J Musculoskel Neuron Interact 2003; 3(4):317-319

Perspective Article

Hylonome

Injectable rhBMP-2/CPM paste for closed fracture and minimally invasive orthopaedic repairs

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Keywords: rhBMP-2, Calcium Phosphate, Cement, Fracture

Introduction

BMPs have been used to accelerate fracture healing, bridge segmental defects and generate spine fusions in numerous animal preclinical models[1-3] and recent human clinical trials[4-7]. In these studies, BMPs were delivered with carriers placed during an open surgical procedure. The advent of minimally invasive surgery for open procedures combined with the fact that the majority of fractures are closed fractures has led to the development of percutaneous injectable delivery vehicles for BMPs. Recent studies have demonstrated the efficacy of rhBMP-2 administered in an injectable calcium phosphate matrix (CPM) to accelerate healing in a rabbit mid-diaphyseal ulna osteotomy screening model[8] and a more clinically relevant canine mid-diaphyseal tibia osteotomy model[9]. The CPM paste used in these studies is an endothermically-setting, biocompatible, biodegradable material that forms a hydroxyapatite crystalline structure similar to bone[10,11].

This rhBMP-2/CPM combination has also been shown to accelerate healing in a non-human primate mid-diaphyseal fibula osteotomy model that more closely mimics the bone healing response expected in humans[12,13]. The rhBMP-2/CPM paste combination induced a concentration-dependent (0.0 to 4.5 mg/mL) 40-50% acceleration of osteotomy healing by 8 to 10 weeks after surgery compared to untreated surgical controls and osteotomies treated with carrier alone. The 1.5 mg/mL rhBMP-2/CPM concentration appeared to be the most effective concentration. Acceleration of osteotomy healing was based on radiographic and histologic evaluation, as well as torsional biomechanical properties. These results were evident whether 1.5 mg/mL rhBMP-2/CPM paste was injected 3 hours to 14 days after osteotomy. Release kinetics were unaffected by time of injection (1 to 7 days). In these studies, ^{125}I-labeled rhBMP-2 was still detected at the site of injection 35 days after treatment. *In vitro* studies indicate that rhBMP-2 extracted from the cement was still 100% bioactive at one month from the initial formulation[8].

Interestingly, administration of rhBMP-2/CPM paste 7 days after osteotomy resulted in the maximum acceleration of healing. Rapid bone induction was observed radiographically as early as 2 weeks after the 7-day delayed treatment (3 weeks after surgery). The osteotomies treated 7 days after surgery were bridged as early as 6 weeks after the initial surgery. The original osteotomy line was no longer visible by 8 weeks. Torsional biomechanical properties of the 7-day rhBMP-2/CPM-treated osteotomies were equivalent to the torsional biomechanical properties of intact fibulae at 8 weeks. The torsional biomechanical properties of osteotomies treated with rhBMP-2/CPM 3 hours or 1 day after surgery were not equivalent to intact fibulae until 10 weeks after surgery. Untreated surgical control osteotomies required 14-16 weeks to reach biomechanical properties equivalent to intact fibulae. Histologic evaluation of untreated osteotomies at 7 days demonstrated a much larger cellular infiltrate surrounding the osteotomy compared to untreated osteotomies evaluated at 3 hours after surgery. A large number of these cells were identified as osteoblast precursors based on CBFA-1 positive immunohistologic staining. At 14 days after surgery, histologic evaluation of osteotomies treated 7 days after surgery with rhBMP-2/CPM demonstrated a combination of direct bone formation without a cartilage intermediate and endochondral bone formation involving a cartilage intermediate. Regions of direct bone formation were accompanied by a dramatic induction of new blood vessels. The extent of new blood vessel formation was confirmed using immunohistologic staining for collagen 5 antibody. This dramatic neovascularization is likely due to BMP-induced upregulation of VEGF. Bone formation in untreated osteotomies and osteotomies treated 3 hours after surgery with rhBMP-2/CPM appeared to be primarily endochondral ossification at 14 days. Osteotomy heal-

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Accepted 1 August 2003

ing was accelerated in the 3-hour rhBMP-2-treated osteotomies compared to the untreated osteotomies even at this early time point. By 21 days after surgery, there was histologic evidence of new bone bridging the osteotomies treated at 7 days with rhBMP-2/CPM. The untreated osteotomies and the osteotomies treated at 3 hours after surgery with rhBMP-2/CPM still contained cartilaginous callus bridging the osteotomy gap at this time point. Osteotomies treated with CPM alone healed in a similar manner to the untreated surgical controls. Resorption of the CPM carrier by TRAP-positive multinucleated osteoclasts and TRAP-negative multinucleated giant cells was more rapid in the BMP-treated osteotomies compared to the carrier alone.

Based on this histologic evaluation, the accelerated healing response appears to be due to an increase in osteoblast precursor cells from the soft tissue envelope surrounding the osteotomy at the time of the 7-day delayed rhBMP-2 treatment compared to the 3-hour rhBMP-2/CPM treatment. In addition, the combination of both direct bone formation and accelerated endochondral bone formation resulted in more rapid bridging of the osteotomy in the 7-day delayed rhBMP-2/CPM-treated limbs.

In addition to accelerated diaphyseal bone healing, rhBMP-2/CPM paste has also been used to accelerate healing following direct injection into non-human primate metaphyseal 3.5 mm core defects located in the proximal femur, distal femur, proximal tibia and distal radius. These studies were designed to evaluate the efficacy of rhBMP-2/CPM to accelerate trabecular bone healing in metaphyseal sites at high risk for fracture. The normal healing response in untreated core defects results in the generation of a trabecular rim around the core defect. The response is primarily anabolic as early as one week after surgery with osteoblasts laying down new bone to repair the damaged trabeculae at the perimeter of the defect. There is very little in the way of initial osteoclast activity and activation of the marrow is confined to the region of the healing response. Once the rim of the core defect was repaired, there was very little bone formation within the core defect or appositional new bone on existing trabeculae outside the core defect. In contrast, previous studies using the same model to evaluate rhBMP-2 delivered in an absorbable collagen demonstrated the surprising result of initial transient bone resorption and concurrent osteoblast suppression lasting approximately 2-3 weeks following treatment[14]. This initial period of bone resorption was followed by extensive new bone formation within the core defect and on existing trabecular bone outside the defect. In addition, there was extensive marrow activation and vascular proliferation in the areas of new bone formation. The initial transient osteoclast resorption could be blocked with systemic administration of bisphosphonates[15]. The mechanism for transient BMP-induced bone resorption is most likely related to upregulation of osteoclasts in response to RANKL and MCSF expression by BMP induced osteoblast precursors. Soluble factors produced by osteoclasts including PDGFbb are most likely responsible for the transient osteoblast suppression[16]. Upregulation of OPG by maturing osteoblasts in combination with osteoclast apoptosis is likely responsible for the reversal of the osteoclast response and the initiation of subsequent new bone formation. Immunohistologic evaluation confirmed the changes in the ratio of RANKL and OPG tone as a function of time.

Core defects treated with rhBMP-2/CPM exhibited a rapid phase of bone induction within 1 week of treatment. This new bone formation was not preceded by the transient osteoclast bone resorption and osteoblast suppression observed when rhBMP-2 was delivered in a collagen sponge carrier. Concurrent vascular proliferation similar to the response to BMP in the osteotomy models and marrow activation was also observed following rhBMP-2/CPM treatment of the core defects. The difference in initial biologic response to rhBMP-2 delivered in CPM compared to the collagen sponge is likely related to the more prolonged release kinetics of rhBMP-2 from CPM compared to the collagen sponge. ^{125}I-labeled rhBMP-2 was still detected at the site of injection up to 49 days after treatment compared to 14 days using the collagen sponge carrier. During the subsequent 8-week time period extensive new bone induction was observed both within the core defects and on adjacent trabecular bone. Core defects treated with CPM alone healed in a similar manner as untreated surgical controls with minimal new bone formation on existing trabecular bone outside the defect. As was the case in the osteotomy model, resorption of the CPM carrier by osteoclasts and multinucleated giant cells was much more rapid in the rhBMP-2-treated core defects compared to the carrier alone.

The results of this study indicate rhBMP-2 delivered in a calcium phosphate paste can induce rapid trabecular bone formation without transient bone resorption and osteoblast suppression. This rapid bone induction provides compelling evidence that rhBMP-2/CPM can be used to accelerate metaphyseal fracture healing. Ongoing studies using a canine proximal tibia osteotomy model designed to mimic articular tibia plateau fractures with subchondral bone collapse demonstrate comparable healing between groups treated with rhBMP-2/CPM compared to autograft.

The combination of rhBMP-2 and calcium phosphate cement has also been used to better integrate the ingrowth regions of total hip replacements in a canine model[17]. In addition to improving ingrowth, the combination of rhBMP-2/CPC resulted in filling in of a surgically created bone gap adjacent to the acetabular component. The percent ingrowth in the acetabular component in the gap region was similar to percent ingrowth in the contact regions of the implant. The combination of rhBMP-2 and CPC and rhBMP-2 alone has also been used successfully in a number of preclinical craniofacial reconstruction models including alveolar ridge augmentation and prosthetic tooth implant fixation[18,19].

The results of these studies indicate rapid bone induction in response to rhBMP-2/CPM in a number of animal models for bone repair. Based on these preclinical findings, rhBMP-2/CPM may be useful as an injectable treatment for diaphy-

seal and metaphyseal fractures, as well as other types of orthopaedic and craniofacial repairs.

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