

Bone morphogenetic proteins: from structure to clinical use

J.M. Granjeiro¹,
R.C. Oliveira³,
J.C. Bustos-Valenzuela²,
M.C. Sogayar² and R. Taga³

¹Departamento de Biologia Celular e Molecular, Instituto de Biologia, Universidade Federal Fluminense, Niterói, RJ, Brasil
²Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brasil
³Departamento de Ciências Biológicas, Faculdade de Odontologia de Bauru, Universidade de São Paulo, Bauru, SP, Brasil

Abstract

Bone morphogenetic proteins (BMPs) are multi-functional growth factors belonging to the transforming growth factor β superfamily. Family members are expressed during limb development, endochondral ossification, early fracture, and cartilage repair. The activity of BMPs was first identified in the 1960s but the proteins responsible for bone induction were unknown until the purification and cloning of human BMPs in the 1980s. To date, about 15 BMP family members have been identified and characterized. The signal triggered by BMPs is transduced through serine/threonine kinase receptors, type I and II subtypes. Three type I receptors have been shown to bind BMP ligands, namely: type IA and IB BMP receptors and type IA activin receptors. BMPs seem to be involved in the regulation of cell proliferation, survival, differentiation and apoptosis, but their hallmark is their ability to induce bone, cartilage, ligament, and tendon formation at both heterotopic and orthotopic sites. This suggests that, in the future, they may play a major role in the treatment of bone diseases. Several animal studies have illustrated the potential of BMPs to enhance spinal fusion, repair critical-size defects, accelerate union, and heal articular cartilage lesions. Difficulties in producing and purifying BMPs from bone tissue have prompted the attempts made by several laboratories, including ours, to express these proteins in the recombinant form in heterologous systems. This review focuses on BMP structure, molecular mechanisms of action and significance and potential applications in medical, dental and veterinary practice for the treatment of cartilage and bone-related diseases.

Key words

- Bone morphogenetic proteins
- Osteogenic protein
- Protein structure
- Meta-analysis
- Clinical trial

Correspondence

J.M. Granjeiro
Departamento de Biologia Celular e Molecular
Instituto de Biologia, UFF
Outeiro de São João Baptista, s/n
24020-150 Niterói, RJ
Brasil
Fax: +55-21-3701-1617
E-mail: jmgranjeiro@vm.uff.br

Presented at SIMEC 2004
(International Symposium on Extracellular Matrix),
Angra dos Reis, RJ, Brazil,
September 27-30, 2004.

Publication supported by FAPESP.

Received February 23, 2005
Accepted June 16, 2005

Introduction

Since the discovery of bone morphogenetic proteins (BMPs) as bone inductive proteins by Urist (1), many investigators have shown that BMPs induce stem and mesenchymal cell differentiation into osteogenic

cells capable of producing bone. Modern molecular biology theories state that BMPs are morphogenetic proteins, namely, molecules which induce the genome to initiate the formation of a morphogenetic area. BMPs diffuse through a concentration gradient, thereby altering the developmental process.

In response to this stimulus, the cells proliferate and differentiate following a pre-defined pattern and spatial arrangement. From a physical and chemical point of view, BMPs are proteins secreted by cells, which act as ligands for receptors present on the plasma membrane of different types of cells (autocrine and paracrine effects), thus establishing cell and tissue organization.

The general role of BMPs in the process of bone formation during the development and repair of fractures has been well established. BMPs are capable of inducing the formation of bone tissue in ectopic sites and in critical-sized bone defects in several animal models. Until recently, however, little was known about the cellular and molecular mechanisms involved in the ectopic ossification induced by BMPs.

Therefore, the objective of the present review was to clarify some of the basic aspects of the cellular and molecular mechanisms of action of BMPs, presenting some potential clinical applications and perspectives for their utilization.

Structure of bone morphogenetic proteins

The study of BMPs began in the 1960s, with the observation that demineralized bone matrix had the capacity to induce endochondral bone formation in subcutaneous and intramuscular pockets in rodents (1,2). This research group subsequently isolated a low-molecular weight glycoprotein from bone and demonstrated that it promoted bone formation when ectopically located (2).

Demineralized bone matrix is the biomaterial of choice for isolation and purification of BMPs, even though these molecules are also present in dentin (3). At the end of the 19th century it was demonstrated that decalcified bovine bone could be used for the treatment of osteomyelitis. In the middle of the 20th century Lacroix raised the hypothesis of the inductive role of bone, naming

it osteogenin. Some years later, Urist (1) transformed the concept of bone repair after accidentally finding that rabbit demineralized lyophilized bone matrix was able to promote new bone formation when implanted into the musculature.

Induction of ectopic bone formation has been consistently demonstrated with the use of rabbit, canine, bovine, and native human xenogenic demineralized bone matrix implanted in mice. However, rat allogenic matrix and native BMPs induce ectopic ossification in rats but not in other species (4). Therefore, restrictions concerning animal species and their responses to BMPs, probably of an immunological nature, must be considered. Another method of demonstrating the inductive ability of BMPs or demineralized bone matrix is their implantation in intramembranous bone defects. Evidence for repair through endochondral ossification confirms the inductive effect of these materials.

Bone morphogenetic proteins belong to the transforming growth factor β (TGF- β) superfamily. These proteins are synthesized as large precursor molecules. After dimerization, these proteins are cleaved proteolytically at a consensus Arg-X-X-Arg site to generate mature dimers. It has been shown that the N-terminal region controls the stability of the processed mature protein and that the downstream sequence adjacent to the cleavage site determines the efficiency of cleavage (5).

BMPs consist of dimers whose chains are connected by disulfide bonds, and this dimerization is a prerequisite for bone induction. BMPs are active both as homodimer (two identical chains) and heterodimer (two different chains) molecules. Fifteen BMPs have been identified and divided into subfamilies according to similarities in amino acid sequence. Also, they are glycoproteins of relative low molecular mass. More specifically, a BMP is a dimeric molecule with two polypeptide chains held together by a single

disulfide bond, and a primary structure 40 to 50% similar to that of TGF- β . In fact, confirmed aligned segments of common sequences include BMPs as part of the TGF- β superfamily (6). However, TGF- β are found in the bone matrix in higher amounts than BMPs, being classified as cytokines rather than morphogenes. Hence, while BMP-2 induce or enhance the expression of alkaline phosphatase and osteocalcin (osteoblast differentiation markers), TGF- β 1 dramatically inhibit the expression of osteocalcin and the activity of alkaline phosphatase (7).

The three-dimensional structure of BMP-7 osteogenic protein 1 (OP-1) was solved (7,8), showing that, although there is limited sequence identity between OP-1 and TGF- β 2, they share a common polypeptide fold. The identity of OP-1 and TGF- β is less than 38%, but it is 60 and 85%, respectively, relative to BMP-2 and BMP-5. The monomer presents three disulfide bonds, the cysteine knot constituting the monomer core, and four strands of antiparallel β -sheet, which emanate from the knot forming two finger-like projections (Figure 1). Tabas et al. (9) confirmed the assignment of the BMP-2A gene to chromosome 20.

The signaling cascade of bone morphogenetic proteins

Osteogenesis comprises a sequential cascade with three critical phases: migration and mitosis of mesenchymal cells, differentiation of mesenchymal cells into chondroblasts, cartilage formation and, finally, substitution of cartilage by bone. These sequential events are triggered by the binding of plasma fibronectin to the demineralized bone matrix, enhancing adhesion and proliferation of mesenchymal cells at 3 days after implantation. Chondrogenesis is observed after 5 days, reaching its peak at 7-8 days. Cartilage hypertrophy and mineralization are observed after 9 days. Osteoblast differentiation depends on angiogenesis and the high-

est level occurs after 10-11 days (10). Sequentially, the newly formed endochondral bone is remodeled and becomes a hematopoietic site. The sequence of morphogenetic events in response to the demineralized bone matrix mimics the initial events of skeletal morphogenesis in embryos and of bone repair in adults.

Accordingly, the key signals for bone morphogenesis have been identified (Figure 2). The BMP, as a signaling molecule of the TGF- β superfamily, binds to a type II specific receptor present on the cell membrane and recruits a type I receptor, forming a complex. These receptors are transmembrane serine/threonine kinase proteins that self phosphorylate after the formation of the BMP-receptor II-receptor I complex and acquire the ability to phosphorylate Smad proteins, a family of TGF- β transducers. Smads are a family of signaling mediators of BMP receptors in vertebrates homologous of Mad (mothers against decapentaplegic, in *Drosophila*) and Sma (related to Mad in *C. elegans*) and can be classified into three subtypes by structure and function, i.e., receptor-regulated Smads (R-Smads), common-mediator Smads, and inhibitory Smads. R-Smads are phosphorylated by activated serine/threonine

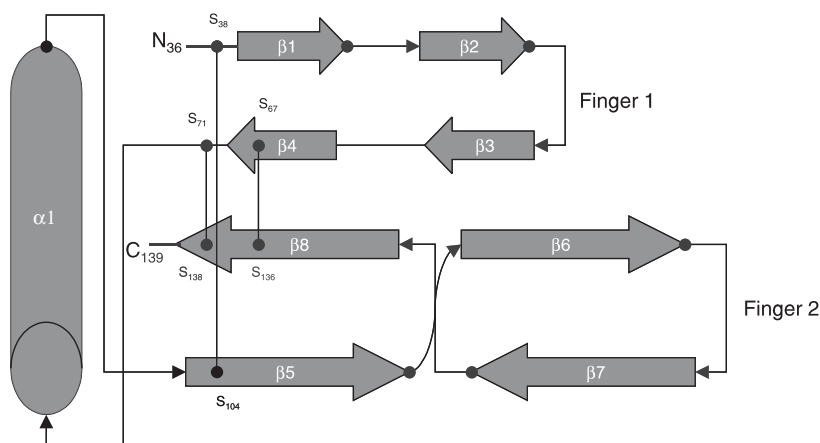


Figure 1. Schematic diagram of bone morphogenetic protein 7 (OP-1/BMP-7). The monomer is stabilized by three disulfide bonds, i.e., Cys-67-Cys-136 and Cys-71-Cys-138 forming a ring through which the third, Cys-38-Cys-104, passes. The cysteine knot constitutes the monomer core from which four strands of antiparallel β -sheets emanate, forming two finger-like projections. Adapted from Ref. 8, with permission.

kinase receptors (BMP-receptor II-receptor I complex). R-Smads interact with common-mediator Smads to form hetero-oligomeric complexes, which then translocate into the nucleus and regulate the transcription of various target genes (for a review, see Ref. 11). It is not clear whether Smads can recognize specific binding sites and bind to DNA by themselves.

Transient co-expression of BMP-2 with BMP-5, BMP-6 or BMP-7, or BMP-4 transiently co-expressed with BMP-7, resulted in more BMP activity than expression of any single BMP, with heterodimeric BMP-2/7

presenting about 20-fold higher specific activity than BMP homodimers (*in vitro* alkaline phosphatase induction assay) (12).

Recent studies have identified specific BMP antagonists (i.e., noggin and chordin) and members of the DAN family (i.e., gremlin). Such antagonists bind to BMP with the same affinity as their specific receptors, blocking signal transduction and thus decreasing bone formation. Therefore, these antagonists may be used therapeutically in pathological conditions characterized by excessive bone formation (13).

Bahamonde and Lyons (14) demonstrated that BMP-3 has an inhibitory effect on osteogenesis, presenting a signaling pathway similar to TGF- β /activin. The ability of BMP-3 to inhibit the activity of BMP-2 seems to result from competition for common signaling components of the TGF- β /activin and BMPs pathways. Since BMP-3 is by far the most abundant BMP in demineralized bone, it probably plays a fundamental role as a modulator of the osteogenic activity of other BMPs *in vivo*.

These findings are of great clinical relevance because of the need to quantitate the amount of BMP-3 when products composed of exogenous BMPs are used to accelerate bone regeneration. The osteogenic potential of BMPs is increased when the antagonists are eliminated. Nevertheless, BMP-3 could be used in the treatment of diseases characterized by bone hypermineralization, such as osteopetrosis.

Immune response to bone morphogenetic proteins

The immune mechanisms activated upon implantation of BMPs are not fully understood or well defined due to controversies in the literature, which are raising some confusion. It seems that the single application of allogeneic BMPs and non-collagenic proteins provides a moderate immune response through the production of immunoglobulins

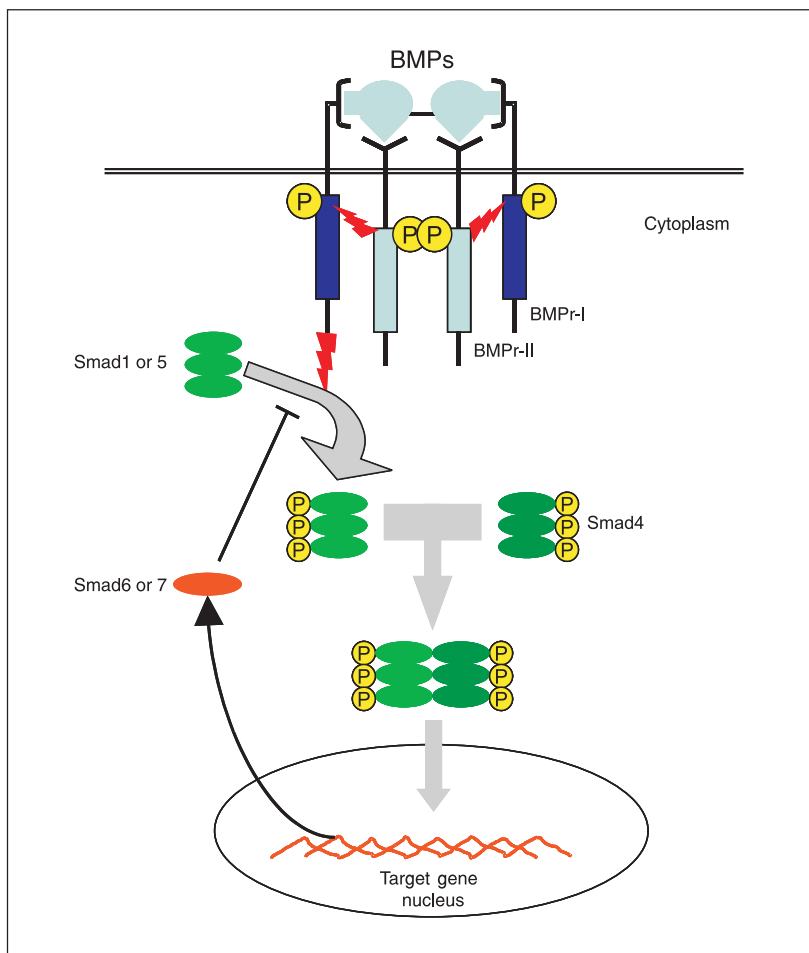


Figure 2. BMP-mediated gene transcription. The transmembrane serine/threonine kinase BMP receptors activated complex, triggers receptor-regulated Smad phosphorylation and binding to common-mediator Smads. After heteromeric complex formation, Smads move into the nucleus and regulate transcriptional activity. BMP = bone morphogenetic protein; Smads = signaling mediators of BMP receptors homologous to Mas (*Drosophila*) and Sma (*C. elegans*) genes. Adapted from Ref. 11, with permission.

G, but does not decrease the osteoinductive capacity of BMP. On the other hand, a single dose of BMP-non-collagenic protein stimulates a high concentration of anti-BMP antibody, which could inhibit the osteoinductive potential of BMP (15).

Implantation of allogenic or xenogenic BMPs appears to promote recruitment of macrophages, lymphocytes and plasma cells, and production of antibodies which may inhibit osteogenesis. Whereas some studies demonstrate a species-specific effect of BMPs (16), others demonstrate that a single dose of up to 100 mg xenogenic BMP would be safe and would not stimulate a detectable immune response (17). However, a later study by Urist and collaborators (18) showed that a second implantation of BMPs results in intensification of the immune response and in reduced efficacy of the xenogenic BMP in the treatment of critical-size lesions in dogs. Analysis of the immune response after implantation of recombinant human BMP (rhBMP) has not yet been thoroughly studied. Nevertheless, preliminary studies have reported that anti-rhBMP-2 was not produced after implantation of this recombinant protein in defects of the mandibles of dogs.

Further studies are certainly necessary to explain the relationship between bone induction and the occurrence of immune responses upon implantation of BMPs, and to ensure safety in their use for orthopedic and dental purposes.

Target cells for bone morphogenetic proteins

Numerous studies have demonstrated the cellular and molecular effects of demineralized bone matrix and purified or recombinant BMPs on several cell lines. At least some pluripotent mesenchymal cell lines, bone marrow cells, osteoblast precursors, myoblasts, fibroblasts, and neural cells respond to BMPs. Numerous markers of bone

metabolism such as alkaline phosphatase, parathyroid hormone receptor, osteocalcin, osteopontin, and osteonectin are modulated by BMPs. In spite of the accumulated evidence that the BMP-mediated response involves the use of specific receptors during the development of cartilage and bone, their signal transduction mechanisms are still unclear. Indeed, BMPs play an important role during the initial stages of organogenesis.

In mesenchymal and embryonic cells, the most impressive effect of BMPs is the ability to induce differentiation of these cells into osteoblasts, stimulating cartilage formation and alkaline phosphatase activity. Other hormones or cytokines cannot modulate the level of these markers of bone metabolism. Noteworthy is the fact that in *in vitro* experiments low concentrations of BMPs promote differentiation of mesenchymal cells into adipocytes, whereas high concentrations of these proteins promote osteoblast differentiation. This emphasizes the need to specify the doses of BMP in order to predict its effect (19). Osteoblasts treated with rhBMP-2 present rapid differentiation, similarly to mesenchymal cells, with an increase in the levels of alkaline phosphatase, osteocalcin, osteopontin, and bone sialoprotein (20).

The effects of BMPs on osteoblasts and periosteal cells have been thoroughly studied in order to obtain a better understanding of the action of BMPs at the cellular level. In general, there is an increase in DNA synthesis activity and in the transcription of genes involved in the synthesis of bone matrix proteins. rhBMP-2 blocks the differentiation of osteoblast precursor cells into myoblasts or adipocytes (21). Sampath and collaborators (22) demonstrated that when OP-1 (BMP-7) is added to cultures of bone cells enriched with osteoblasts at different stages of differentiation it stimulates cell proliferation, collagen synthesis, induction of alkaline phosphatase, parathyroid hormone-mediated production of cAMP, and osteocalcin synthesis.

Since many types of BMPs can induce endochondral ossification, chondroblasts should also be natural targets of these proteins. In fact, many BMPs have been shown to induce cell proliferation and synthesis and activity of alkaline phosphatase of chondroblasts and chondrocytes of the growth plate. The nature of the chondrocytes for *in vitro* culture has a significant role in the effect of BMPs, showing that the stimuli for these proteins are tissue-specific (23).

Bovine BMPs induced an increase of DNA and protein synthesis and also of alkaline phosphatase activity in NIH-3T3 fibroblasts in a dose-dependent manner (24). In contrast, rhBMP did not induce an increase in alkaline phosphatase in these cells. BMP-2 promoted differentiation of BALB/c-3T3, Swiss-3T3 and 3T3-L1 fibroblasts into adipocytes and osteoblasts (20).

Usually, target cells for BMPs differentiate into osteoblast-like cells and produce alkaline phosphatase and mineralized tissue. On the other hand, Kaneko et al. (25) examined the direct effect of BMPs on osteoclastic bone-resorbing activity in a culture of highly purified rabbit mature osteoclasts. BMP-2 and -4 increase in bone resorption pits excavated by the isolated osteoclasts. BMP-2 also elevated the messenger RNA expression by cathepsin K and carbonic anhydrase II, which are key enzymes for the degradation of organic and inorganic bone matrices, respectively.

Purified versus recombinant bone morphogenetic proteins

As with every growth factor, BMPs act at very low doses in the tissues, existing in nanograms or micrograms. However, in order to isolate a couple of micrograms of BMPs, kilograms of demineralized bone matrix are needed (26). Once isolated, different BMPs may be identified by their amino acid sequence. Purification of BMPs from the demineralized bone matrix can be car-

ried out by four distinct methods: 1) enzymatic digestion, since they resist collagenase; 2) ethylene glycol extraction, due to the hydrophobic nature of the BMP molecule; 3) 6 M urea plus 0.5 M CaCl₂, since BMPs can be dissociated from other non-collagen proteins in chaotropic solvents; 4) concanavalin A affinity chromatography due to their hydrophobic nature and to carbohydrates present in their structure.

In spite of these methods, purification of BMPs is an extremely laborious process and the yields are low. Preparations must be initiated with a minimum of 100 kg of washed fresh cortical bone free of bone marrow (26). Since their molecular weight ranges from 15 to 30 kDa, partial purification of BMPs yields 57 mg of the pool of BMPs per kg of fresh bone (26). Isolation of a particular native BMP yields even smaller amounts of the order of µg/kg tissue. The small amounts of BMPs resulting from such a laborious purification process have stimulated the application of molecular biology techniques for the cloning and expression of these proteins.

The molecular cloning of the first genes encoding BMPs took place at the end of the 1980's and more than 30 members of the BMP family have been described (27). The study of different BMPs revealed that their expression pattern and their biological functions are not restricted to skeletal development. Other functions have been identified, such as cell proliferation and differentiation, apoptosis, morphogenesis of various organs, including the skeleton, and organogenesis.

Alternatively, a number of laboratories have isolated bioactive proteins which induce cartilage and/or bone formation at the sites implanted, but the yields were low and the purification process was very laborious (28). In addition, the potential risk implicated in their origin from allogeneic donor bone reduced their clinical application (29). cDNAs for different BMPs have been identified and cloned. The sequences deduced from these cDNAs have indicated that these

proteins are members of the TGF- β superfamily, except for BMP-1, which has been identified as procollagen C proteinase (30). The molecular cloning of BMP-encoding genes and their identification as TGF- β relatives has enhanced the interest in these proteins and has permitted expression and functional studies.

In view of the osteoinductive properties of BMP-2 and BMP-7, we set out to isolate the human cDNA counterparts of these molecules to clone them into appropriate transducing vectors in order to produce and purify these recombinant proteins using heterologous bacterial, mammalian and baculovirus expression systems (Bustos-Valenzuela JC and Sogayar MC, unpublished data). The recombinant proteins obtained are being used in blind cDNA cloning strategies to identify and characterize novel potential regulators of the osteoblast differentiation process, to better understand the molecular mechanisms involved in bone formation and to gain new therapeutic insights (drug design and gene therapy).

The osteoinductive properties of recombinant BMPs are reduced compared to purified BMPs and require the characterization of BMPs by genetic engineering techniques. Bessho et al. (31) have analyzed in detail the effects of purified versus recombinant bovine BMP. On the basis of Ca²⁺ content and radiographic aspects, they observed that maturation of bone tissue ectopically formed in rat muscle was as much as 10 times greater when bovine BMP was used.

Several hypotheses have been proposed and tested to explain such discrepancies. There are reports about differences in the amino acid sequence of recombinant and purified bovine BMP. In addition, it has been suggested that these cytokines operate coordinately in bone repair, emphasizing the need for several recombinant BMPs simultaneously to further facilitate the repair. Another consideration is the type of carrier used to transport the BMP into the defect, with collagen-derived materials being ex-

cellent candidates (31).

Osteogenic protein-1/bone morphogenetic protein-7

OP-1, or BMP-7, was cloned in the 1990's. The gene encoding OP-1 was found in placenta, hippocampus and osteosarcoma cDNA libraries, using a consensus probe based on the polypeptide chain of bovine BMP-enriched preparations. OP-1 promotes an increase in chondrocyte proliferation and induces chondrogenic differentiation. OP-2 (BMP-8), however, is only expressed during embryogenesis. Like BMP-2 to 6, OP-1 is a homodimeric glycoprotein stabilized by disulfide bonds, with molecular weight ranging from 32 to 36 kDa. The amino acid sequence of OP-1, initially purified from bovine bone matrix, is approximately 60% similar to that of BMP-2.

Recombinant human OP-1 produced by the expression of the entire cDNA in mammalian cells was able to induce bone formation *in vitro*, in a manner similar to preparations of highly purified bovine OP-1 (22). During the initial stages of treatment, OP-1 elicits an intense chondrogenic response and stimulates an osteoblastic response during the course of treatment.

Investigations regarding the biological effects of OP-1 have been carried out, mainly concerning its potential for osteogenesis. It has been demonstrated that OP-1 stimulates cell proliferation, collagen synthesis and osteoblast differentiation. Studies on animal models have shown that OP-1 is capable of inducing endochondral ossification in segmental osteoperiosteal defects (32).

The FDA of the United States has recently approved the use of OP-1 for the treatment of spinal fusion, emphasizing its use in orthopedic and dental surgeries (33).

Clinical applications

A search of PubMed retrieved 2,975 hits

for the descriptor “bone morphogenetic protein”, but only 20 citations for Clinical Trials in Humans. The highest level of scientific evidence arises from meta-analysis and randomized controlled trials. Systematic reviews synthesize studies related to the side effects and benefit of treatments. They are based on good quality papers obtained from correct databases and focusing on a specific question. Avoiding or minimizing bias, systematic reviews are very helpful in decision making. In meta-analysis, the quantitative combination of results from different studies yields an estimate of the expected effect of a treatment (34). Table 1 summarizes the papers found in PubMed related to clinical trials, randomized clinical trials and meta-analysis, emphasizing the clinical applications proposed for BMPs.

The first studies were clinical trials, one of which a multi-center study, analyzing the effect of BMPs (rhBMP-2) for local alveolar ridge preservation or augmentation (54) or maxillary sinus floor augmentation (53). The great majority of studies concerned spinal fusion (Table 1), with all of them agreeing about the benefit of BMPs. Burkus et al. (43) conducted an integrated analysis of multiple clinical studies involving 679 patients and concluded that rhBMP-2-treated subjects had statistically significant outcomes regarding duration of surgery, blood loss, hospital stay, reoperation rate, median time to return to work, and fusion rates at 6, 12, and 24 months. The safety and effectiveness of 1.5 mg/mL rhBMP-2 applied to an absorbable collagen sponge was demonstrated for the treatment of 450 patients with open tibial fractures.

Table 1. List of publications related to human clinical application of bone morphogenetic proteins (BMP), classified as clinical trial (CT), randomized clinical trial (RCT), multi-center (MC), and meta-analyses (MA).

Type	Author	Objective	Sample size	Recommend BMP use?
RCT	Haid Jr. et al. (35)	rhBMP-2 on anterior lumbar interbody fusion	67	Yes
CT	Kuklo et al. (36)	BMP on transforaminal lumbar interbody fusion	22	Yes
CT	Lanman and Hopkins (37)	rhBMP-2 on transforaminal lumbar interbody fusion	43	Yes
CT	Mummaneni et al. (38)	BMP on transforaminal lumbar interbody fusion	40	Yes
RCT	Baskin et al. (39)	rhBMP-2 for the treatment of human cervical disc disease	33	Yes
RCT	Burkus et al. (40)	rhBMP-2 on anterior lumbar interbody fusion	42	Yes
RCT	Jung et al. (41)	rhBMP-2 on lateral ridge augmentation	11	Yes
MA	Giannobile and Somerman (42)	Growth and amelogenin-like factors in periodontal wound healing A systematic review	511	Partially
MA	Burkus et al. (43)	Is INFUSE bone graft superior to autograft bone? An integrated analysis of clinical trials using the LT-CAGE lumbar tapered fusion device	679	Yes
RCT, MC	Boden et al. (44)	rhBMP-2 on lumbar arthrodesis	25	Yes
RCT, MC	Burkus et al. (45)	BMP (INFUSE bone graft) on lumbar interbody fusion	46	Yes
RCT, MC	Groeneveld and Burger (46)	rhBMP-2 on open tibial fracture	450	Yes
MA	Salata et al. (47)	Recent outcomes and perspectives of the application of bone morphogenetic proteins in implant dentistry		Insufficient data
RCT, MC	Friedlaender et al. (48)	OP-1 (BMP-7) in the treatment of tibial nonunion	122	Yes
CT	Cochran et al. (49)	(rhBMP-2) loaded in an absorbable collagen sponge for alveolar ridge augmentation	12	Yes
CT, MC	Schedel et al. (50)	rhBMP-2 to treat avascular necrosis of the femoral head	6	Yes
CT	van den Bergh et al. (51)	OP-1 in sinus floor elevation	6	Partially
RCT	Geesink et al. (52)	OP-1 in tibial osteotomy	24	Yes
CT	Boyne et al. (53)	rhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation	12	Yes
CT, MC	Howell et al. (54)	rhBMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation	12	Yes

rhBMP = recombinant human BMP-2; OP-1 = osteogenic protein 1.

Significantly superior results compared to standard care were observed in terms of reducing the frequency of secondary interventions and the overall invasiveness of the procedures, with accelerated fracture and wound-healing, and a reduced rate of infection.

In dentistry, BMPs have been tested in periodontal (regeneration of lost bone tissue due to periodontal disease), implant (increase in bone volume for placement of implants, maxillary sinus augmentation) and restorative-endodontic (pulpotomies) procedures.

Several animal studies have been carried out to evaluate the efficacy of BMPs for maxillary sinus augmentation, and studies in both animals and humans have demonstrated similar, but still unsatisfactory, results, when compared to other procedures. Animal assays using rhBMP-2 associated with a carrier (collagen foam) in 3-sided intrabone defects in dogs have demonstrated an increase in the rate of bone formation without side effects such as ankylosis or apical bone resorption (55). A clinical trial studying 6 patients (3 of them used as control) indicated that the OP-1 (2.5 mg in 1 g collagen carrier) has the potential to initiate bone formation in the human maxillary sinus within 6 months after a sinus floor elevation operation (51). However, the behavior of this material is not fully predictable.

Barboza et al. (56) have used BMPs as an aid to increase bone crest height prior to the placement of implants. However, Salata et al. (47), in a meta-analysis of 379 scientific reports concerning the use of BMPs on implant dentistry, concluded that the number of studies is too small to establish clinical protocols for the improvement of a recipient bone bed prior to implant placement or to enhance the integration process of an implant.

Giannobile et al. (57) have shown promising and encouraging results with rhBMP-7 implanted in type III furcation defects in dogs, with no signs of side effects such as ankylosis of the affected roots. However, in a systematic review published later (42) these investigators observed that the majority of the reports available had a low-quality evidence rating and that most reports were case studies or case series without controls. They concluded that there were insufficient data to conduct a meta-analysis and that pre-clinical and initial clinical data for growth factors appear promising but are insufficient to draw definitive conclusions, mainly for long-term evaluation.

BMPs have been tested in pulp capping procedures for more than a decade, and have presented enhanced potential as effective agents in the induction of a mineralized barrier in the pulp (58). Jepsen et al. (59) used recombinant BMP-7 as a capping agent in minipigs and detected the formation of a thicker dentin barrier in the group treated with recombinant BMP-7 than in the group treated with Ca(OH)_2 . Ren et al. (60) tested recombinant BMP-2 associated with fibrin as a capping agent in the pulps of dogs (molars and premolars) and observed the formation of a dentin barrier after one week, this being a better result than for the control group treated with Ca(OH)_2 or the experimental group treated with BMP-2 only.

Knowledge of the cellular and molecular basis of BMP signaling pathways and the development of appropriate carriers will certainly stimulate a great revolution in dentistry, allowing the predominance of regenerative over cicatricial processes. However, it is clear that well-designed blind and randomized clinical trials are required to identify the effective applications of BMPs in medical and dental clinics.

References

- Urist MR (1965). Bone: Formation by autoinduction. *Science*, 150: 893-899.
- Urist MR, Nogami H & Mikulski A (1976). A bone morphogenetic polypeptide. *Calcified Tissue Research*, 21 (Suppl): 81-87.
- Bessho K, Tanaka N, Matsumoto J et al. (1991). Human dentin-matrix-derived bone morphogenetic protein. *Journal of Dental Research*, 70: 171-175.
- Urist MR, Grant TT, Lindholm TS et al. (1979). Induction of new-bone formation in the host bed by human bone-tumor transplants in athymic nude mice. *Journal of Bone and Joint Surgery*, 61: 1207-1216.
- Constam DB & Robertson EJ (1999). Regulation of bone morphogenetic protein activity by prodomains and proprotein convertases. *Journal of Cell Biology*, 144: 139-149.
- Wozney JM, Rosen V, Celeste AJ et al. (1988). Novel regulators of bone formation: molecular clones and activities. *Science*, 242: 1528-1534.
- Spinella-Jaegle S, Roman-Roman S, Faucheu C et al. (2001). Opposite effects of bone morphogenetic protein-2 and transforming growth factor-beta1 on osteoblast differentiation. *Bone*, 29: 323-330.
- Griffith DL, Keck PC, Sampath TK et al. (1996). Three-dimensional structure of recombinant human osteogenic protein 1: structural paradigm for the transforming growth factor beta superfamily. *Proceedings of the National Academy of Sciences, USA*, 93: 878-883.
- Tabas JA, Zasloff M, Wasmuth JJ et al. (1991). Bone morphogenetic protein: chromosomal localization of human genes for BMP1, BMP2A, and BMP3. *Genomics*, 9: 283-289.
- Pacicca DM, Patel N, Lee C et al. (2003). Expression of angiogenic factors during distraction osteogenesis. *Bone*, 33: 889-898.
- Sakou T (1998). Bone morphogenetic proteins: from basic studies to clinical approaches. *Bone*, 22: 591-603.
- Israel DI, Nove J, Kerns KM et al. (1996). Heterodimeric bone morphogenetic proteins show enhanced activity *in vitro* and *in vivo*. *Growth Factors*, 13: 291-300.
- Groppe J, Greenwald J, Wiater E et al. (2002). Structural basis of BMP signalling inhibition by the cystine knot protein noggin. *Nature*, 420: 636-642.
- Bahamonde ME & Lyons KM (2001). BMP3: to be or not to be a BMP. *Journal of Bone and Joint Surgery*, 83-A (Suppl 1): S56-S62.
- Urist MR, Nilsson OS, Hudak R et al. (1985). Immunologic evidence of a bone morphogenetic protein in the milieu interieur. *Annales de Biologie Clinique*, 43: 755-766.
- Johnson EE, Urist MR, Schmalzried TP et al. (1989). Autogenic cancellous bone grafts in extensive segmental ulnar defects in dogs. Effects of xenogeneic bovine bone morphogenetic protein without and with interposition of soft tissues and interruption of blood supply. *Clinical Orthopaedics and Related Research*, 243: 254-265.
- Nilsson OS, Urist MR, Dawson EG et al. (1986). Bone repair induced by bone morphogenetic protein in ulnar defects in dogs. *Journal of Bone and Joint Surgery. British Volume*, 68: 635-642.
- Nilsson OS & Urist MR (1991). Immune inhibition of repair of canine skull trephine defects implanted with partially purified bovine morphogenetic protein. *International Orthopaedics*, 15: 257-263.
- Carrington JL, Chen P, Yanagishita M et al. (1991). Osteogenin (bone morphogenetic protein-3) stimulates cartilage formation by chick limb bud cells *in vitro*. *Developmental Biology*, 146: 406-415.
- Wang EA, Israel DI, Kelly S et al. (1993). Bone morphogenetic protein-2 causes commitment and differentiation in C3H10T1/2 and 3T3 cells. *Growth Factors*, 9: 57-71.
- Chen TL, Bates RL, Dudley A et al. (1991). Bone morphogenetic protein-2b stimulation of growth and osteogenic phenotypes in rat osteoblast-like cells: comparison with TGF-beta 1. *Journal of Bone and Mineral Research*, 6: 1387-1393.
- Sampath TK, Maliakal JC, Hauschka PV et al. (1992). Recombinant human osteogenic protein-1 (HOP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. *Journal of Biological Chemistry*, 267: 20352-20362.
- Chen P, Vukicevic S, Sampath TK et al. (1993). Bovine articular chondrocytes do not undergo hypertrophy when cultured in the presence of serum and osteogenic protein-1. *Biochemical and Biophysical Research Communications*, 197: 1253-1259.
- Vukicevic S, Latin V, Chen P et al. (1994). Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes. *Biochemical and Biophysical Research Communications*, 198: 693-700.
- Kaneko H, Arakawa T, Mano H et al. (2000). Direct stimulation of osteoclastic bone resorption by bone morphogenetic protein (BMP)-2 and expression of BMP receptors in mature osteoclasts. *Bone*, 27: 479-486.
- Gao T, Lindholm TS, Martinen A et al. (1996). Composites of bone morphogenetic protein (BMP) and type IV collagen, coral-derived coral hydroxyapatite, and tricalcium phosphate ceramics. *International Orthopaedics*, 20: 321-325.
- Reddi AH (1997). Bone morphogenetic proteins: an unconventional approach to isolation of first mammalian morphogens. *Cytokine and Growth Factor Reviews*, 8: 11-20.
- Wang EA, Rosen V, Cordes P et al. (1988). Purification and characterization of other distinct bone-inducing factors. *Proceedings of the National Academy of Sciences, USA*, 85: 9484-9488.
- Kirker-Head CA (2000). Potential applications and delivery strategies for bone morphogenetic proteins. *Advanced Drug Delivery Reviews*, 43: 65-92.
- Hofbauer LC & Heufelder AE (1996). Updating the metalloprotease nomenclature: bone morphogenetic protein 1 identified as procollagen C proteinase. *European Journal of Endocrinology*, 135: 35-36.
- Bessho K, Kusumoto K, Fujimura K et al. (1999). Comparison of recombinant and purified human bone morphogenetic protein. *British Journal of Oral and Maxillofacial Surgery*, 37: 2-5.
- Sampath TK, Rashka KE, Doctor JS et al. (1993). *Drosophila* transforming growth factor beta superfamily proteins induce endochondral bone formation in mammals. *Proceedings of the National Academy of Sciences, USA*, 90: 6004-6008.
- Vaccaro AR, Patel T, Fischgrund J et al. (2003). A pilot safety and efficacy study of OP-1 putty (RhBMP-7) as an adjunct to iliac crest autograft in posterolateral lumbar fusions. *European Spine Journal*, 12: 495-500.
- Sacks HS, Berrier J, Reitman D et al. (1987). Meta-analyses of randomized controlled trials. *New England Journal of Medicine*, 316: 450-455.
- Haid Jr RW, Branch Jr CL, Alexander JT et al. (2004). Posterior lumbar interbody fusion using recombinant human bone morphogenetic protein type 2 with cylindrical interbody cages. *Spine Journal*, 4: 527-538.

36. Kuklo TR, Rosner MK & Polly Jr DW (2004). Computerized tomography evaluation of a resorbable implant after transforaminal lumbar interbody fusion. *Neurosurgical Focus*, 16: E10.
37. Lanman TH & Hopkins TJ (2004). Lumbar interbody fusion after treatment with recombinant human bone morphogenetic protein-2 added to poly(L-lactide-co-D,L-lactide) bioresorbable implants. *Neurosurgical Focus*, 16: E9.
38. Mummaneni PV, Pan J, Haid RW et al. (2004). Contribution of recombinant human bone morphogenetic protein-2 to the rapid creation of interbody fusion when used in transforaminal lumbar interbody fusion: a preliminary report. Invited submission from the Joint Section Meeting on Disorders of the Spine and Peripheral Nerves, March 2004. *Journal of Neurosurgery. Spine*, 1: 19-23.
39. Baskin DS, Ryan P, Sonntag V et al. (2003). A prospective, randomized, controlled cervical fusion study using recombinant human bone morphogenetic protein-2 with the CORNERSTONE-SR allograft ring and the ATLANTIS anterior cervical plate. *Spine*, 28: 1219-1225.
40. Burkus JK, Dorchak JD & Sanders DL (2003). Radiographic assessment of interbody fusion using recombinant human bone morphogenetic protein type 2. *Spine*, 28: 372-377.
41. Jung RE, Glauser R, Schärer P et al. (2003). Effect of RhBMP-2 on guided bone regeneration in humans. *Clinical Oral Implants Research*, 14: 556-568.
42. Giannobile WV & Somerman MJ (2003). Growth and amelogenin-like factors in periodontal wound healing. A systematic review. *Annals of Periodontology*, 8: 193-204.
43. Burkus JK, Heim SE, Gornet MF et al. (2003). Is INFUSE bone graft superior to autograft bone? An integrated analysis of clinical trials using the LT-CAGE lumbar tapered fusion device. *Journal of Spinal Disorders and Techniques*, 16: 113-122.
44. Boden SD, Kang J, Sandhu H et al. (2002). Use of recombinant human bone morphogenetic protein-2 to achieve posterolateral lumbar spine fusion in humans: a prospective, randomized clinical pilot trial: 2002 Volvo Award in Clinical Studies. *Spine*, 27: 2662-2673.
45. Burkus JK, Transfeldt EE, Kitchel SH et al. (2002). Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. *Spine*, 27: 2396-2408.
46. Groeneveld EH & Burger EH (2000). Bone morphogenetic proteins in human bone regeneration. *European Journal of Endocrinology*, 142: 9-21.
47. Salata LA, Franke-Stenport V & Rasmusson L (2002). Recent outcomes and perspectives of the application of bone morphogenetic proteins in implant dentistry. *Clinical Implant Dentistry and Related Research*, 4: 27-32.
48. Friedlaender GE, Perry CR, Cole JD et al. (2001). Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *Journal of Bone and Joint Surgery*, 83-A (Suppl 1): S151-S158.
49. Cochran DL, Jones AA, Lilly LC et al. (2000). Evaluation of recombinant human bone morphogenetic protein-2 in oral applications including the use of endosseous implants: 3-year results of a pilot study in humans. *Journal of Periodontology*, 71: 1241-1257.
50. Schedel H, Schneller A, Vogl T et al. (2000). Dynamic magnetic resonance tomography (MRI): a follow-up study after femur core decompression and instillation of recombinant human bone morphogenetic protein-2 (RhBMP-2) in avascular femur head necrosis. *Röntgenpraxis*, 53: 16-24.
51. van den Bergh JP, ten Bruggenkate CM, Groeneveld HH et al. (2000). Recombinant human bone morphogenetic protein-7 in maxillary sinus floor elevation surgery in 3 patients compared to autogenous bone grafts. A clinical pilot study. *Journal of Clinical Periodontology*, 27: 627-636.
52. Geesink RG, Hoefnagels NH & Bulstra SK (1999). Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. *Journal of Bone and Joint Surgery. British Volume*, 81: 710-718.
53. Boyne PJ, Marx RE, Nevins M et al. (1997). A feasibility study evaluating RhBMP-2/absorbable collagen sponge for maxillary sinus floor augmentation. *International Journal of Periodontics and Restorative Dentistry*, 17: 11-25.
54. Howell TH, Fiorellini J, Jones A et al. (1997). A feasibility study evaluating RhBMP-2/absorbable collagen sponge device for local alveolar ridge preservation or augmentation. *International Journal of Periodontics and Restorative Dentistry*, 17: 124-139.
55. Choi SH, Kim CK, Cho KS et al. (2002). Effect of recombinant human bone morphogenetic protein-2/absorbable collagen sponge (RhBMP-2/ACS) on healing in 3-wall intrabony defects in dogs. *Journal of Periodontology*, 73: 63-72.
56. Barboza EP, Duarte ME, Geolas L et al. (2000). Ridge augmentation following implantation of recombinant human bone morphogenetic protein-2 in the dog. *Journal of Periodontology*, 71: 488-496.
57. Giannobile WV, Ryan S, Shih MS et al. (1998). Recombinant human osteogenic protein-1 (OP-1) stimulates periodontal wound healing in class III furcation defects. *Journal of Periodontology*, 69: 129-137.
58. Nakashima M (1994). Induction of dentin formation on canine amputated pulp by recombinant human bone morphogenetic proteins (BMP)-2 and -4. *Journal of Dental Research*, 73: 1515-1522.
59. Jepsen S, Albers HK, Fleiner B et al. (1997). Recombinant human osteogenic protein-1 induces dentin formation: an experimental study in miniature swine. *Journal of Endodontics*, 23: 378-382.
60. Ren WH, Yang LJ & Dong SZ (1999). Induction of reparative dentin formation in dogs with combined recombinant human bone morphogenetic protein 2 and fibrin sealant. *Chinese Journal of Dental Research*, 2: 21-24.