J Orthopaed Traumatol (2007) 8:43–48
DOI 10.1007/s10195-007-0162-0

TISSUE-SPARING SURGERY SECTION

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Bone morphogenetic proteins and growth factors: emerging role in regenerative orthopaedic surgery*

Received: 28 September 2006
Accepted: 21 January 2007
Published online: 5 March 2007

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Abstract Bone morphogenetic proteins (BMPs) were discovered by Urist and colleagues in 1965 and later defined as multifunctional cytokines involved in osteoinduction. BMPs are members of the transforming growth factor-β superfamily, with the exception of the BMP-1. Presently, at least 20 BMPs have been identified and studied, but only BMP 2, 4 and 7 have been able in vitro to stimulate the entire process of stem cell differentiation into osteoblastic mature cells. In preclinical and clinical studies, BMPs have demonstrated potential in osteoinduction and have been approved for clinical use in treating open fractures of the long bones and nonunions and in vertebral arthrodesis. Additional clinical uses of these molecules are under investigation and the possibility of using gene therapy in selected pathologies seems the most appealing.

Key words Bone morphogenetic proteins • Bone engineering • Growth factors

Introduction

Bone morphogenic proteins (BMPs) were discovered by Urist et al. [1] in 1965. Since then, more than 20 BMPs have been identified and, with the exception of BMP-1, all are members of the transforming growth factor (TGF)-β superfamily. BMPs are now defined as multifunctional cytokines involved in osteoinduction. They are probably the most important growth factors in bone formation and healing [2]. They share action with a number of other molecules, all members of the TGF-β superfamily, but their effects are superior [3] and more specific, as extensively shown by several clinical trials [4, 5].

Spinal fusion

Subsequent to animal studies that attributed high fusion rates to the use of osteoinductive proteins in noninstrumented posterolateral fusions, Johnsson et al. [4] randomized 20 patients to fusion with either BMP-7 (OP-1 Implant, Stryker) or autograft bone from the iliac crest. At surgery, 0.8-mm metallic markers were positioned in L5 and in the sacrum, enabling radiostereometric follow-up analysis for 1 year. The patients kept the trunk straight for 5 months after surgery with the aid of a soft lumbar brace. The three-dimensional vertebral movements were calculated with an accuracy of 0.5–0.7 mm and 0.5°–2.0°. The

* Proceedings of the Consensus Conference 'TSS in hip and knee replacement" (Rapallo, 22–24 June 2006)

study failed to show a significant difference between the OP-1 Implant and fusion with autograft bone. A significant relation between reduced vertebral movements and better bone formation was demonstrated. No adverse effects of the OP-1 Implant occurred, while persistent minor pain at the iliac crest was noticed in one patient in the autograft group. In conclusion there was no significant difference between the two fusion versions. The same outcome of comparable results with OP-1 added to autograft bone was reported by Vaccaro et al. [5] who, in a pilot clinical trial, observed a successful fusion in 55% of patients (which was not significantly different from the 45% fusion rate obtained in a historical control group of autograft alone).

Boden et al. [6] reported the results of lumbar interbody arthrodesis for 14 patients with a single-level lumbar degenerative disc disease. Patients were treated with tapered cylindrical threaded fusion cage filled with rhBMP-2/collagen sponge or autogenous iliac crest bone. They were evaluated with radiography, computed tomography (CT), and the Short Form-36 (SF-36) and Oswestry outcome questionnaires. All 11 patients who received rhBMP-2 were judged by three independent radiologists to have solid fusions at the 6-month postoperative visit, whereas only 2 of the 3 control patients were deemed to be fused. The Owestry Disability Questionnaire scores of the rhBMP-2 group improved sooner (after 3 months) than those of the autograft group, but both groups demonstrated similar improvements at 6 months. SF-36 scores continued to improve up to 24 months. In conclusion, arthrodesis occurred more reliably in patients treated with rhBMP-2-filled fusion cages than in controls with autogenous bone graft, and there were no adverse events related to the rhBMP-2 treatment. We note, however, that the sample size of this study was limited.

A larger number of patients were evaluated by Burkus et al. [7]. In a prospective randomized study, they investigated 42 patients who underwent a single-level anterior lumbar interbody fusion using cylindrical interbody fusion cages. Two groups were formed: 22 patients underwent interbody fusion using two tapered cylindrical fusion cages (LT-CAGE) and rhBMP-2 on an absorbable collagen sponge, and 20 patients (control group) underwent the procedure receiving the devices and autogenous iliac crest bone graft. Plain radiographs and CT scans were used to evaluate the pattern of osteoinduction in the interbody space and the progression of fusion 6, 12, and 24 months after surgery. All the patients who received rhBMP-2 showed radiographic evidence of osteoinduction in the interbody cages 6 months after surgery. New bone formation occurred in the disc space outside the cages by 6 months in 18 of the patients in the investigational group (82%). By 24 months, all patients in the investigational group showed new bone formation outside the cages. In the autograft control group, 10 patients (50%) showed evidence of bone formation outside the cages. Therefore definite evidence of osteoinductive properties of rhBMP-2 was supported by these data that showed in a clinical setting accelerated spinal fusion and new bone apposition under the influence of the morphogenetic protein.

Fracture and nonunions

Friedlaender et al. [8] enrolled 122 patients (124 tibial nonunions) in a pilot randomized controlled, partially blinded, multicenter clinical trial and followed them at frequent intervals over 24 months. Each patient was treated by insertion of an intramedullary rod, accompanied by rhOP-1 in a type I collagen carrier or by fresh bone autograft. Assessment criteria included the severity of pain at the fracture site, the ability to walk with full weight-bearing, the need for surgical retreatment of the nonunion during the course of this study, plain radiographic evaluation of healing, and physician satisfaction with the clinical course. In addition, adverse events were recorded, and patients were screened for antibodies to OP-1 and type I collagen at each visit. At 9 months following the operative procedures, 81% of the OP-1-treated nonunions (n=63) and 85% of those receiving autogenous bone (n=61) were judged by clinical criteria to have been treated successfully (p=0.524). By radiographic criteria, at this same time point, 75% of those in the OP-1-treated group and 84% of the autograft-treated patients had healed fractures (p=0.218). These clinical results continued at similar levels of success throughout 2 years of observation, and there was no statistically significant difference in outcome between the two groups of patients at this point (p=0.939). More than 20% of patients treated with autografts had chronic donor site pain following the procedure. The conclusion of the authors were that rhOP-1 (BMP-7), implanted with a type I collagen carrier, was a safe and effective treatment for tibial nonunions. This molecule provided clinical and radiographic results comparable with those achieved with bone autograft, without donor site morbidity.

Govender et al. [9] presented the data resulting from a randomized controlled, single-blind study on 450 patients. Patients with an open tibial fracture were randomized to receive either intramedullary nail fixation and routine soft-tissue management or the same treatment plus an implant containing either 0.75 mg/ml rhBMP-2 (total dose, 6 mg), or 1.50 mg/ml rhBMP-2 (total dose, 12 mg). The rhBMP-2 implant (rhBMP-2 applied to an absorbable collagen sponge) was placed over the fracture at the time of definitive wound closure. At the 12-month follow-up, 421 (94%) of the patients were controlled. RhBMP-2 group had significantly faster fracture-healing (p=0.0022) than did the control patients and significantly more patients treated with 1.50 mg/ml rhBMP-2 had healing of the fracture at the postoperative visits from ten weeks through twelve months (p=0.0008). Patients treated with 1.50 mg/ml rhBMP-2 also

Table 1 Effect of BMP-2 on bone regeneration and healing

Reference	Material	Results
Sciadini, Johnson [11]	Dog radial defect; external fixation and different doses of BMP-2	Healing of defects treated with BMP-2, no healing of untreated controls. Better mechanical performance of lower dosage of BMP-2
Bostrom, Camacho [12]	Fracture healing of rabbit ulnae	Accelerated healing of BMP-2 treated fractures compared to controls
Burkus et al. [7]	Single level lumbar fusion in humans; BMP-2 + cages vs. autograft	Accelerated spinal fusion and increased bone formation inside and outside the cages in BMP-2-treated group
Govender et al. [9]	Healing of open fractures in 450 patients	BMP-2 treated group showed faster bone and wound healing, less need for re-operation, lower infection rate

Table 2 Effect of BMP-7 (OP-1) on bone regeneration and healing

Reference	Material	Results
Cook et al. [13]	Long bone defect, BMP-7 at different doses	Healing of treated defect except for lowest dosage; mechanical testing similar to intact side, no healing in control group
den Boer et al. [14]	Fracture healing in goats	Faster healing with OP-1 independent from collagenic carrier
Mizumoto et al. [15]	Distraction osteogenesis in rats	Accelerated osteogenesis in OP-1 group, with more bone formation also after treatment
Johnsson et al. [4]	Lumbar fusion in humans	No differences between OP-1 and autograft
Friedlaender et al. [8]	Tibial nonunions in humans	No differences between OP-1 and autograft

had significantly fewer infections and faster wound-healing. The authors concluded that rhBMP-2 implant was safe and, when 1.50 mg/ml was used, significantly superior to the standard of care in reducing the frequency of secondary interventions and the overall invasiveness of the procedures, accelerating fracture and wound healing, and reducing the infection rate in patients with an open fracture of the tibia.

Studies on the effects of BMP-2 and BMP-7 on bone regeneration and healing are summarized in Tables 1 and 2, respectively.

Personal experience

Between 2000 and February 2006, we have treated 82 cases of large bone defects with a combination of homologous bone, growth factors (platelet-derived growth factors, PDGF) and fresh bone marrow. From this series of patients, we retrospectively selected and reviewed those with cavitary defects and those with orthopaedic problems, mainly pseudoarthroses, to assess the safety, results and complications of the procedure. Among the 82 cases, 42 (37 patients) were treated for healing of large defects: 20 males and 17 females. The mean age of the patients was 19 years (range, 6–54). The lesions were located in 22 cases in the femur, 11 cases in the humerus, 4 cases in the tibia, 2 cases each in the scapula and calcaneus, and in one case in the fibula. The original diagnosis for surgery was an aneurysmal bone cyst in 18 cases, unicameral bone cyst in 11 cases, fibrous dysplasia in 6 cases, a giant cell tumor in 4 cases, chondroblastoma in 2 cases and one case of benign fibrous histiocytoma. In 14 cases (9 patients), the lesion was treated percutaneously with an infiltration of autologous fresh bone marrow associated with demineralized bone matrix in only one case. The percutaneous technique was repeated in 3 patients twice and three times in one patient who fractured two years after the obtained healing in a motorcycle accident, sustaining a pertrochanteric fracture. The 26 patients operated with standard

technique of curettage and grafting healed at 121 days on average (range, 58–279). In some cases the patients lived far from our institution and did not manage to come to all the fixed follow-up visits. Therefore we considered the healing time based on the date of the first follow-up in which the clinician assessed personally the clinical and radiological healing. The medium follow-up is 25 months (range, 6–39). We had two patients with recurrent disease: one patient with an aneurysmal bone cyst of the proximal humerus and one patient with a giant cell tumor of the elbow, 10 and 26 after the original surgeries. The first patient has already been operated with another curettage, while the second one has had needle biopsy to confirm the recurrence. All the remaining 24 patients experienced a successful result both clinically and radiologically. We did not experience any surgical site infection or wound complication in this series. No additional recovery time was needed for the procedure, and the absence of autologous bone graft harvesting surgical procedure obviously was associated with the total lack of additional complications such as pain and blood loss. The surgical time was prolonged by one hour on average, which was the time required for the iliac crest bone marrow harvest and for operating field changing, for during bone marrow processing and bone graft preparation surgery proceeded without differences.

The 40 patients with orthopaedic pathology were treated for osteonecrosis of the femoral head in 3 cases and pseudoarthrosis in 37 cases (Figs. 1 and 2). Femoral head necrosis was treated with vascularized fibula in one case and vascularized iliac crest in 2 cases (Fig. 3). In all 3 cases we added homologous morcellized bone mixed with platelet-rich plasma (PRP) and concentrated fresh bone marrow. Treatment of pseudoarthrosis was done by the standard of care (SOC), i.e. appropriate osteosynthesis and addition of allograft enriched by PRP and concentrated fresh bone marrow or BMP-7. The 3 cases of femoral osteonecrosis healed without complications. In the 37 cases of pseudoarthrosis, 7 affected the humerus and 30 affected the lower limb: 16 cases of the femur, 13 cases of the tibia and one was a subtalar arthrodesis of the talus.

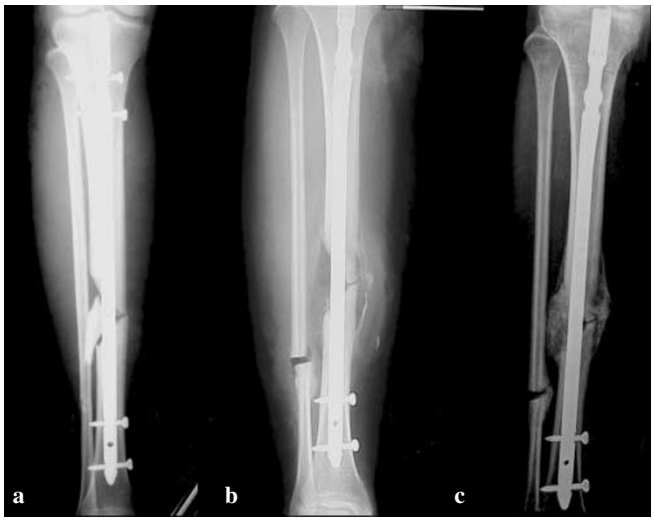

Fig. 1a-c *A 35-year-old woman with an open grade I tibial fracture*. **a** Despite immediate stabilization with an unreamed tibial nail (titanium), at the 6-month follow-up there was evident nonunion. **b, c** Treatment was dinamization of the nail and apposition of allograft and OP-1. Radiographs at one month (**b**) and 6 months (**c**) show complete healing

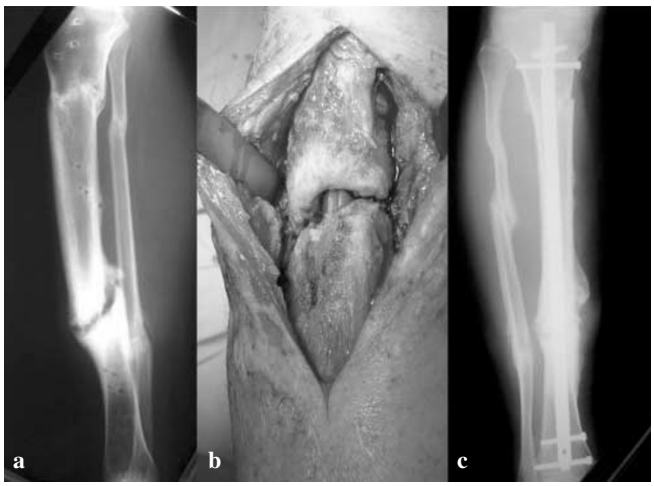

Fig. 2a-c *Male patient with multiple fractures*. **a** Treatment of the right tibia with external fixion for an open grade III fracture resulted in nonunion. **b** Intraoperative image after closed reaming and nailing and exposition of the nonunion site for insertion of OP-1. **c** Radiograph at 4 months shows healing of the nonunion

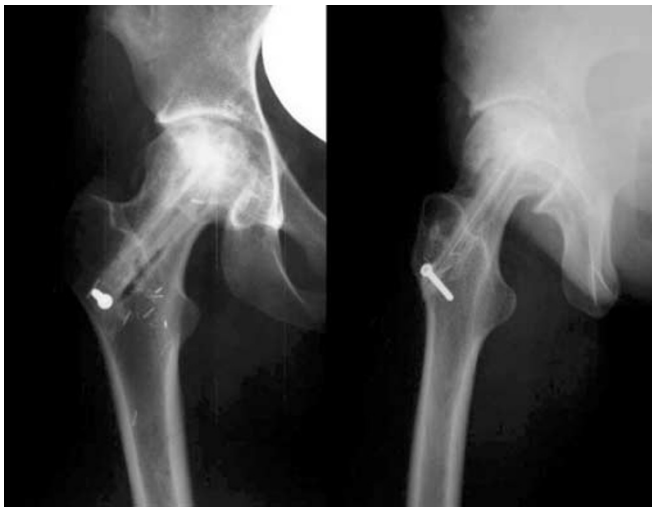

Fig. 3 An 18-year-old woman with idiopathic osteonecrosis of the femoral head, treated with vascularized fibula, growth factors and stem cells. Radiographs at the 2-year follow-up show good conservation of the femoral head

The average age was 30 years (range, 16–65). In 5 cases we used bone marrow with a percutaneous technique with a 60% healing rate. The two failures were in the same patient at the tibia and the femur. In 32 cases we used open surgery and added bone graft plus PDGF and bone marrow in 16 cases, and OP-1 in other 16 cases. The healing rate was similar for both techniques with a success of 87.5%. Even if there was a higher number of previous surgeries in the patients treated with OP-1, still the BMP-7 proved to be effective in these cases with a success rate similar to less complicated cases.

Conclusions

The satisfactory results obtained by the use of rhBMPs in clinical settings do not address completely the concerns regarding safety and cost. BMP-7 have been shown to elicit a subclinical immune response in one-third of patients. BMP-2 does not seem to produce immune responses but its utility in the healing process has been limited by experimental studies only to the initial phases. What is still challenging is the migration from experimental in vitro studies to preclinical and clinical research. The results of the studies are often contradictory and too many variables are present that can influence the outcomes of BMP use: the matrix or carrier used, delivery method, timing of adding BMPs, and contamination from naturally delivered growth factors in animal and human trials. Delivery methods for BMPs and other growth factors are currently under investigation. Recent research by Wildemann et al. [10] examined the possibility of coating osteosynthesis devices with growth factors; by showing the efficacy of this technique, they introduced a new concept of growth factors delivery in addition to established fixation devices. This concept could lead to the development of specialized fixation devices for nonunion which already deliver in a controlled way the necessary growth factor creating true bioactive plating or nailing.

However, there are also other areas that stimulate great interest for future applications of growth factors to enhance bone healing: acceleration of fracture healing (particularly in patients who are at high risk for nonunion) and treatment of established nonunions by injectable preparations of BMPs; enhancement of primary spinal fusion and treatment of established pseudoarthrosis of the spine; enhancement of prostheses fixation to bone and gap filler in revision arthroplasty. Growth factors are not the only strategy available to enhance bone repair in the future: mesenchymal stem cell research and gene therapy have already arrived to preclinical stage.

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