Bone morphogenetic proteins in orthopaedic surgery

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Summary
Bone morphogenetic proteins (BMPs) are growth factors which induce new bone formation. They are an increasingly important adjunct in the treatment of certain musculoskeletal disorders. Their underlying basic science and role in bone healing is explained. Delivery systems, safety issues and current evidence-based clinical applications of BMPs in orthopaedic surgery are described.

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Introduction

In 1965 Urist reported that intramuscular injection of demineralised bone matrix elicited new bone formation. This led to research into a family of growth factors known as "bone morphogenetic proteins" (BMPs). These are proteins that act as cellular signaling agents and BMPs play a crucial role in cell signaling in bone growth and healing. When they bind to their mesenchymal cell surface receptors, they activate a signaling cascade to the cell nucleus. Genes are then expressed leading to synthesis of macromolecules involved in bone/cartilage repair, and the mesenchymal cell differentiates towards the chondrocyte or osteoblast phenotype.

BMPs are of interest to the orthopaedic surgeon for many reasons. In the treatment of tibial nonunion, BMPs have been shown to be equally effective as allograft, possibly obviating the need to harvest allograft. BMPs are also becoming an important adjunct in treatment of segmental defects, fresh fractures, cartilage, and intervertebral disc repair, allograft incorporation, impact bone grafting, spinal fusion, acceleration of regenerate ossification in callotasis, and enhancement of tendon incorporation in ligament reconstruction. Hence orthopaedic surgeons should understand the basic science to utilise their full therapeutic potential.

Osteoinduction, osteogenesis and osteoconduction

- Osteoinduction implies the recruitment of immature cells and the stimulation of these cells to develop into preosteoblasts, leading to de novo bone formation in—usually—mesenchymal tissue.
- Osteogenesis is new bone formation from osteocompetent cells in connective tissue or cartilage.
- Osteoconduction is the process of bone formation on a three-dimensional implant or graft through ingrowth of capillaries, mesenchymal tissue and osteoprogenitor cells from the recipient host.
**Basic science**

Osteoinduction was initially thought to be due to one protein in the bone matrix, i.e. "BMP". However more than 40 BMPs have been identified to date. Most are distinguished by their potential to induce new bone formation. Of these, recombinant human (rh) BMP-2 and rhBMP-7 (also known as Osteogenic Protein-1 or OP-1) appear to be the most effective, and are the only ones that have currently been developed for clinical use.

BMPs are part of the transforming growth factor-β (TGF-β) superfamily, a group of growth factors that play an important role during embryogenesis and postnatal tissue repair. Within this group, BMPs appear to be the most selective for, and have the greatest effect upon, osteogenesis. They are dimeric molecules with two polypeptide chains of over 400 amino acids linked by a single disulphide bond and on X-ray crystallography exhibit a characteristic cysteine knot. Genes expressing BMPs 2, 7 and 14 are located on chromosome 20.

**BMP in the bone morphogenesis cascade**

Expression of BMPs is a complex intra- and extracellular signaling process which is variable throughout the bone morphogenesis cascade. BMPs 2 and 4 are expressed early by primitive mesenchymal stem cells, and throughout the cascade. BMP-2, 6, and 9 are involved at the early stage of differentiation of mesenchymal progenitor cells to pre-osteoblasts. BMP-7 is expressed by osteogenic cells from day 7, peaking at 2–4 weeks. BMPs 2, 4, 6, 7 and 9 increase osteocalcin expression and alkaline phosphatase expression in pre-osteoblasts, leading to mineralisation. BMP-3 has been shown to be osteoinductive, but may also be inhibitory in the presence of BMP-2 and 7, acting as a negative regulator of bone formation.

Animal and human studies of BMPs demonstrate a typical sequence of events in bone morphogenesis; chemotaxis and mitosis of mesenchymal cells, differentiation of these cells into cartilage, and replacement of cartilage with bone. What initiates this cascade is unclear, but it has been hypothesised that plasma fibronectin bonds to implanted demineralised matrix, facilitating recruitment and proliferation of mesenchymal cells (maximal on day 3). The mesenchymal cells differentiate into chondrocytes (days 5–8) which then hypertrophy. Calcification of the cartilage matrix ensues (day 9). Angiogenesis and vascular invasion occurs simultaneously with differentiation of osteoblasts and bone formation (days 10–11). Finally the newly formed woven bone remodels into trabecular and cortical bone and bone marrow formation occurs. In a callus this remodelling is not homogeneous and depends on ‘zonal ossification’—broadly speaking the medul lary and inter-cortical areas create ‘soft callus’ and enchondral ossification occurs, whilst the subperiosteal area and soft tissues surrounding the fracture form ‘hard callus’ and bone through intramembranous ossification.

**BMP receptors and Smads**

Only certain cell types are able to respond to BMPs. Their actions are mediated via specific BMP receptors that activate intracellular messenger proteins called Smads. The activated Smads are then translocated to the cell nucleus, activating BMP responsive genes, resulting in transcription of macromolecules involved in bone and cartilage formation.

**Clinical applications of BMPs**

The best evidence for the clinical use of BMPs is from randomised, controlled trials involving BMP-2 and BMP-7, which show that BMP-7 is at least as effective as autograft in treatment of nonunion. BMP-2 enhances healing of open tibial fractures, and BMP-2 is at least as effective as autograft used in anterior interbody spinal fusions in degenerative disc disease. Other BMPs have not been subject to this level of evidence testing, nor have other growth factors. Evidence for other clinical applications is taken from smaller uncontrolled observational trials, and animal models.

**Potential applications of BMPs in bone**

**Treatment of non-union**

In 2001, a prospective randomised controlled multicentre trial compared rhBMP-7 (also known as OP-1) with fresh bone autograft in 122 patients with 124 tibial nonunions. At 9 months, 81% of the rhBMP-7 group and 85% of the autograft group achieved clinical union, and 75% of the rhBMP-7 group and 84% of the autograft group were deemed to have united radiologically. There was no statistical significance in outcome between the two groups at 2 years. The authors concluded that rhBMP-7 was equally effective to autograft in the treatment of tibial nonunion. However 20% of those treated with autograft experienced chronic donor site pain, and the incidence of postoperative osteomyelitis was significantly greater in the autograft treated group (21%) compared to the rhBMP-7 treated group (3%). On the basis of these data, the United States Food and Drug Administration (FDA) licensed rhBMP-7 for use as an alternative treatment to autograft in long bone nonunions.

An observational, non-randomised study assessed clinical application of rhBMP-7 in 653 patients treated for a variety of conditions across the UK. Of these patients, 395 (60.5%) had a fracture nonunion. Mean time from initial injury to BMP-7 application in this nonunion group was 19.7 months, and mean number of procedures performed previously was 3.5. The authors showed an overall success rate of 82% clinically and radiologically using rhBMP-7 in a wide variety of clinical scenarios.

An observational study of treatment of femoral nonunion using allograft and partially purified human BMP demonstrated that 24 out of 30 femoral nonunions healed at an average of 6 months (follow up 55 months). In 26 of these
femurs, a one stage lengthening technique was used to correct shortening deformity.24

Healing of fresh fractures

In 2002, the BESST study group7 compared treatment of 450 patients with open tibial fractures using intramedullary nail fixation alone, or with 6 mg (0.75 mg/mL) or 12 mg (1.5 mg/mL) of rhBMP-2 in a prospective, controlled, randomised trial. Randomisation was stratified according to the severity of the open fracture. The main outcome measure was whether a patient required secondary intervention within 12 months due to delayed union or nonunion. Ninety-four percent (n = 421) of patients were followed up at 12 months. The group receiving the higher dose of rhBMP-2 (12 mg) had a significantly greater rate of healing, fewer infections and invasive interventions and lower rate of nonunion compared to the control group.

Autograft substitute in spinal surgery

RhBMP-2 was licensed for use by the United States FDA for single-level interbody fusions of the lumbar spine in 2001. This decision was based upon data from a prospective, randomised, non-blinded multicentre study of 279 patients with degenerative lumbar disc disease undergoing anterior interbody fusion with use of two tapered threaded fusion cages.14 The control group received autograft (n = 136) whilst the treatment group (n = 143) received rhBMP-2 on a collagen sponge. Radiologically fusion rates were higher for the rhBMP-2 group compared to the autograft group at 24 months (94.5% vs. 88.7%) while clinically outcome was similar in both groups.

More recently a prospective, randomised, multicentre study of 131 patients compared the use of rhBMP2 with autograft cortical struts (ACS) (n = 79) against autograft (n = 52) in anterior lumbar spinal arthrosis.29 Radiologically the patients receiving rhBMP-2/ACS achieved significantly higher rates of fusion, and improved functional disability scores compared to the autograft group. The authors concluded that rhBMP-2/ACS is equally effective as autograft and avoids the morbidity associated with autograft harvest.

Pilot clinical trials have all taken place comparing use of rhBMP-2 to autograft in posterior lumbar interbody fusion, anterior cervical discectomy and fusion and posterolateral lumbar fusion, and await further study.30

Healing of segmental/critical-sized defects (CSDs)

A CSD is the smallest sized defect in a bone, which will not heal spontaneously. CSDs have been established in animal models using long bone, cranial and mandibular defects. Studies of healing of CSDs have been carried out using BMP-2 in the rat femur and BMP-7 in the canine ulna with promising results.5,6 However direct comparisons of humans with animal models should be made with caution.

Impaction grafting/ primary and revision arthroplasty

Several observational studies in animal models have evaluated the effect of BMPs upon osteoconduction. In a canine model, uncemented total hip arthroplasties were performed on 15 dogs.31 A 2 mm deep artificial defect was created behind the acetabular component to mimic bone loss. Five dogs received rhBMP-2 in a carrier (xBSM), five dogs received xBSM alone, and five were controls. At 12 weeks the control group showed no bone formation in the defect, whereas the rhBMP-2 group showed bone formation and filling of the defect.

In another study using a canine model (n = 28), 3 mm defects were created between a titanium implant and the proximal humerus.13 These defects were treated with rhBMP-2 alone, rhTGF-β/2 alone, both rhBMP-2 and rhTGF-β/2, or autogenous grafting. The greatest implant strength was obtained using combined rhBMP-2 and rhTGF-β/2 and was equivalent to autogenous grafting alone.

Treatment of congenital pseudo-arthritis of the tibia

Congenital pseudo-arthritis of the tibia is a rare disorder which is difficult to treat and often has an unsatisfactory outcome. In 2006 an isolated case report described usage of BMP-7 in treatment of this disorder in a 13-year-old child. He had been treated unsuccessfully with nine previous operations. Intramedullary nailing was performed using autograft and 3.5 mg of BMP-7. Bony union was noted at 5 weeks, and full weight bearing allowed at 5 months. Fracture union did not occur until 28 months, and despite a 5 cm leg length discrepancy, he and his family were reportedly satisfied with the result.32

A case series of five children with congenital pseudo-arthritis of the tibia treated with rhBMP-7 (3.5 mg combined with allograft) was also reported in 2006. There was evidence of radiographic union in only one out of the five cases (follow up 12–18 months). The authors concluded that use of BMPs alone may not be enough to overcome the poor healing environment associated with congenital pseudo-arthritis of the tibia.33

Treatment of avascular necrosis of the femoral head

A retrospective analysis was performed on 15 patients involving 17 hips with avascular necrosis of the femoral head.34 Fifteen of the hips were classified at Ficat Stage IIA. Treatment consisted of core decompression combined with allograft and 50 mg of partially purified BMP. Average follow up was 53 months. Fourteen of the 15 Ficat Stage IIA hips treated were judged clinically and radiologically to be a success.

Potential applications of BMPs in musculoskeletal tissue

Cartilage repair

In vitro and in vivo studies have demonstrated that BMPs are required for chondrocyte differentiation and regulation of
SOX proteins required for type II collagen expression during chondrogenesis.\textsuperscript{35} In a rat model, BMP-4 delivered locally through genetically engineered muscle-derived stem cells (MDSCs) has been shown to enhance chondrogenesis and improve repair of articular cartilage.\textsuperscript{8} In a canine model rhBMP-7-induced hyaline cartilage like repair of full-thickness osteochondral defects.\textsuperscript{9}

Degenerative intervertebral disc repair

A number of studies in different animal models have evaluated the role of BMPs, in particular BMP-7, in treatment of degenerative disc disease. Injection of BMP-7 into iatrogenically compressed rat intervertebral disc has been shown histologically to enhance discal extracellular matrix and clinically to inhibit pain-related behaviour.\textsuperscript{10}

Disc degeneration was iatrogenically created in a rabbit model (\(n = 90\)) by puncture of intervertebral discs with an 18 gauge needle. Radiographic evaluation showed induction of disc degeneration at 4 weeks. Following injection with BMP-7 into the disc, histological and radiographic evaluation showed significant improvement in disc height at 6 weeks compared to a control group, which remained until end of follow up at 24 weeks.\textsuperscript{36}

BMP specific antagonists

Over expression of BMP pathways has been implicated in heterotopic bone formation in fibrodyplasia ossificans progressiva (FOP), for example in the posterior longitudinal ligament of the spine.\textsuperscript{37} BMP specific antagonists exist (e.g. ‘noggin’, ‘gremlin’) which bind to BMPs themselves.\textsuperscript{38,39} In a mouse model, under-expression of noggin, the extracellular BMP antagonist, has been shown to result in heterotopic ossification in FOP in vivo.\textsuperscript{40} It has been hypothesised that BMP specific antagonists may be of therapeutic value in treatment of pathologies with excessive bone formation.

BMPs are pleiotropic

BMPs also have actions upon development of nonmusculoskeletal tissue. Knockout of BMP genes in mice have resulted in developmental defects in the heart (BMP-2), eye and kidney (BMP-7).\textsuperscript{31,42}

Delivery

When the osteoinductivity of individual recombinant human BMPs (rhBMPs) was discovered, it was found that this follows a dose-response ratio\textsuperscript{43} and that the dose of BMPs must reach a threshold value before osteoinduction can occur. It followed that local concentration of BMPs was the most important factor, more than patient characteristics or total dose. As systemic clearance of BMPs is high, local application of BMPs is essential.

From primate models, it has been estimated that the human therapeutic dosage is 0.88 mg/mL of sterile saline solution for rhBMP-7, and 1.50 mg/mL of sterile water for rhBMP-2.\textsuperscript{44} The concentrations of BMPs used in current clinical applications are several orders of magnitude greater than BMPs occurring naturally in the body (e.g. 3.5 mg in a single dose of BMP-7 vs. several nanograms). The expense of BMPs may be a prohibitive factor for combination therapies of BMPs and means that development of efficient, cost-effective delivery systems remains all important, until the complex cascade of fracture healing is better understood and can be replicated.

Several methods of delivering BMPs have been tried, e.g. direct application of the recombinant protein to the regeneration site by injection, with or without a carrier such as bone matrix or formulation buffer, local vs. systemic application, and gene therapy, in which a protein is delivered indirectly by its encoding gene.

Direct application of rhBMPs

BMP in a buffer solution may be lost to surrounding tissue, but by suspending BMP in a carrier it may possible to retain the agent in the required location for a longer time. Combinations of BMPs with carrier vehicles such as gelatin foam, collagen or calcium phosphate directly applied to a regeneration site give BMP retention rates of 15–55%, compared to <5% retention using a buffer delivery system alone.\textsuperscript{45} One disadvantage of solid carriers is that they require an open procedure whereas injectable carriers of BMPs are easier to administer and preferable where open surgery would not otherwise be necessary (e.g. closed treatment of fractures).

Gene therapy

Gene therapy is still at an early stage, with promising results in animal models.\textsuperscript{46} Either genes expressing BMPs can be delivered to the regeneration site directly resulting in transfection of cells and protein expression (in vivo transduction), or through transfection of cultured cells (in vitro transduction) which are then implanted and express the required protein. Advantages such as percutaneous delivery, relatively low cost of manufacture and high local concentration must be weighed against immunogenicity, inflammatory response, and variation of gene expression.

Safety of BMPs

The concentration of BMPs used in clinical application is often at least four to six orders of magnitude greater than the concentration of naturally occurring BMPs. Ectopic bone formation and overgrowth has been reported after the use of extremely high BMP doses.\textsuperscript{43} This appears to be regulated by the limited number of mesenchymal stem cells which express BMP receptors, as well as osteoclastic activity stimulated by high doses of BMPs.\textsuperscript{47} However, using current therapeutic doses, there appears to be a relatively small risk of excessive bone formation, and it is usually eventually remodelled to the natural contour of the bone.\textsuperscript{48}

Systemic clearance of rhBMPs is rapid and human studies have not demonstrated any systemic toxicity so far. Collagen carrier materials have been shown to induce antibody formation in 5–20% of patients in one series,\textsuperscript{7} while antibodies against BMPs developed in 6–10% patients in another.\textsuperscript{4} Currently, use of BMP on more than one occasion in
the same patient is contra-indicated. It is unknown whether immunogenic reactions will occur if higher doses of BMPs or collagen are used in initial applications, or when used again in a previously sensitised patient.

Long-term genetic effects in humans are unknown. No studies have demonstrated any evidence of carcinogenicity. Usage of BMPs during pregnancy is contraindicated, and must be used with caution in children.

Discussion

BMPs show great clinical potential in the treatment of musculoskeletal disorders. However a particular criticism leveled at current usage of BMPs is that the method of using a huge concentration of a single growth factor is a crude (yet effective) tool. BMPs are just one type of growth factor in a complex healing cascade which is not fully understood. Nonetheless single BMPs seem to be able to initiate the normal fracture healing cascade in practice and there is good evidence for the clinical application of BMPs in orthopaedic surgery. In particular BMP-7 has been shown to be of benefit in the treatment of nonunion, whilst BMP-2 is effective in fresh fractures and interbody spinal fusion.

BMPs are not an orthopaedic panacea, and further evidence is required to demonstrate other areas of therapeutic potential as, despite promising results for potential clinical applications in animal models, extrapolation of these results to the human model requires caution and further study. Combinations of BMPs may be more effective. Other growth factors such as fibroblast growth factor and growth hormone have also shown promise in animal models, particularly in tandem with BMPs.

The main problems are to refine delivery methods, optimise delivery time and therapeutic dosages. Greater understanding of the potential applications of BMPs combined with meticulous surgical technique will enhance usage of this therapeutic tool. In future BMPs may also be used in combination with other growth factors, mesenchymal stem cells and gene therapy.

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Bone morphogenetic proteins in orthopaedic surgery


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