: References 9, 19, 22, 24, and 32 are level I/II prospective, randomized studies. The remaining references are case-control cohort studies, basic research studies, or expert opinion.

Citation numbers printed in bold type indicate references published within the past 5 years.

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