Bone morphogenetic protein in spinal fusion: overview and clinical update

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The widespread use of fusion procedures in the management of spinal disorders has led investigators to explore the use of growth and differentiation factors in such procedures. As an adjuvant to allograft bone or as a replacement for harvested autograft, bone morphogenetic proteins (BMPs) appear to improve fusion rates after spinal arthrodesis in both animal models and humans, while reducing the donor-site morbidity previously associated with such procedures. The use of recombinant genetic technology in the production of BMP has improved the efficiency, cost effectiveness, and safety of producing and using such materials. Recombinant human BMP-2 (rhBMP-2), as one of the first factors identified in the process of endochondral bone formation, has been extensively researched over the past decade. The efficacy and dose profile of this differentiation factor in the context of various carrier substrates has been investigated. Based on the encouraging results of preliminary studies, the future role of rhBMP-2 may lie in its replacement of autologous bone grafting and, consequently, the reduced need for instrumented fixation, while concurrently improving overall fusion rates. The authors provide an overview of BMP and review its use in clinical and laboratory settings.

KEY WORDS • bone morphogenetic protein • spinal fusion • osteogenesis

MOLECULAR BIOLOGY

Each of the six known osteoinductive BMPs shares significant molecular similarities. Synthesized within the cell in precursor form, each molecule has a hydrophobic leader or secretory sequence with the mature portion of the protein at the carboxy terminus marked by a highly conserved, seven-cysteine repeat. Each mature BMP begins as two monomers of 120 amino acids each, which undergo disulfide linkage dimerization to form either a homologous or a heterologous protein chain. In the case of BMP-2 and BMP-7, the specific structure was first identified by isolating the bovine protein from bone extracts. Oligonucleotide probes were then used to obtain the human cDNA sequence. The cDNA clones were then spliced into a viral expression vector and transfected into a carrier cell in a process called recombination. In the case of BMP-2, the cells used were Chinese hamster ovary cells, which produce the pure recombinant differentiation factor rhBMP-2 in large quantities in a process similar to fermentation. This process avoids potential complications related to the transmission of infectious materials from human donor bone tissue and eliminates the possibility of xenograft interactions with human recipients of BMP derived from bovine sources.

OSTEOINDUCTIVE ROLE OF BMP

To function as a suitable graft for bridging bone defects or fusing fracture lines and unstable motion segments, the prospective material would ideally possess three charac-
characteristics. First, the material would provide a source of primitive osteoprogenitor cells that, under the appropriate influence, would form osteoblasts and osteocytes (osteopromotion). Such precursor cells are, unfortunately, relatively scarce. Bone marrow, for example, contains only one osteoprogenitor cell to approximately 50,000 nucleated cells in a young adult. This ratio may drop to 1:200,000 cells in an elderly individual afflicted by degenerative spinal disease. Despite techniques to concentrate marrow extracts, successful efforts have only resulted in a fivefold increase of the unfavorable cellular ratio. Second, the graft material would produce local growth factors to stimulate bone growth and vascularity in the area (osteoinduction). There are numerous reports in which investigators detail the complex interaction of various autocrine and paracrine growth factors released from fibroblasts, platelets, and even local hematoma at the site of injury. Finally, the third property of the graft material would be its ability to act as a scaffold for bone ingrowth (osteocoinduction).

The osteoinductive role of BMP is multifaceted. Bone morphogenetic protein acts as a chemotactic agent, a growth factor, and a differentiation factor. As a chemotactic factor, it initiates the recruitment of progenitor and stem cells toward the area of bone injury. Analysis of in vivo studies of the local effects of BMP indicates an initial migration of mesenchymal stem cells to the area of implantation far in excess of that supplied by bone marrow grafting. As a growth factor, it stimulates both angiogenesis and the proliferation of stem cells from surrounding mesenchymal tissues. As a differentiation factor, it promotes maturation of stem cells into chondrocytes, osteoblasts, and osteocytes.

Some cells respond to the growth factor aspect of BMP by altering their rates of proliferation. Yamaguchi et al. have demonstrated this in vitro by quantifying cellular proliferation of the rat C26 calvarial osteoprogenitor cells after treatment with BMP-2. This BMP-2 effect, however, appears to maintain specificity for certain cell types. For example, although BMP-7 has been shown to be mitogenic for a human osteosarcoma cell line (TE-85), treatment with BMP-2 showed no measurable effect on proliferation. In contrast, treatment of an osteoblast cell line (MC3T3-E1) with BMP-4 results in a growth inhibition and a globally diminished proliferative index. Because of their effects on both mature and immature cell types, the complex interaction of BMPs clearly must be involved in the regulation of bone growth and maintenance.

Bone morphogenetic proteins also may initiate the differentiation of stem cells into a specific phenotype. For example, the rat calvarial stem cell line (C26) is considered multipotential, as such cells may be precursors for adipocytes, muscle cells, or osteoblasts. When BMP-2 is added to the culture medium, such cells become mature osteoblasts with increased surface expression of receptors for parathyroid hormone, alkaline phosphatase, and calcitonin. This effect may also be observed in bone marrow cells. For example, the mouse line of marrow cells (W-20-17) may differentiate into either adipocytes or osteoblasts, depending on the specific hormonal influence. Treatment of such cells with BMP-2 results in both the differentiation of the cells into osteoblasts and the surface expression of receptors normally seen on mature cells.

**SOURCES OF BMP**

At present there are three ways to obtain growth and differentiation factors: extraction of the factors from animal or human bone matrix, production of a single factor by cellular hosts by using recombinant technology, and direct delivery to cells at the site of desired bone formation of the DNA encoding for the factor.

The first of these was initially used by Urist and colleagues. From massive quantities of bovine bone, the group was able to extract a mixture of proteins found to stimulate bone growth in vivo. Commercially available today as NeOsteo (Sulzer Spinetech, Wheat Ridge, CO), this mixture of BMPs is derived through a well-engineered process of isolation from bovine sources. The precise combination of factors comprising this substance has not been fully characterized, but it appears to be reproducible through the manufacturer’s process. This substance has shown promise in bridging both segmental skeletal defects in dogs and in inducing spinal fusion in animal models of posteroateral arthrosis. Like other growth and differentiation factors, BMPs require a carrier substance to maintain adequate concentrations at the site of fusion. Substances such as natural coral, collagen, and calcium sulfate have been investigated. The second method of obtaining bone growth and differentiation factors has previously been discussed (see Molecular Biology). The process of obtaining rhBMPs such as rhBMP-2 (Medtronic Sofamor Danek, Memphis, TN, and Genetics Institute, Cambridge, MA) and rhBMP-7 (Stryker Biotech, Hopkinton, MA) has been described. Such proteins differ from mixtures of extracted substances mainly in terms of purity of product. In original studies of these substances the authors focused on animal models of segmental bone defects in the appendicular skeleton of rats, sheep, and dogs. With considerable success, studies have compared rhBMP in a carrier with autologous grafting in a skeletal defect model. Gerhart et al. showed the utility of rhBMP in healing segmental femoral defects in sheep. Shortly thereafter, recombinant BMP was applied in animal models of spinal fusion and, later, in humans.

The third strategy for engineering bone formation involves gene therapy, or the delivery of the appropriate gene or cDNA encoding for BMP to the local cells, rather than the actual factor. There are two obvious benefits to this strategy compared with recombinant technology. First, the cost of genetic manipulation is significantly less than that required to produce and market the purified rhBMP. Second, the potential for prolonged local production of the factor is greater when using gene therapy compared with the relatively short-lived effect of the rhBMP/carrier complex. Attempts to introduce BMP-2 cDNA into animal models are preliminary but have met with limited success.

**EXPERIMENTAL EVIDENCE**

Analysis of early work in the isolation of proteins with osteoinductive activity suggested that BMP-2 and BMP-7 were primarily responsible for the effects observed in vivo. As a result, rhBMP-2, produced in a Chinese
hamster ovary cell line, was the first of these molecules studied in detail. Implantation of the recombinant factor in a rat model resulted in ectopic bone formation with a dose–effect relationship temporally identical to that of bone-derived extracts; however, the amount of pure rh-BMP-2 required to induce formation of a given amount of bone was approximately 10-fold greater than that required of the bone extract.5,13,14,45 Such results implied an in vivo synergy in the mixture of differentiation factors obtained through the extraction process.

The ability to produce bone at ectopic sites, however, had little application in current spinal fusion techniques. Realizing the limitations inherent in autogenous and allogeneic bone grafting, investigators began applying BMP technology to animal models of spinal fusion.25,31,29,30,33,34,44,48,54,55,60 The authors of numerous studies, involving various concentrations of BMP in a variety of carrier substrates, have shown remarkable results. Early work by Boden and colleagues,12–14 Holliger and associates,39 and Schimandle, et al.,54 compared rhBMP-2 with autologous bone graft in a rabbit posterolateral lumbar fusion model. Remarkably, all BMP-treated animals attained solid fusions across the operated level that were biomechanically stiffer and stronger than the autograft-only fusions observed in 42% of the control animals. In similar studies in a canine model the authors have also confirmed the efficacy of rhBMP-2 in producing mature fusion masses.25 The canine study published by David, et al.,25 demonstrated a dose dependence to the BMP effect, with greater concentrations producing greater effects; however, this finding contradicts work reported by Sandhu, et al.,51–53 in a similar canine model, which showed BMP to be more effective than autologous bone graft but in a dose-independent manner.25 Most investigators developed the opinion that bone induction was a relatively simple process in lower species but was only indirectly applicable to human models. Consequently, research focused on developing spinal fusion models in primates. As a developmentally higher species, primates provide a more realistic test environment for evaluating the effectiveness of BMP.36,37 With this in mind, Boden, and coworkers10 studied a nonhuman primate model of intertransverse spinal fusion and demonstrated effective fusion rates after delivery of rhBMP-2 on a collagen sponge carrier via a minimally invasive approach (Fig. 1). Sandhu and colleagues53 went a step fur-
ther, demonstrating clinically, mechanically, and radiographically equivalent rates of rhBMP-2–induced spinal arthrodesis without having to decorticate the prospective fusion bed. Boden and colleagues,7,15,45 by adding rhBMP-2 to autograft, were able to demonstrate subsequent induction of BMP-6, osteocalcin, and collagen within the graft itself.

In posterolateral fusion models some researchers attempted to replace autograft with a BMP/carrier complex, but internal fixation was still required. Attention was then focused on interbody spinal fusion techniques, which could possibly obviate the need for both autograft and fixation.18,31,41,42,50,56,57 Zdeblick, et al.,64 performed three-level anterior cervical fusions in goats; they used a BAK cage filled with either local autograft or a collagen-imregnated rhBMP-2 sponge.43,64 In three of seven animals in the autograft group there was radiographic evidence of pseudarthrosis, whereas in none of the BMP group did failure occur. The biomechanical stiffness of the BMP construct was equal to that of an autograft/cervical-plated level. Boden and associates8 performed the same procedure in the lumbar spine of primates: rhBMP-2 on a collagen carrier in both titanium threaded interbody cages and threaded bone dowels was delivered laparoscopically; fusion rates were improved over empty and autograft-filled cages (Figs. 2 and 3).

Preliminary results obtained in clinical studies have been encouraging. In a recent report of a randomized, prospective controlled clinical pilot study, Boden and colleagues8 demonstrated that solid bone fusions had been achieved, by both clinical and radiographic criteria, in 11 patients who underwent anterior lumbar interbody fusion procedures in which they placed a tapered, threaded titanium cage filled with a rhBMP-2–impregnated collagen sponge (Fig. 4). Pain scores, as documented by the Oswestry Disability and the Short Form–36 questionnaires, improved concomitantly as fusion progressed.8,27,35,46,50,58,61

Fig. 3. Images demonstrating bone dowel interbody device implanted into the lumbar spine of a primate. The control group received a dowel with autograft. The experimental group received a bone dowel with an rhBMP-2–impregnated sponge placed inside. The images show the actual spines at 6 months postsurgery. A solid fusion is shown in one control animal (upper left), and resorption of the bone dowel/autograft construct is shown in another control animal (lower left). Mature bone bridging the interspace with resorption of the dowel is shown in both animals that received a bone dowel treated with BMP-2–impregnated sponge (upper and lower right).

Fig. 4. Computerized tomography reconstructions obtained in a patient who received a threaded titanium cage with rhBMP-2 on a collagen sponge carrier. Upper: Sagittal scans of the left cage at 6, 12, and 24 months. Center: Scans obtained at 6, 12, and 24 months. Lower: Coronal scans obtained at 6, 12, and 24 months. All scans demonstrate fusion with increasing bone density over time.
Overview of bone morphogenetic protein

SUMMARY

Over the past decade research has shown the utility of using the differentiation factor BMP-2 to promote bone growth at the site of bone loss or injury. The in vivo roles of BMP-2 and its complex interactions with other growth and differentiation factors remain to be clarified. The use of BMP-2 as a means of replacing harvested autograft and obviating the need for internal fixation, each with its attendant morbidity, appears likely, based on results obtained in both animal and human studies. Although dose relationships and carrier substrates may provide continued investigational challenges, the use of recombinant technology and gene therapy in the field of bone fusion have been firmly established. It seems unlikely that Marshall Urist, who first first coined the term bone morphogenetic protein, could have envisioned the monumental strides and clinical progress researchers in the field have achieved over the past 30 years.

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References
