Inflammation, Bone Healing, and Anti-Inflammatory Drugs: An Update

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Summary: Fracture healing is a unique multifaceted process requiring the presence of cells, molecular mediators, and angiogenic factors. The state of inflammation dominates the initial phase, but the ideal magnitude and duration of the process for an optimal outcome remains obscure. Biological response modifiers, such as platelet-rich plasma (PRP) preparations, have been used to reconstitute the desirable early inflammatory state, but the results obtained remain inconclusive. Ongoing research to characterize and quantify the inflammatory response after bone fracture is essential in order to better understand the molecular insights of this localized reaction and to expand our armamentarium in the management of patients with an impaired fracture healing response. Non-steroidal anti-inflammatory drugs frequently administered for analgesia after trauma procedures continue to be a cause of concern for a successful bone repair response.

Key Words: inflammation, fracture healing, cytokines, mesenchymal stem cells

(*J Orthop Trauma* 2015;29:S6–S9)

INTRODUCTION

Bone healing is a complex, though well-orchestrated, biological phenomenon involving the interaction of molecular mediators and cellular elements.[1] The 2 forms of fracture healing are the primary or intramembranous, and the secondary or indirect or endochondral bone healing.[2] In indirect bone healing, which is the most common pathway through which fractures heal, formation of the initial hematoma is followed by the acute inflammatory response, the recruitment of mesenchymal stem cells (MSCs), the formation of cartilaginous and osseous callus, the revascularization and neoangiogenesis of the fracture site, and finally the mineralization of the cartilaginous callus and remodelling.[3]

Accepted for publication September 17, 2015.

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The authors report no conflicts of interest.

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INFLAMMATORY-RELATED CYTOKINES: WHAT ROLE DO THEY HAVE IN HEALING?

The initial local inflammation generated at the site of injury is considered a key factor, playing a significant role in the healing cascade; thus, the in-depth understanding of its regulation has gained considerable attention. Initially, the influx of inflammatory cells (neutrophils, lymphocytes, and macrophages) and the release of various cytokines and growth factors take place. Cytokines are cell-signaling molecules that assist communication between cells and stimulate the movement of cells toward sites of inflammation, infection and trauma. Cytokines play an essential role in the induction of downstream responses after injury, signaling chemotaxis of inflammatory cells, enhancing extracellular matrix synthesis, stimulating angiogenesis, and recruiting endogenous fibrogenic cells to the injury site. Their expression peaks within the first 24 hours after fracture and then rapidly declines during the period of cartilage formation.[4] Cytokine expression increases again during the bone remodeling phase.[5]

In general terms, fracture of bone stimulates expression of several inflammatory cytokines, including interleukin-1a (IL-1a), IL-1b, IL-6, IL-18, and tumor necrosis factor alpha (TNF-α). These cytokines are released in a temporally and spatially regulated manner.[6] The expression of these cytokines is critical for the inflammatory response that triggers osteogenesis. Tumor necrosis factor alpha promotes MSC recruitment, induces apoptosis of hypertrophic chondrocytes, and stimulates osteoclasts. Bone healing is significantly delayed in tumor necrosis factor alpha-receptor-deficient mice, with a delayed resorption of mineralized cartilage that prohibits new bone formation.[7]

Other studies have shown that femur fractures cause immunosuppression of the Th-1 pathway that is modulated by a decrease in interleukin-12 (IL-12). IL-12 serves as the gatekeeper for innate immunity and may be the key to normalizing function of the immune system against infection in the trauma setting. Lindsey et al,[8] using a murine open femur fracture model, showed that systemic administration of IL-12 could be used to immunomodulate the environment and actually aid bone formation. While several studies have attempted to identify a single cytokine's impact on fracture healing, these factors interact in a highly

complex physiologic system with a great deal of cross-talk and feedback.

INFLAMMATION AND HEALING: WHEN IS TOO MUCH A BAD THING?

During the initial phase of bone repair, both the magnitude and length of inflammation have been thought to affect the outcome. For instance, overly activated inflammatory cells have been linked to a compromised healing process and subsequent delayed healing. Mesenchymal stem cell function has been shown to be compromised by reduced bone morphogenetic protein-2 (BMP-2) production of macrophages after systemic and local administration of lipopolysaccharide in an experimental rat model, suggesting that conditions promoting excessive proinflammatory activity of the macrophage might impede osseous healing.[9,10]

Schmidt-Bleek et al[11] reported on the immune cell composition and expression pattern of angiogenic factors in a sheep bone osteotomy model compared to a mechanically induced impaired/delayed bone healing group. Delayed revascularization was observed in the delayed healing group and was attributed to the continued proinflammatory processes in this group. Moreover, another experimental study indicated that upregulation of anti-inflammatory IL-10 expression in recombination activating gene 1 knockout ($RAG1^{-/-}$) mice lacking an adaptive immune system led to acceleration of fracture healing, suggesting that immunomodulation directed toward minimizing the destructive effects of inflammation may promote fracture repair.[12]

In the clinical setting, prolonged inflammation such as that observed in chronic inflammatory conditions, polytrauma, and infection is considered to inhibit the bone repair process.[13] Increased inflammation could delay fracture healing through a variety of suggested mechanisms, including neutrophilia/altered neutrophil priming,[14] and prolongation of osteoclast life along with decreased chrondrocyte and osteoblast lifespans.[15] Overall, one could argue that contemporary emerging evidence is suggestive of a negative effect of prolonged and excessive inflammation to fracture healing/bone repair.

CAN PRPS MODULATE THE INFLAMMATORY RESPONSE DURING HEALING?

The development of a compromised fracture healing response expressed in the form of delayed union and/or nonunion has also been the focus of intense experimental and clinical research activity among orthopaedic traumatologists and basic scientists because of significant burden that it poses to individuals, societies, and healthcare systems.[16,17] Manipulation of the initial cascade of events with biological response modifiers has been at the centre of our interventions. One of the strategies that has attracted great attention is the delivery of platelet-rich plasma (PRP) to the site of the injury.

Platelets, along with fibrin, are responsible for the formation of the initial hematoma in the acute phase of a fracture. Activated platelets subsequently release cytokines and growth factors that are involved in the development of the inflammation phase of bone healing.[18] Increased concentration of platelets within PRP is thought to provide a local supraphysiological burst of cytokines and growth factors to the fracture site that may speed up the regeneration process.[19] It has been demonstrated that PRP with platelet concentrations more than 10^6/L provides a 3-to 5-fold increase in growth factor concentrations.[20] The increased number of growth factors is thought to derive from the degranulation of the α-granules of platelets.[21] In addition to growth factor release, PRP has been linked to increased proinflammatory cytokines[22] and angiogenic factor release.[23–25] Marx et al[26] reported that the first successful clinical application of PRP in 1988 was in the management of mandibular effects. Since then, various PRP applications have been reported in different fields of regenerative medicine, including the management of skin, soft tissue, and nerve healing.[22] In experimental models, PRP has been shown to promote the quality of bone healing.[27] It has also been demonstrated that the efficiency of PRP is increased when used in combination with an autologous cancellous graft and biocompatible matrix.[28]

Despite sound basic research evidence for the role of PRP in fracture healing, clinical evidence is inconclusive regarding an optimal delivery method and its relevant efficiency. Reported outcomes of clinical studies utilizing PRP for the management of bone defects are wide-ranging. There is a paucity of high-quality evidence to support its specific indications, safety, and effectiveness. Bielecki et al[29] studied the application of percutaneous PRP on established long-bone delayed unions and nonunions and had promising results when PRP injection was performed within 11 months from the index operation. In a prospective randomized study of 162 long-bone nonunions treated either with recombinant human bone morphogenetic protein (rhBMP-7) or PRP, Calori et al reported superior results with the use of rhBMP-7.[30] Despite the fact that PRP application is theorized to promote fracture healing through modulation of the inflammation process, there is weak clinical evidence to support its use in everyday clinical practice. Practical issues that need to be addressed in the near future are the clarification of PRP indications, the best combination of PRP with other grafting material, and the optimal volume/concentration of PRP.

ANTI-INFLAMMATORIES: HOW AND WHEN CAN THEY BE USEFUL IN ORTHOPAEDIC TRAUMA?

The impact of anti-inflammatory medications on fracture healing has been a topic of intense discussion for some years. Anti-inflammatory medications are widely prescribed in orthopaedic trauma surgery and include steroids and nonsteroidal anti-inflammatory drugs (NSAIDs). Their pharmacological properties have analgesic, anti-inflammatory, and antipyretic effects, making them potentially valuable medications for orthopaedic trauma patients. The mechanisms of action, pharmacokinetics, and bioavailability of these medications are well understood. Equally understood, therefore, are the side-effect profiles for these medications. Striking a balance between the potential positive benefits of anti-inflammatories and the patient morbidity engendered by side effects continues to pose a clinical challenge. The most common indication for

NSAID use in orthopaedic trauma relates to analgesia. NSAIDs have very desirable analgesic properties, including both peripheral and central activity on pain and swelling. These combined effects result in a substantially decreased need for opiate analgesia.

Conversely, the effect of ongoing NSAID use on bone healing is concerning. Multiple animal studies have demonstrated increased rates of nonunions, weaker unions, and delayed unions when animals were treated with NSAIDs. In a retrospective review by Giannoudis et al,[31] nonunion was associated with the use of NSAIDs after fracture of the femoral diaphysis ($P = 0.000001$). The effect of dose, timing, and duration of therapy on fracture healing remains unclear. Isolating a therapeutic window for the use of NSAIDs as an analgesic remains controversial. In an experimental study, Pountos et al[32] investigated the effect of NSAIDs on bone marrow MSC proliferation and osteogenic and chondrogenic differentiation, and evaluated both cyclooxygenase-1 (COX-1) and COX-2 specific drugs. The effects of 7 COX-1 and COX-2 inhibitors on MSC proliferation and osteogenic and chondrogenic differentiation were tested using Vybrant, sodium 3'-{1-[(phenylamino)-carbonyl]-3, 4-tetrazolium}bis (4-methoxy-6-nitro)benzenesulfonic acid hydrate (XTT), and functional and quantitative assays of MSC differentiation. The MSC expression of COX-1, COX-2, and prostaglandin E2 (PGE-2) levels was serially evaluated during lineage differentiation by quantitative PCR and ELISA. The authors reported that NSAIDs inhibited bone formation via blockage of MSC chondrogenic differentiation, which is an important intermediate phase in normal endochondral bone formation. Based on these findings, one can speculate that the administration of NSAIDs could be considered safe in the setting of anticipated primary bone healing (absolute stability) and during the first week of healing via callous formation (secondary bone healing).

An additional indication for anti-inflammatory medication in orthopaedic trauma exploits the effect on bone growth in heterotopic ossification. The subset of orthopaedic injuries that result in an increased risk of developing heterotopic ossification, including acetabular fractures and severe elbow trauma, have been effectively treated with anti-inflammatories. A Cochrane review in 2004 demonstrated a 59% risk reduction in the development of heterotopic ossification after hip surgery.[33] Once again, the literature on heterotopic ossification remains controversial, as the need for NSAID prophylaxis is comparatively uncommon.

CONCLUSIONS

During the initial phase of fracture healing, a local inflammatory response plays a significant role in the subsequent cascade of events leading to successful bone repair. It seems that the length and magnitude of inflammation could influence the outcome. Emerging evidence is suggestive of a negative impact of prolonged and excessive inflammation to fracture healing. PRP has been used as a biological response modifier to promote an enhanced fracture healing response, as it contains growth factors, proinflammatory factors, and angiogenic factors. Nonetheless, recent clinical evidence is inconclusive regarding its optimal dose, delivery method, and effectiveness. Prolonged administration of NSAIDs has been shown to interfere with a successful bone healing response, but current evidence supports the view that NSAID administration is safe in the setting of anticipated primary bone healing (absolute stability) and during the first week of healing via callous formation (secondary bone healing).

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