The use of bone morphogenetic proteins (BMPs) in long-bone non-unions

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Summary

The use of bone morphogenetic proteins (BMPs) in treating the clinical challenge posed by fracture delayed and non-union is still under investigation. When used to supplement basic surgical management clinical trials while few in number have demonstrated that BMPs are effective and safe for human application and have an efficacy comparable with that of autologous bone-grafting and eliminate donor site morbidity and reduces the risk of infection at the recipient site. Level one evidence has shown that recombinant human bone morphogenetic protein-7 (rh-BMP-7 or OP-1) is a reasonable alternative to autologous bone grafting in the treatment of long bone non-unions.

Treatment of aseptic non-unions depends on an accurate assessment and classification of the non-union, including the mechanical stability in the fracture area (internal or external fixation), assessment and enhancement of the bone and surrounding tissue biology (biological stimulation).

Autologous cancellous bone-grafting (ABG) is the gold standard biological method to promote union by stimulating the local micro-environment at the non-union site. However, clinical studies have demonstrated up to a 30% rate of unsatisfactory results after surgical treatment of segmental bone defects caused by complicated fractures and its limited availability, donor site morbidity such as pain, neurovascular injury, or infection have driven the search for alternative methods of biological stimulation used either alone or in combination such as allografting, bone marrow injections, electrical, ultrasound, and shockwave stimulation, and bone graft substitutes, with either osteoconductive or both osteoconductive and osteoinductive properties.
Bone morphogenetic proteins (BMPs) and platelet-derived growth factors, also, appear to be safe and efficacious. After their osteoinductive properties their genetic sequences had been identified, they have been produced by recombinant gene technology. Recombinant BMPs (rh-BMPs) are alternatives or adjuncts in the treatment of cases where bone regeneration is not anticipated.

The bone morphogenetic proteins

BMPs were first described by Dr. Marshall Urist in 1965. They are members of the transformation growth factor-b (TGF-b) superfamily and have great osteoinductive potential. Having been isolated from different species, and due to the lack of standardization of these purifications, several of the BMPs have alternate names that are often used interchangeably, e.g. BMP-7 is OP-1, BMP-8 OP-2, BMP-12 is Growth and Differentiation Factor 7 (GDF-7), and BMP-13 is both GDF-6 and CDMP-2. At least 40 different sub-types have been described to date and these have been divided into groups according to their primary amino acid sequence as below:

- BMP 2/4: BMP-2 and BMP-4 (BMP-2b).
- BMP 7/OP1: BMP-5, -6 (Vgr-1), -7(OP1), -8(OP2), -8b(OP3).
- BMP 3: BMP-3 (osteogenin) and BMP-3b (GDF-10).
- CDMP: BMP-12, -13, -14 (CDMP1-3).
- Others: Growth/differentiation (GDF) 5, 6 and 7, BMP-15, BMP-16.

Their role in inducing differentiation of fibroblasts to convert to osteoblasts thus form bone has been established in vivo. Work continues to clearly define the biological activity of each of these molecules but BMPs at tiny concentrations strongly induce new bone formation both within osseous lesions and at ectopic sites, such as skeletal muscle. They stimulate chemotaxis, proliferation and differentiation of mesenchymal cells into chondrocytes and osteoblasts.

Meyer et al. in 2001, showed in rats that the expression of BMPs and their receptors peaks at one to two weeks after fracture and then sharply decreases to very low or undetectable levels in young rats, in which fractures heal, and in old rats, in which non-unions develop. In particular BMP-2, BMP-4, and BMP receptor type IA (BMPR-IA) mRNA peaked at one to two weeks after fracture and then fell to very low levels at four to six weeks after fracture. Kloen et al. using immuno-histochemical analysis to investigate the expression of BMPs and the activation of BMP-signaling components in human fracture non-unions to determine whether BMPs, BMP receptors, or SMAD1 (one of the signal-transducing, receptor-regulated Smad proteins) disappear from the involved tissues during the development of a fracture non-union and whether their disappearance contributes to the development of the non-union. They studied delayed unions or non-unions with an average duration of 22 months in 21 adult patients. BMP-2, BMP-4, BMP-7, and their BMP receptors (BMPR-IA, BMPR-1B, and BMPR-II) as well as Smad1 were examined. The site of all of the BMP-signaling components was demonstrated in 81% of the patients, and the staining patterns showed co-localization of the BMPs with their receptors. Thus, the concept that the expression and activation of BMPs and their signaling components are lacking at the sites of delayed unions or non-unions was not supported by this study. However, although delayed unions and non-unions of long duration showed persistent expression of BMPs, their receptors and signaling components, the authors accepted that the concentrations of the BMPs and/or their receptors may not be sufficient to obtain normal healing. This raises questions about fundamental differences among critical-sized segmental bone defects, which heal in the presence of exogenous BMPs but do not heal spontaneously, fractures that heal readily in the presence of endogenous BMPs, and fractures with a propensity for non-union, which do not heal despite an initial increase in the levels of BMPs and their receptors and about the persistent presence of BMPs in the specimens of non-union.

BMPs facilitate the repair of critical-sized segmental bone defects in experimental animals by stimulating the migration of mesenchymal stem cells from muscle, periosteum, endosteum, and bone marrow into the defect, by the proliferation and differentiation of the mesenchymal stem cells, and by the formation of bone through endochondral ossification. Recently much has been learned about the cellular and molecular mechanisms by which the BMPs induce bone formation. Most of these studies have involved critical-sized segmental osteoperiosteal defects in experimental animals (Table 2). In the experimental operative procedure used to create the osteoperiosteal defect, the soft tissues surrounding the defect are generally preserved and there is an abundant source of mesenchymal stem cells, particularly in the surrounding muscle. The experimental defect is stabilized by internal fixation or by an intact radius or tibia when the defect is created in the ulna or fibula, respectively.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Commerically available rh-BMP-2 and rh-BMP-7.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Commercially available product</strong></td>
<td><strong>Composition</strong></td>
</tr>
<tr>
<td>OP-1™ Implant Stryker Biotech</td>
<td>rh-BMP-7 with Type-1 collagen</td>
</tr>
<tr>
<td>InFUSE® bone graft</td>
<td>Genetically engineered human protein (rh-BMP-2)</td>
</tr>
</tbody>
</table>
Human fracture may differ greatly from those in experimental animals in geometry and complexity which may be as important in determining whether a non-union will occur as the concentration of endogenous or exogenous BMPs at the fracture site. Also in response to the chemotactic stimulus of BMPs, mesenchymal stem cells may have too great a distance to migrate from muscle and periosteum to the interstices of the fracture fragments and in open fractures, particularly after debridement with loss of muscle and periosteum, there may be an inadequate supply of mesenchymal stem cells.

**BMP manufacture**

Cadaver bone provides only small quantities purified human BMP (hBMP), limiting commercial production. Thus, industry turned to recombinant gene technology and focussed on those with the greatest potential for bone induction.\(^8\)

Recombinant human BMP-2 and BMP-7 (OP-1) have been approved for limited clinical use,\(^{41}\) but their application and efficacy has been reduced by delivery problems. As they have very short biological half-lives and are difficult to retain at sites of local application, large bolus doses are required to induce bone healing.\(^{73,74,30}\) Under these conditions, release of growth factors is not uniform. Instead, there is an initial rapid "out-flux" saturating surrounding tissue with supra-physiological concentrations of growth factor, leading to systemic exposure. Subsequent release, although slower, provides much lower, suboptimal concentrations.\(^{75–77}\) Another disadvantage of using recombinant proteins is their high cost.\(^{78}\)

These products have been investigated as an alternative to bone autografting in a variety of clinical situations, including spinal fusions, internal fixation of fractures, treatment of bone defects, and reconstruction of maxillo-facial conditions. Rh-BMPs are delivered to the bone-grafting site as part of a surgical procedure. A variety of carrier and delivery systems have been investigated.

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### Table 2 Segmental bone defects in animal models treated with BMP-preparations.

<table>
<thead>
<tr>
<th>Implant/carrier</th>
<th>Animal model</th>
<th>Bone</th>
<th>Defect size</th>
<th>Analysis methods</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>bBMP</td>
<td>Rat</td>
<td>Femur</td>
<td>1.0 cm</td>
<td>Ro, histology</td>
<td>Tagaki and Urist(^{48})</td>
</tr>
<tr>
<td>bBMP</td>
<td>Dog</td>
<td>Ulna</td>
<td>2.5 cm</td>
<td>Ro, histomorphometry</td>
<td>Nilsson et al.(^{49})</td>
</tr>
<tr>
<td>bBMP/PLA</td>
<td>Dog</td>
<td>Radius</td>
<td>0.3 cm</td>
<td>Ro, histomorphometry</td>
<td>Heckman et al.(^{50})</td>
</tr>
<tr>
<td>rh-BMP-2/DBM</td>
<td>Rat</td>
<td>Femur</td>
<td>0.5 cm</td>
<td>Ro, torsion test, histology, radio-isotope boneimaging</td>
<td>Yasko et al.(^{51})</td>
</tr>
<tr>
<td>rhOP-1/collagen</td>
<td>Rabbit</td>
<td>Ulna</td>
<td>1.5 cm</td>
<td>Ro, torsion test, histology</td>
<td>Cook et al.(^{52})</td>
</tr>
<tr>
<td>rhOP-1/collagen</td>
<td>Dog</td>
<td>Ulna</td>
<td>2.5 cm</td>
<td>Ro, torsion test, histology</td>
<td>Cook et al.(^{53})</td>
</tr>
<tr>
<td>rhOP-1/collagen</td>
<td>Green</td>
<td>Ulna and monkey tibia</td>
<td>2.0 cm</td>
<td>Ro, torsion test, histology</td>
<td>Cook et al.(^{54})</td>
</tr>
<tr>
<td>rh-BMP-2/PGA</td>
<td>Rabbit</td>
<td>Ulna</td>
<td>2.0 cm</td>
<td>Ro, torsion test, histology</td>
<td>Boström et al.(^{55})</td>
</tr>
<tr>
<td>sBMP/TCP</td>
<td>Sheep</td>
<td>Tibia</td>
<td>1.6 cm</td>
<td>Ro, torsion test, histology</td>
<td>Gao et al.(^{56})</td>
</tr>
<tr>
<td>MBMP/coral</td>
<td>Sheep</td>
<td>Tibia</td>
<td>1.6 cm</td>
<td>Ro, torsion test, histology</td>
<td>Gao et al.(^{57})</td>
</tr>
<tr>
<td>bBMP/DBM</td>
<td>Dog</td>
<td>Radius</td>
<td>2.5 cm</td>
<td>Ro, torsion test, histology</td>
<td>Sciadini et al.(^{58})</td>
</tr>
<tr>
<td>bBMP/coral</td>
<td>Dog</td>
<td>Radius</td>
<td>2.5 cm</td>
<td>Ro, torsion test, histology</td>
<td>Sciadini et al.(^{59})</td>
</tr>
<tr>
<td>rh-BMP-2/PLA</td>
<td>Rabbit</td>
<td>Radius</td>
<td>2 cm</td>
<td>Ro-morphometry, histomorphometry</td>
<td>Zegzula et al.(^{60})</td>
</tr>
<tr>
<td>rh-BMP-2/PLA</td>
<td>Rabbit</td>
<td>Radius</td>
<td>1.0 cm</td>
<td>Ro</td>
<td>Zellin and Linde(^{61})</td>
</tr>
<tr>
<td>rh-BMP-2/PDLLA</td>
<td>Dog</td>
<td>Ulna</td>
<td>2 cm</td>
<td>Ro, histomorphometry</td>
<td>Itoh et al.(^{62})</td>
</tr>
<tr>
<td>rhOP-1/collagen</td>
<td>Dog</td>
<td>Ulna</td>
<td>2.5 cm</td>
<td>Ro, torsion test, histology</td>
<td>Cook et al.(^{63})</td>
</tr>
<tr>
<td>rh-BMP-2/PDLLA</td>
<td>Sheep</td>
<td>Femur</td>
<td>2.5 cm</td>
<td>Ro, histology</td>
<td>Kirker-Head et al.(^{64})</td>
</tr>
<tr>
<td>rh-BMP-2/PDLLA</td>
<td>Rabbit</td>
<td>Femur</td>
<td>0.5 cm</td>
<td>Ro, torsion test</td>
<td>Lane et al.(^{65})</td>
</tr>
<tr>
<td>rh-BMP-2/PDLLA</td>
<td>Rabbit</td>
<td>Radius</td>
<td>2.0 cm</td>
<td>Ro</td>
<td>Texeira and Urist(^{66})</td>
</tr>
<tr>
<td>Rh-BMP-2/PLA</td>
<td>Rabbit</td>
<td>Radius</td>
<td>2.0 cm</td>
<td>Radiomorphometry, torsion test</td>
<td>Wheeler et al.(^{67})</td>
</tr>
<tr>
<td>cBMP/PLA</td>
<td>Dog</td>
<td>Radius</td>
<td>0.3 cm</td>
<td>Ro, histomorphometry</td>
<td>Heckman et al.(^{68})</td>
</tr>
<tr>
<td>rh-BMP-2/PGA</td>
<td>Rat</td>
<td>Femur</td>
<td>0.5 cm</td>
<td>Ro, histology</td>
<td>Isobe et al.(^{69})</td>
</tr>
<tr>
<td>rh-BMP-2/TCP- MCPP</td>
<td>Rat</td>
<td>Femur</td>
<td>0.5 cm</td>
<td>Ro, torsion test</td>
<td>Ohura et al.(^{70})</td>
</tr>
<tr>
<td>rh-BMP-2/collagen</td>
<td>Dog</td>
<td>Radius</td>
<td>2.5 cm</td>
<td>Ro, histology, biomechanical</td>
<td>Sciadini–Johnson(^{71})</td>
</tr>
<tr>
<td>Ad.BMP-2/gene</td>
<td>Rat</td>
<td>Femora</td>
<td>5 mm</td>
<td>Ro-morphometry, histomorphometry</td>
<td>Betz(^{72})</td>
</tr>
</tbody>
</table>

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\(^8\)This technology is viewed by the FDA as being associated with risk, thus recombinant BMPs are classified as Class-III devices.
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allograft, help to maintain the concentration of the rh-BMP at the treatment site, provide temporary scaffolding for osteogenesis and prevent ectopic bone formation. The rh-BMP and carrier may be placed to the recipient area via a delivery system, which may also provide mechanical support e.g. for interbody spinal fusion, delivery systems have included interbody fusion cages. As carrier and delivery systems are important variables, and different clinical applications require different dosages of rh-BMP with different carriers and delivery systems, the results of one clinical application cannot be extrapolated to others.

Preclinical animal studies

Numerous preclinical studies have been published on the use of BMPs on large critical-sized diaphyseal segmental defects (i.e., defects that cannot heal without exogenous osteogenic stimulation) in rats, rabbits, dogs, sheep and non-human primates to evaluate the osteoinductive properties of BMP. These studies have shown that the implantation of BMPs with carrier matrices in bone defects led to biomechanical and biologically sound bone formation. In such animal models, BMP was equivalent to or better than autologous bone-grafting, the standard treatment in clinical practice. This demonstrated the potential for BMPs to be used as an alternative to autologous bone-grafting. This has been investigated intensively as bone-graft harvest causes morbidity, such as persistent pain, numbness, or hypersensitivity at the donor site. Einhorn et al. studied a single percutaneous injection of rh-BMP in 144 male Sprague—Dawley rats, torsional biochemical testing showed that stiffness of rh-BMP treated fractures was twice that of control groups at 2, 3 and 4 weeks. This showed that a single injection of BMP-2 would accelerate fracture repair and suggested their use in fractures not requiring operative treatment or treatment without direct exposure of the fracture site. BMP was also shown to accelerate bone formation and repair in non-critical size defects in closed fracture models with an early return of strength and stiffness. A few preclinical animal studies are set out in Table 2.

Clinical use of BMPs in bone defects and non-unions

Delayed and non-unions remain a clinical challenge. While BMPs have been studied, prospective randomized controlled studies are lacking. We review the first clinical studies by anatomical site and various clinical trials are summarized in Table 3.

Tibial non-union

Johnson et al. evaluated several small series of resistant non-unions and segmental long-bone defects had been treated with hBMP. Urist had purified the protein in his laboratory, and Johnson and colleagues used the protein in clinical settings. These uncontrolled retropective series (Level-IV evidence) gave encouraging results and led to further studies. As recombinant BMPs were not yet available, a purified mixture of BMP proteins (hBMP) was used, in combination with insoluble non-collagenous proteins, both derived from human donor bone. At that time the risk of immunogenicity associated with alloimplants was becoming apparent, but it remained unknown which specific proteins were responsible for the osteoinductive activity and how this activity should be managed to develop an applicable clinical product. However, these three small case series were the first studies to assess purified BMPs clinically and they demonstrated that these implants were tolerated and could be useful in the management of difficult non-unions.

In his small series of six cases attempted to enhance bone regeneration by augmenting autogenic cancellous bone graft with human BMP implants. The patients had segmental tibial bone loss ranging from 3 to 17 cm and five had a history of infection. Four had had previous treatment with external factors, and three had failed previous autogenic cancellous grafting. The latter three patients had also failed pulsed electromagnetic bone growth stimulation.

An rh-BMP strip was placed under the medial periosteal sleeve of the defect while autogeneic cancellous bone filled the intercalary defect between the proximal and distal shaft fragments. They concluded that rh-BMP can be implanted without any adverse effects.

Johnson’s second series of four patients distal tibial metaphyseal non-unions with residual anterior cortical bone loss. They had open tibial fractures and had significant posterior bowing deformities with fibular malunion with bony overgrowth prevented manual correction of the tibial deformity. They had had an average of 5.8 previous surgical procedures (six plate stabilizations and seven autogeneic cancellous iliac crest bone grafts). They underwent reduction and fixation of the posterior tibial cortex and rh-BMP implants were positioned across the anterior tibial cortical defect in contact with the residual freshened bone of the distal tibial metaphysis. All four non-unions united at an average of 4.4 months.

In 1992, the United States Food and Drug Administration (FDA) approved a human clinical trial for the evaluation of OP-1 in treatment of tibial non-union. In this large prospective, randomized, controlled, partially blinded, multicenter study, Friedlaender et al. assessed the efficacy of the OP-1 Device (3.5 mg of rh-BMP-7 in a bovine bone-derived type-I collagen-particle delivery vehicle; Stryker Biotech, Hopkinton, Massachusetts) comparing it to autografting in the treatment of 122 patients with a total of 124 tibial non-unions. All non-unions were at least nine months old and had shown no progress toward healing for the three months prior to the patient’s enrollment in the study. All the patients were treated withreamed intramedullary nailing and were then randomized to have either autograft bone or OP-1 implanted at the non-union site. Despite randomization, there were more smokers in the OP-1 group. Nine months after the surgery, 81% of the 63 non-unions treated with OP-1 and 85% of the 61 treated with autograft had clinical evidence of union. Radiographic assessments suggested healing of 75% and 84% of these non-unions, respectively. As statistical analysis of these...
results showed equivalent efficacy between OP-1 and autograft. The authors concluded that OP-1 was a safe and effective alternative to bone graft in the treatment of tibial non-unions. A limitation of the study was that the investigators could not control for the potential healing effects produced by reamed intramedullary nailing of tibial non-unions but it is noteworthy that half of the non-unions treated in this study were of fractures that had failed to heal following reamed nailing as a primary treatment. Friedlaender et al. did show that the risk of infection at the implantation site was significantly lower in the rh-BMP-7 group than it was in the group treated with autologous bone-grafting ($p = 0.002$). The authors concluded that BMPS were an effective alternative to autografting if the morbidity associated with harvesting of the graft is taken into consideration.30

Table 3  Overview of clinical trials on use of BMP in long-bone non-unions.

<table>
<thead>
<tr>
<th>Author</th>
<th>Anatomic Site</th>
<th>No.</th>
<th>Type of study</th>
<th>Material</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Johnson et al.31</td>
<td>Tibial bone loss</td>
<td>6</td>
<td>Case series (small)</td>
<td>rh-BMP strip</td>
<td>Healing without any adverse effects</td>
</tr>
<tr>
<td>Johnson et al.31</td>
<td>Femoral non-union</td>
<td>12</td>
<td>Case series (small)</td>
<td>Partially purified BMP extract with or without autogenous or an allogenic bone graft</td>
<td>Healing in 11/12 cases after single intervention</td>
</tr>
<tr>
<td>Johnson et al.82</td>
<td>Distal tibial defect non-union</td>
<td>4</td>
<td>Case series (small)</td>
<td>rh-BMP strip</td>
<td>All 4 non-unions united (aver. 4.4 months)</td>
</tr>
<tr>
<td>Johnson et al.83</td>
<td>Femoral non-union</td>
<td>30</td>
<td>Case series</td>
<td>Partially purified BMP extract ± autogenous graft</td>
<td>24 of 30 (80%) healed</td>
</tr>
<tr>
<td>Geesink et al.84</td>
<td>Fibular defect of critical size</td>
<td>6</td>
<td>Case series (small)</td>
<td>rh-BMP-7</td>
<td>5 out of 6 pts</td>
</tr>
<tr>
<td>Friedlaender et al.30</td>
<td>Tibial non-unions</td>
<td>124</td>
<td>Prospective, randomized, controlled, partially blinded, multicenter study</td>
<td>OP-1 vs. autograft</td>
<td>Comparable clinical outcome of OP-1 and autograft (81% vs. 85%)</td>
</tr>
<tr>
<td>McKee et al.85</td>
<td>Tibial, calvarias humeral, ulnar, femoral non-unions</td>
<td>31</td>
<td>Case series</td>
<td>OP-1</td>
<td>Good osteogenesis in all patients</td>
</tr>
<tr>
<td>Susarala et al.56</td>
<td>Humeral non-unions</td>
<td>6</td>
<td>Case series (small)</td>
<td>OP-1</td>
<td>Union achieved in 11/12 cases</td>
</tr>
<tr>
<td>Dimitriou et al.87</td>
<td>Tibial non-unions, femoral, humeral, ulnar, patellar, and clavicular non-union</td>
<td>17</td>
<td>Case series</td>
<td>rh-BMP-7 ± autologous graft</td>
<td>Union in 8/9 cases managed only with rh-BMP-7 and in 16/17 cases treated with combination therapy</td>
</tr>
<tr>
<td>Giannoudis and Tzioupis88</td>
<td>Atrophic persistent long-bone non-unions, various anatomic sites</td>
<td>395</td>
<td>Multicenter, retrospective study, non-randomized study</td>
<td>OP-1 alone 74%</td>
<td>Successful outcome 82%</td>
</tr>
<tr>
<td>Jones et al.89</td>
<td>Tibial diaphyseal fracture with defect</td>
<td></td>
<td>An observational, retrospective, non-randomized study</td>
<td>OP-1+ autograft 23% rh-BMP-2 and autogenous or an allogenic bone graft</td>
<td>Better results with rh-BMP-2 [13/15] than with bone graft [11/15]</td>
</tr>
<tr>
<td>Ronga et al.75</td>
<td>Long-bone non-union, 46 tibia, 26 femur, 20 humerus, 12 forearm, 2 clavicle</td>
<td>106</td>
<td>An observational, retrospective, non-randomized study</td>
<td>rh-BMP-7 vs. rh-BMP-7+autograft</td>
<td>Successful outcome in 88/105 patients (83.8%)</td>
</tr>
</tbody>
</table>
BMP for the treatment of femoral non-union

Johnson reported a series of 12 patients with intractable non-union of the femoral diaphyseal or metaphyseal–diaphyseal shaft with an average duration of non-union of 31.6 months. All had radiographic discontinuity gaps with abnormal mobility and pain. Eleven patients had previously failed 43 procedures and pulsed electromagnetic field bone growth stimulation. Seven patients were stabilized with plate osteosynthesis and five received intramedullary nailing. The BMP was used to aid in fracture union and was positioned in the peri-non-union area. BMP was thought to have favorably influenced the healing in 11 of 12 patients.31

Johnson in 2000, reported a series of 30 patients average age 47 years with femoral diaphyseal or diaphyseal–metaphyseal reconstructions augmented with rh-BMP and allogeneic, autolyzed antigen-free bone carrier alloimplants.83 The series included 24 shortened atrophic femoral non-unions, four equal length atrophic non-unions, and two longstanding shortened malunions. They had had an average of two previous failed procedures and an average of two failed autogenous cancellous bone-grafting per patient and the average time from initial femoral fracture to rh-BMP implantation was 39 months.

Patients with shortened femoral non-unions underwent standard deformity correction and restoration of the mechanical axis. In addition, the non-unions were lengthened with a one-stage distraction creating an intercalary defect at the non-union site. The rh-BMP allograft bridged the medial aspect of larger intercalary defects greater than 2 cm. Autograft material was required to bridge these defects and allow host bone induction and remodeling. Thirteen patients received additional autogenous cancellous bone grafts to the intercalary segmental defect after one-stage lengthening. The interface between the implant and the host bone above and below the defect was not in contact with any cancellous graft material. Twenty-four of 30 femora healed with rh-BMP allogeneic implants and plate osteosynthesis with an average time to healing of 6 months. The mean percentage of increased length over the pre-treatment shortened femur was 7%. Six patients had fatigue failure of the plate implant of which four patients underwent revision surgery due to persistent distal metaphyseal non-unions. Three patients required additional cancellous grafting to the anterior and posterior aspects of the intercalary defects at an average of 5 months after lengthening.

Osseous defects

While there are many preclinical animal studies of BMPs in osseous defects, Geesink et al. were the first to demonstrate clinically the osteoinductive capacity and effectiveness of rh-BMP-7 in humans in inducing healing of cortical bone defects.84 They investigated the osteogenic activity of OP-1 in bridging fibular defects made at the time of tibial osteotomy for varus or valgus deformity of the knee. Radiological and DEXA (dual energy X-ray absorptiometry) evaluation showed that in patients in whom the defect was left untreated no formation of bone occurred, whereas in five of six patients, rh-BMP-7 bound to collagen particles was shown to be effective with a critically sized fibular defect.

BMP use in other non-unions

Other studies of various anatomic sites have confirmed the above evidence.75,85–89 In a study of 31 patients with 6 tibial, 9 calvarial, 10 humeral, 2 ulnar, and 4 femoral non-unions undergoing standard internal fixation supplemented with OP-1, McKee et al. found abundant new bone formation in all 31 patients and fracture healing at a mean of 13 weeks without any adverse effects.85 A retrospective study of 12 humeral non-unions treated with OP-1 by Susarala et al. showed clinical and radiographic evidence of union in 11 of the 12 patients at an average of 162 days (Table 3).86

Giannoudis et al. in a recent retrospective, non-randomized, multicenter study evaluated the use of recombinant BMP in 653 patients in different clinical scenarios.88 Three hundred and thirty-five patients with atrophic non-unions in various anatomic sites were treated with a single application of 3.5 mg OP-1 with an appropriate surgical procedure. OP-1 was used alone in 74% of the non-unions and combined with autograft in 23%. The overall success rate was up to 82% in terms of clinical and radiological non-union with no systemic complication or adverse effects.88

In another study, conducted by Dimitriou et al., fracture non-unions were treated with rh-BMP-7 including 10 tibial, eight femoral, three humeral, three ulnar, one patellar, and one clavicular non-unions.87 Seventeen had autologous bone graft in addition to rh-BMP-7 and nine cases received only rh-BMP-7. Sixteen cases (94.1%) managed with combination of BMP and bone graft while eight cases (88.9%) treated exclusively with rh-BMP-7 achieved clinical and radiological union (Table 3).87

In a prospective, randomized, controlled trial by Jones et al., 30 adult patients with a tibial diaphyseal fracture and a residual cortical defect received either autogenous bone graft or allograft (rh-BMP-2/cancellous bone chips) for staged reconstruction of the tibial defect.89 Ten patients in the autograft group and 13 patients in the rh-BMP-2/allograft group had healing without further intervention. In another observational, retrospective, non-randomized study by the BMP-7 Italian Observational Study (BIOS) Group on the use of BMP-7, 105 non-unions in various sites were treated with additional grafts if deemed necessary clinically.75 The mean follow-up was 29.2 months by radiographic and clinical assessment of the two groups, groups: BMP-7 with autograft or BMP-7 alone. There was an 88.8% success rate with an average healing time of 7.9 months. This demonstrated the efficacy of BMP-7 both with and without bone-grafting for the treatment of long bone non-unions.75

Open tibial fractures and rh-BMP-2

There is a growing clinical interest in rh-BMPs in open tibial fractures. Riedel reported a series examining the effect of rh-BMP-2 on extraarticular, open tibial fractures (Gustilo-Anderson II or higher) in 12 patients.93 Patients underwent surgical procedures that including fracture reduction within 24 h of injury, repeated wound debridement, and fracture coverage within 14 days of the injury. Median time between
injury and rh-BMP-2/ACS implantation was 4 days. Eleven patients completed 9-month follow-up and their fractures healed without further intervention. The remaining three patients required second surgical interventions for delayed union and underwent bone-grafting 16–17 weeks following injury. Two patients had positive antibody titers to rh-BMP-2. He concluded that the implantation of rh-BMP-2 with an absorbable collagen sponge is surgically feasible and safe.

More recently, the BMP-2 Evaluation in Surgery for Tibial Fractures (BESTT) study group reported the results of a large multicenter, prospective, randomized, controlled study of the effects of INFUSE (rh-BMP-2 on an absorbable type-1 collagen sponge; Medtronic Sofamor Danek, Memphis, Tennessee) in open tibial fractures. Four hundred and fifty patients were initially managed with irrigation, débridement, and intramedullary nail fixation. At the time of definitive wound closure, the patients were randomized to one of three groups: standard closure, standard closure and the addition of 6 mg of rh-BMP-2 to the fracture site, or standard closure and the addition of 12 mg of rh-BMP-2 to the fracture site. The primary outcome measure in this study was the rate of secondary interventions i.e., further operative treatment. The group treated with the higher dose of rh-BMP-2 had fewer secondary interventions. Interestingly, although not used as primary outcome measures, an accelerated time to union, improved wound-healing, and a reduced infection rate were also found in the patients treated with the high dose of rh-BMP-2. This could be in part related to increased vascular supply at the fracture site induced by BMP-2.

In a similar study to that of the BESTT group, McKee et al. investigated the use of OP-1 in the treatment of open tibial fractures. Fractures were treated initially with irrigation, débridement, and locked intramedullary nailing. At the time of definitive wound closure, the patient was randomized to be managed with either standard closure or standard closure with 3.4 mg of OP-1 to the fracture site. One hundred and twenty two patients with a total of 124 tibial fractures were enrolled in the study. There was a significant decrease in the rate of secondary interventions for delayed unions and non-unions (the primary outcome measure) in the OP-1-treated group (p = 0.02). There was a corresponding improvement in patient function, with 80% of the OP-1 group having no or mild pain with activity at 12 months post-injury compared with 65% of the control group (p = 0.04).

Miniscalco et al. conducted a randomized controlled pilot study to examine the effect of OP-1 in fresh tibial fractures. Fourteen patients with type A1 or A2 closed tibial shaft fractures treated with a monolateral external fixator were randomized into two groups. One group received OP-1 while the other acted as a control group. Time to bone healing in the OP-1 group was similar to the control group. Hence he did not recommend OP-1 for fresh fractures of the tibia. As most fresh fractures are adequately managed by other conventional techniques, the routine use of BMP may not be justified in such cases.

These studies suggest that the use of rh-BMP-2 in open tibial fractures accelerates the time to union, lowers the rate of secondary interventions, improves wound-healing and reduces the infection rate. However, McKee and Miniscalco et al. have shown that OP-1 does not accelerate fracture healing of open and fresh tibial fractures, but might decrease the rate of secondary interventions for delayed unions and non-unions and improve the patient’s function.

Discussion

While it took almost 40 years from the discovery of BMPs by Urist for them to become available for clinical application, they have led to a major step forward in the understanding of bone physiology and the evolution of more advanced surgical techniques. They have the unique potential to induce new bone formation, even at extraskeletal sites. However, the factors such as optimal therapeutic dosage, delivery system, and local conditions for bone repair are still under investigation and basic surgical management to provide adequate environmental conditions of the implantation site, such as soft-tissue coverage, host-bed vitality, and biomechanical stability, remains essential. Clinical trials, although still limited in number compared with preclinical studies, have demonstrated that BMPs are effective and safe for human use and have an efficacy comparable with that of autologous bone-grafting. Growth-factor therapy with BMPs offers a new surgical approach that can augment or even replace bone-grafting procedures. However, despite contrary results in animal studies, treatment of fresh fractures with BMP in humans has not resulted in a significantly higher rate of bone healing compared with that achieved by the current treatment techniques such as autologous bone-grafting and BMPs must be given in much higher doses to accomplish osteoinductive activity in humans.

Clinical studies of BMP-2 and 7 have shown that bone formation is not consistent, possibly due to the relative osteoinductivity of the applied BMPs in the presence of responding cells and the time at which the BMPs are presented locally by their carrier. Better understanding of these mechanisms is needed to develop better carrier systems and possible combination therapies with other BMPs or growth factors.

Production of individual recombinant human BMPs (rh-BMPs) has led to two important conclusions. Firstly, single BMPs are osteoinductive by themselves and secondly, the osteoinductivity of a single BMP has a dose–response ratio, unmodified by individual patient characteristics as they act locally. Hence the concentration of BMPs at the site of implantation is more important than the total dose. The dose must overcome a threshold before effective induction of bone formation; if the dose of BMPs is too low there will be inadequate bone formation, and if it is too high there will be more bone formation and more rapid osteoinduction than desired. This increased bone formation eventually results in direct (intramembranous) ossification, bypassing the intermediate phase of endochondral ossification that occurs when lower doses are used. However, with high doses of BMPs, initial localized resorption of bone can be seen as a result of increased osteoclastic activity, as BMPs also stimulate osteoclastogenesis and osteoclastic activity. At this point, a higher dose of BMPs is ineffective, as no further acceleration of bone formation occurs. Local overdoses of BMPs could be expected to lead to heterotopic ossification, but this phenomenon has not been shown to occur under physiological
conditions. In a mouse model of BMP-4-induced heterotopic ossification, Glaser et al. demonstrated in vivo that heterotopic ossification in fibrodysplasia ossificans progressiva may not be due to the genetic overexpression of BMP-4 but rather to the underexpression of the extracellular antagonist of BMPs, noggin. Excessive ossification in this animal model could be prevented by local delivery of noggin, illustrating the highly regulated negative feedback mechanisms for BMPs that prevent abnormal or heterotropic bone formation even with high doses. In addition, the dose of BMP needed for clinical efficacy must overcome a threshold, and the dose–response curve becomes steeper as one progresses from rodent to non-human primate models. The latter species, most closely related to humans, was used to derive the human therapeutic dosage of 3.5 mg/4 mL of sterile saline solution or 0.88 mg/mL of sterile saline solution for rh-BMP-7 and 12 mg/8 mL of sterile water or 1.50 mg/mL of sterile water for rh-BMP-2. 

Rh-BMPs are expensive and are used in current clinical applications at concentrations that are ten to 1000-fold higher than those of native BMPs. These high doses of BMPs are used in an attempt to produce a clinical effect comparable with that shown to be osteoinductive in animal studies. The strongly regulated signaling mechanisms and the rapid local and systemic clearance of BMPs in higher species also necessitate higher doses. It has also been assumed that higher species have fewer responding cells than do lower species which raises important questions regarding combination therapies of BMPs and the development of more efficient and more cost-effective delivery systems.

Currently two recombinant BMPs, rh-BMP-2 and rh-BMP-7 (also known as osteogenic protein-1 [OP-1]) are available for clinical use. Each has been evaluated in randomized controlled trials involving trauma patients, providing Level-I evidence.

BMPs 2 and 7 have been studied in treating non-union of long bones. Several randomized controlled trials have compared BMP to autograft in the treatment of both open and closed tibial fractures. Patients with closed tibial fractures showed similar outcomes regardless of treatment regimen. Tibial non-unions also showed similar outcomes. Data from one randomized controlled trial suggested that open tibial fractures benefited from treatment with BMP compared with standard treatment. Patients who received the BMP implants tended to have shorter operative times and shorter hospital stays, but the differences were not statistically significant. In addition, patients with BMP implants did not experience donor site pain in comparison to autograft patients. Two small series of femoral non-union have been published. Patients had positive outcomes following BMP treatment, but it is uncertain if BMP had a significant effect on outcomes as there was no comparison group. Randomized controlled trials of BMP in spinal fusions compared to autograft have shown similar outcomes regardless of treatment but patients with BMP implants did not experience donor site pain.

Rh-BMP-7 has received FDA approval through the Humanitarian Device Exemption process as an alternative to bone autograft in recalcitrant long-bone non-unions. The data presented to the FDA has now been published.

Osteogenic protein-1 (OP-1) is a growth factor, which is one of the components of the autologous bone graft. It plays an important role in bone formation by stimulating stem cells to differentiate into osteoblasts. The Friedlaender et al. randomized controlled trial of OP-1 on tibial non-unions demonstrated equivalence between autologous bone-grafting and OP-1. The rate of clinical and radiological success in tibial non-unions was comparable to the autologous-bone grafting when used in conjunction with intramedullary rod fixation. In addition, the rate of post-operative infection at the recipient site was significantly lower in those treated with OP-1. This study represents the cornerstone for the rh-BMP-7 use. Based on this trial, the FDA issued a Humanitarian Device Exemption to authorize the marketing of OP-1.

In the Friedlaender et al. study both treatment groups received an intramedullary rod and compared rh-BMP-7 with autologous grafting improved. The authors chose a difficult population of patients with non-union to study; the majority had had prior bone-grafting and intramedullary rod fixation before inclusion in the study and about half of the patients had undergone a prior attempt at fusion with an intramedullary rod and from 30–40% had undergone a prior autograft. The union rate was similar in the two groups, which also did not differ with regard to clinical or radiographic healing characteristics. Although comparative efficacy was demonstrated, the goal of the BMP therapy, a higher healing rate, was not achieved. It is interesting to speculate that the rh-BMP-7 might have been more effective, as the percentage of patients with atrophic non-union was higher in the rh-BMP-7 group (41%) than it was in the group treated with autologous bone-grafting (25%). Unfortunately, the authors did not conduct a power analysis for their study, and the lack of differences between the groups may have been the result of a type-II statistical error. No statistical analysis with adjustment for atrophic non-union was performed.

Other methods such as percutaneous injection of autologous bone marrow which contains stem cells, unspecialized cells that can differentiate and produce mature osteoblasts and capable of producing bone, for the treatment of long-bone non-unions have reported success rates ranging from 75% to 95.

It would be sensible to wait for any randomized control trials to compare it to the gold standard.

Non-union and delayed union sites have been shown to possess all the components of the BMP-signaling pathway. However, it still remains unclear as to why union does not occur in some cases despite the presence of the BMP-signaling pathway. Some have hypothesized that the levels of the BMPs and/or their receptors at the sites of delayed unions and non-union might be suboptimal while others believe that BMP inhibitors like noggin which bind to the same receptors as BMP might play a role.

Regardless of the micro-environment, it is well established that exogenous BMPs can drive osteogenesis or chondrogenesis and they will undoubtedly play an instrumental role in bone healing at non-union and delayed union sites. BMP may appear a cost effective tool in the surgeon’s armamentarium since it reduces the number of secondary interventions and sickness payment expenses, however, a sound economic
model is needed to assess the cost-effectiveness and budget impact of rh-BMP. At the present time, two rh-BMPs and associated carrier/delivery systems have received FDA approval are available in the market (Table 1).

OP-1 has received FDA approval through the Humanitarian Device Exemption process.

Conclusion

Based on level one evidence, OP-1 is as effective as autologous bone-grafting and offers the advantages of eliminating donor site morbidity and reducing the risk of infection at the recipient site. Therefore, OP-1 is a reasonable alternative to autologous bone-grafting in the treatment of long bone non-unions and the decision to use it should be left to the discretion of treating physicians.

Non-unions and delayed unions are one of the most difficult scenarios encountered in orthopaedic practice. Judicious use of BMP in certain clinical scenarios can revolutionize management of non-unions and delayed unions. The major constraints for routine use of BMP are inadequate clinical trials in humans and the need for further evaluation of cost benefit in various clinical situations. The expression and activation of BMPs from the surrounding tissues and their participation in the quite complex mechanisms of healing have just started to be understood. Future preclinical and clinical research must clarify issues regarding the relative effectiveness of BMPs, the interaction between BMP sub-types, on delayed unions, non-unions and bone defects and the characteristics of responding cells in much greater detail. BMPs have great clinical potential, but in the next decades we will have to determine whether there is a single pathway to efficiently treat non-unions or whether different clinical situations require different formats. Other emerging technologies and modalities, like gene therapy and or percutaneous techniques like autologous bone marrow grafting, have to be assessed in the future to individualize non-union scenarios and treatment options.

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