

REVIEW

Bone morphogenetic proteins in human bone regeneration

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Abstract

Recently, the first clinical reports on bone regeneration by two recombinant human bone morphogenetic proteins (rhBMPs), BMP-2 and BMP-7 (also named osteogenic protein-1, OP-1) have been published (1–4). Although both BMPs were able to support bone regeneration, a significant variation in individual response was observed with both proteins. Animal studies and laboratory experiments reveal a number of conditions that influence the osteoinductivity of BMP, such as BMP concentration, carrier properties and influence of local and systemic growth factors and hormones. In this paper, these studies and the clinical reports are reviewed, and the conditions that modulate the BMP-dependent osteoinduction are discussed. The information may provide clues as to how the performance of recombinant human BMP as bone-graft substitute in humans can be improved.

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BMP and demineralized bone matrix

The history of bone morphogenetic proteins (BMPs) began with the observation that demineralized bone matrix (DBM) is able to induce ectopic bone formation in subcutaneous and intramuscular pockets in rodents (5, 6). This bone induction process has been studied extensively (7–13). Histological and biochemical analyses showed that cartilage appears 5–10 days after implantation of active DBM (8). This cartilage mineralizes by day 7–14 and is subsequently replaced by bone (7–9). After 21 days, haematopoietic bone marrow formation can be observed (12). These cellular events observed after DBM implantation mimic embryonic bone development and normal fracture repair (11). As DBM-related bone formation was observed to occur at ectopic sites, it was assumed that pluripotent mesenchymal cells are attracted to the site of implantation. Isolation of the bone-inducing substance revealed that certain proteins were responsible, which were termed bone morphogenetic proteins (BMPs) or osteogenic proteins (OPs).

The use of DBM in treating bone defects has proven beneficial for bone regeneration both in animals and in humans (14–17). DBM has become widely accepted as a bone-graft substitute in clinical practice (18–22), but its bone inductive capacity has been questioned (23, 24). In several studies, including our own, histology revealed that new bone was generated by osteoconduction rather than osteoinduction (25–27) – that is, bone regeneration occurred by growth of existing host bone on the DBM granules, which acted as a scaffold, rather

than by *de novo* differentiation of bone, independent of pre-existing bone. Lack of inductive properties of DBM may be related to the procedures of production of commercially available DBM, as preservation of osteoinductive activity can be affected by the processing (28–30) or sterilization procedures (31). It is also possible that DBM of human origin, which is preferred for use in clinical practice, is less osteoinductive than DBM derived from animals, which is commonly used in animal studies. Several studies show that DBM from long-lived species such as baboon and human was not able to induce bone differentiation in short-lived animals such as rats, mice and guinea pigs (8, 32, 33). Allogenic intramuscular implantation of DBM in adult monkeys also failed to induce the formation of bone (34), or produced only strongly delayed bone formation, 72 days after subcutaneous implantation (35). Sampath and Reddi (8) suggested that this limited cross-reactivity was caused by immunogenic factors in crude BMP extracts, which inhibited the bone induction process. Indeed when BMPs were purified and reconstituted with inactive DBM, bone induction was observed in different species (8, 36, 37). Purified human BMP has been used in a number of clinical cases, to repair resistant non-unions and segmental defects of long bones (38–42).

Identification of single BMPs and their role in osteoinduction

BMPs belong to the transforming growth factor- β (TGF β) superfamily, which consists of a group of related

peptide growth factors. More than 40 related members of this family have been identified, including BMPs, growth and differentiation factors (GDFs), inhibins/activins, TGF β s and Müllerian inhibiting substance (43–47). Members of the TGF β superfamily are synthesized as large precursor molecules, and the mature protein is released from a propeptide region by proteolytic cleavage (47). BMPs consist of dimers that are interconnected by seven disulphide bonds (47); this dimerization is a prerequisite for bone induction (43). BMPs are active both as homodimer molecules that consist of two identical chains, and as heterodimers consisting of two different chains. Fifteen BMPs have currently been identified (7, 43, 48–59) (Table 1), and they are further divided into subfamilies according to their amino acid sequence similarities. BMPs-2 and -4 form one subgroup, BMPs-5–8 form a second subgroup, and a third subgroup contains BMP-3 and GDF-10 (60), a related growth factor. Members of each subgroup have shown osteoinductivity (Table 1), with an identical mechanism as observed after ectopic implantation of osteoinductive DBM. BMP-1 is not related to the BMP family. It does not show osteoinductivity (61), and has recently been identified as procollagen-C-proteinase (62).

In vitro studies of the effects of BMP support the theory that multipotent cells play a role in bone induction *in vivo*. Multipotent cells, either from pre- or postnatal animals or from animal and human bone marrow, showed responsiveness to various BMPs. Newborn rat calvarial cells and rat osteosarcoma cells showed osteogenic differentiation after treatment with recombinant human (rh)OP-1 (63, 64). The fibroblastic cell line, C3H10T1/2, established from an early mouse embryo, showed osteoinductive responses to rhBMP-2 (65, 66). Rat and mouse bone marrow have shown responsiveness to rhBMP-2 by an increase in osteoblastic parameters (67, 68), human bone marrow has shown an increase in osteoblastic parameters after rhBMP-2 or rhOP-1 treatment (69–72). Treatment of primary human bone marrow stromal cells with rhOP-1 resulted in a concentration dependent increase of the osteogenic parameter alkaline phosphatase (72).

The differentiation stage of multipotent cell populations was found to be an important determinant of the effects of BMP (66, 73–75). Whereas myoblasts retained the ability to change their differentiation pathway to express osteoblast parameters (76–78), several studies have shown that BMPs do not stimulate mature osteoblasts (66, 69, 79); moreover, mature fibroblasts could not be induced to express osteogenic parameters after treatment with BMP-2 (65). These results indicate that the osteogenic influence of BMPs is directed towards immature and multipotent cells. Mature cells seem to lose their responsiveness.

In healing fractures, which contain many immature cells, expression of native BMPs has been demonstrated (80, 81), indicating that these proteins have a local

regulatory role during fracture repair. As cells derived from non-healing fractures did show an osteogenic response to BMP (82), it is possible that failure of fracture healing may derive from an insufficient BMP supply.

Few studies have compared the effects of various BMPs directly within one experiment. Mayer and coworkers (83) showed that different BMPs varied in their mitogenic capacity in cultures of periosteal cells and epiphyseal and sternal chondrocytes derived from chick embryos. DNA synthesis increased more after treatment with BMPs-2 and -4 than after BMPs-5 or -6 treatment; the effects of OP-7 were marginal, and BMP-3 had no effect (83). Differences in osteogenic effect have been demonstrated in some studies, but the results are somewhat conflicting. Takuwa and coworkers (61) showed that BMP-2 was able to stimulate alkaline phosphatase activity and collagen synthesis in MC3T3-E1 cells, whereas BMP-3 stimulated collagen synthesis only. BMP-6 was found to increase the osteoblastic phenotype in secondary rat calvarial cell cultures more than twice as much as BMPs-2 or -4, and BMP-2 was more potent than BMP-4 (84). BMP-6 was a more potent stimulator of bone formation than BMPs-2 and -4 in rat osteoblasts (85). However, in several bone marrow cell lines, BMP-6 showed less osteogenic potential than BMPs-2 and -4 (73). Some studies showed that heterodimers were much more osteoinductive than homodimers. For example, BMPs-2/7 and BMP-4/7 heterodimers were found to be more active than BMPs-2, -4 and -7 homodimers (86–88). However, the *Xenopus* BMP-4/7 heterodimer was less inductive than the homodimeric recombinant human BMP-2 (89). These data suggest that more potent BMP formulations are possible. However, *in vivo* osteoinduction studies with these BMPs have not been reported.

The identification of BMP receptors and intracellular signal transduction after ligand binding is an area of intensive research that has been reviewed recently (90–93). Receptors for BMPs are complexes of two different types of membrane-bound serine/threonine kinases: type I BMP receptors, BMPR-1A and BMPR-1B, and type II receptors. After ligand binding, the type II receptor phosphorylates the type I receptor. The activated type I receptor then phosphorylates a member of the Smad family of intracellular proteins, which are the functional signal transducers of the TGF β /BMP family (94). The Smad superfamily can be subdivided into classes I–III. After binding of a BMP to its receptor, Smad 1 and 5 (class I Smads) form heteromeric Smad–Smad complexes with Smad 4 (class II Smad). The complexes regulate molecular transcriptional responses directly. Smads 6 and 7 (class III Smads) are inhibitors of TGF β /BMP signalling (93).

Bone healing in animal studies using BMP

The osteoinductive properties of rhBMP-2, rhOP-1 and purified BMP-3 have been studied in bone defects in

Table 1 Overview of identification and cloning of bone morphogenetic proteins (BMPs) and their role in osteoinduction.

BMP	Cloning (ref.)	Bone induction (ref.)
BMP-2 (BMP-2a)	1988 (43)	Bone induction in subcutaneous pockets in rats (7, 43)
BMP-3 (osteogenin)	1988 (43)	Bone induction in subcutaneous pockets in rats (7, 43)
BMP-4 (BMP-2b)	1988 (43)	Bone induction in subcutaneous pockets in rats (7, 43)
BMP-5	1990 (48)	No report on osteoinductivity. Role in embryology in the initiation of formation of particular skeletal elements (49)
BMP-6 (Vgr-1)	1990 (48)	Bone induction in subcutaneous pockets in nude mice (50)
BMP-7 (OP-1)	1990 (48, 51)	Bone induction in subcutaneous pockets in rats (52)
BMP-8 (OP-2)	1992 (53)	No report on osteoinductivity
BMP-9 (GDF-2)	1994 (54)	No report on osteoinductivity
BMP-10	1995 (55)	No report on osteoinductivity
BMP-11	1995 (55)	No report on osteoinductivity
BMP-12 (GDF-11, CDMP-3)	1995 (55)	No report on osteoinductivity. Inhibition of terminal myoblast differentiation (56)
BMP-13 (GDF-6, CDMP-2)	1994 (57)	No report on osteoinductivity. Inhibition of terminal myoblast differentiation (56). Ectopic induction of tendon and ligament in rats (58)
BMP-14 (GDF-5, CDMP-2)	1994 (57)	No report on osteoinductivity. Ectopic induction of tendon and ligament in rats (58)
BMP-15 (CDMP-1)	1996 (59)	No report on osteoinductivity

OP, osteogenic protein; GDF, growth and differentiation factor; CDMP, cartilage-derived morphogenetic protein.

animal models. These studies comprise the grafting of a segmental bone defect that does not heal spontaneously during the lifetime of the animal; such a defect is also called a critical-size defect. Complete healing of critical-size defects in rat calvariae was observed after grafting fleeces of bovine bone derived collagen that had been soaked in a rhBMP-2 solution (95). Calcifying cartilage and remineralization of the collagen carrier were observed after 1 week, which was followed by rapid bone formation (95). Yasko and coworkers (96) reported healing of femur defects by rhBMP-2 in rats. In sheep, femoral defects showed new bone formation 1 month after grafting with rhBMP-2, and complete radiographic bone union was observed 3 months after grafting; 1 year after grafting, histology showed the presence of woven and lamellar bone (97). In dogs, mandibular defects were restored by rhBMP-2 within 3 months. The bone quality as measured by biomechanical strength, degree of mineralization and bone thickness improved significantly during the next 3 months (98). Complete fusion of vertebrae in experimental spinal fusion in dogs was achieved 3 months after grafting with rhBMP-2 (99). Combination of rhBMP-2 with other carriers such as polymers of poly lactic acid (PLA) or poly glycolic acid (PGA), or a combination of both, have also led to healing of bone defects in rabbits and rats (100, 101).

Use of rhOP-1 showed similar results. Cook and coworkers (102, 103) showed that rhOP-1, linked to a carrier of collagen particles derived from bovine bone, restored critical-sized segmental ulnar defects in rabbits and dogs. Complete radiographic union was observed after 2 months in both species. Histology showed that, after 2 months, lamellar bone had formed, with marrow elements and signs of remodelling. The average torsional strength of the unions was comparable to that of intact bone. The same research group showed

similar results after rhOP-1 grafting in spinal fusions in dogs and in ulnar and tibial defects in monkeys (103, 104). Ripamonti and colleagues (105) found complete regeneration of calvarial defects 3 months after implantation of rhOP-1 linked to a bovine collagen carrier in baboons. As early as 15 days after surgery, new bone formation was observed.

A few studies have demonstrated that purified BMP-3 has osteoregenerative capacities. Purified bovine BMP-3 on a bone collagen carrier aided healing of femoral defects in rats (106), and purified baboon BMP-3 induced complete regeneration of critical-size calvarial defects in baboons within 3 months (37).

BMPs were also beneficial in maxillofacial surgery in animal studies. In dental extraction sites and in sinus augmentation in chimpanzees, rhOP-1 aided bone formation (107, 108). In goat maxillary sinus floor elevations, implantation of rhBMP-2 on a collagen carrier showed increased radio-opacity, histological examination revealing the presence of dense trabeculae and bone marrow, but no cortical bone, 12 weeks after surgery (109). These data show that rhBMP-2, rhOP-1 and purified osteogenin are able to repair critical-sized defects in various species within a period of 3 months.

Human studies with rhBMP-2 or rhOP-1

Recently, the first human pilot studies have been published using rhBMP-2 or rhOP-1 on a collagen carrier for bone reconstruction (1–4). The results of these studies show a large variation among the responses of individual patients. Boyne and co-workers (1) implanted collagen sheets soaked in rhBMP-2 solution in the maxillary sinuses of 12 edentulous or partially edentulous patients with severe atrophy of the maxilla. The subsequent increase in height of the treated maxilla varied between 2.3 and 15.7 mm. However,

rhBMP-2 grafts were unable to form bone when applied in mandibular ridge augmentation (2). In tooth extraction pockets, where the graft is surrounded by bone, all grafts were replaced with newly formed bone tissue (2). We performed a histological evaluation of the effect of rhOP-1 coupled to a collagen carrier, grafted in the maxillary sinuses of three patients with maxillary atrophy (Fig. 1). Excellent bone formation was found in one of the three patients, but the two other patients showed little or no bone formation after 6 months. In these two patients, persistent device remnants were found, surrounded by fibrous tissue (3). Bulstra and coworkers (4) used rhOP-1 for grafting of six human fibula osteotomies. Five of the six patients showed bone healing, but one patient did not respond to the rhOP-1-containing graft.

The inconsistent results from these clinical pilot studies suggest that certain factors, which are currently unknown, negatively affect the BMP-dependent bone

induction process in humans. For a better performance of BMP-containing bone-graft substitutes, these factors need to be elucidated. In the remainder of this review, factors that may influence BMP-dependent bone induction will be discussed. Modulating factors, known from animal studies and *in vitro* experiments, are BMP concentration, carrier properties and influence of local and systemic growth factors and hormones.

BMP concentration and bone induction

In vitro studies have shown that femtomolar concentrations of BMP initiate chemotaxis of several cell types. Chemotaxis of monocytes occurs by such concentrations of BMPs-3 and -4 and OP-1 (110, 111). BMP-2 was also found to be chemotactic for mature osteoblasts (112). BMP doses in the nanogram range have shown mitogenic and osteogenic effects in cell culture experiments (82, 83). However, macroscopic quantities of

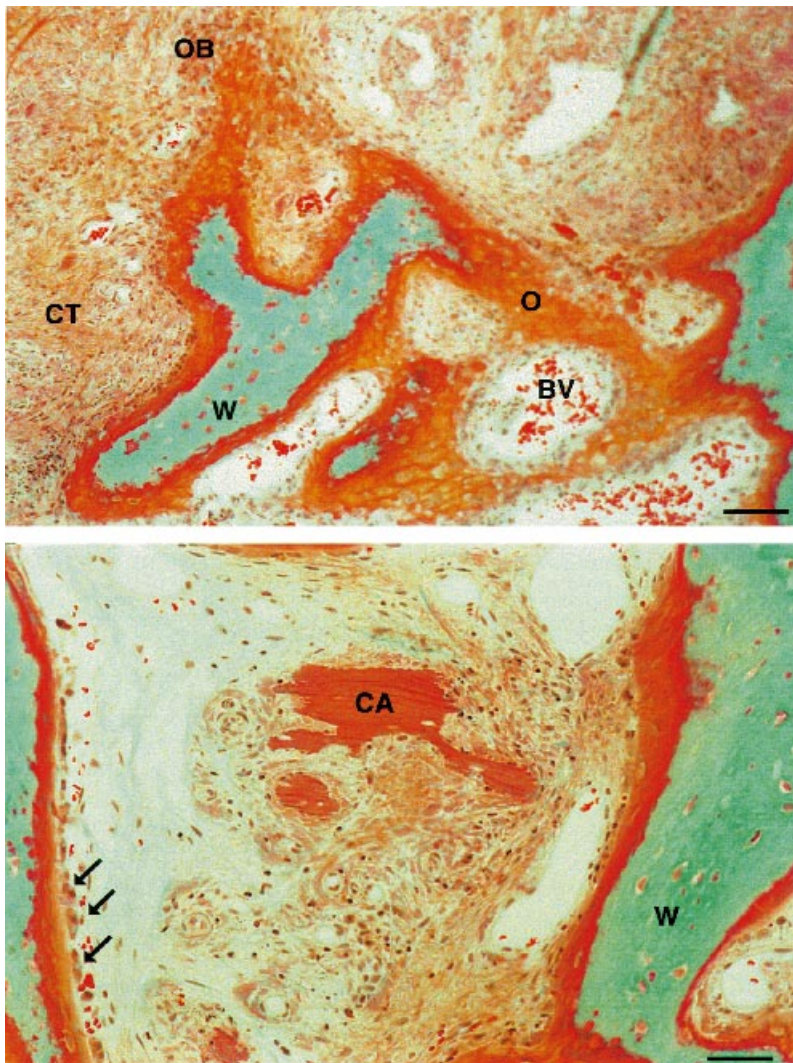


Figure 1 Overview (upper figure) and detail (lower figure) of bone induction in the human maxillary sinus by rhOP-1. Undecalcified histological sections, stained with Goldner's trichrome stain. Calcified bone stains green, osteoid stains red, connective tissue stains brown. The upper figure shows very rapid bone formation, as indicated by thick osteoid seams that seem to spread from bone trabeculae consisting of woven bone (W). OB, osteoblasts; O, osteoid; CT, connective tissue; BV, blood vessel. Bar represents 100 μ m. The lower figure shows a remnant of the OP-1 carrier (CA), lying between trabeculae of rapidly forming bone. Note that bone has developed independently of the carrier surface. The carrier consists of decalcified bone matrix, as indicated by its red colour and the few empty osteocyte lacunae, visible as small white slits. The piece of carrier is in the process of resorption, as indicated by its scalloped appearance. W, woven bone; arrows, osteoblasts. Bar represents 45 μ m.

bone *in vivo* are induced only by milligram quantities of purified BMP (113), or doses of rhBMP in the microgram range (96). These data indicate that the threshold dose of BMP for *in vivo* bone induction is several orders of magnitude greater than that for cell responses *in vitro*. In addition, differences in threshold doses have been observed between species. Yasko and coworkers (96) reported that the use of 1.4 μg BMP-2 in rat segmental defects of the femur did not result in union, whereas a dose of 11 μg was sufficient for complete union. In the rabbit, 3.13 $\mu\text{g}/125\text{ mg}$ carrier was still insufficient to heal segmental ulnar defects (102). In a subcutaneous bone induction bioassay in the rat, concentrations of 0.04–0.2 μg osteogenin/mg collagen were sufficient to induce bone, whereas a concentration of 0.8 μg osteogenin/mg collagen – up to 20 times as much – was needed to achieve the same effect in baboons (37).

Numerous studies have shown that, within one species, the amount of bone formation is dependent on the BMP dose used (95, 96, 100, 101, 103). A plateau in bone volume is reached with a range of effective doses, but the larger doses of BMP seem to reach this plateau more rapidly. Zegzula and colleagues (100) used different doses of BMP-2 on a PLA carrier and found a concentration-dependent difference in radiopacity only in the first 2 weeks. After 4 weeks, all concentrations used showed similar radio-opacities (105). Ripamonti *et al.* (105) implanted different doses of OP-1, on a bovine collagen carrier, in large calvarial defects in baboons. Concentration-dependent differences in bone volume were observed up to 3 months after implantation. Analysis of the implants after 1 year no longer showed a dosage dependency. When the induction process is accelerated with larger doses of BMP, cartilage and bone formation seems to occur simultaneously (46).

Excessive bone formation, spreading outside the original contour, has been reported after use of very high doses of BMP. The threshold for such excessive bone formation was also dependent on host species: in baboons this dose was 500 μg rhOP-1 in 1 g carrier (105); in rabbits, 1200 μg rhOP-1 in 500 mg carrier was used (103), and a concentration of only 10 μg rhBMP-2 in 100 μl produced excessive bone formation in rats (114). The excessive bone volume eventually remodels to follow the original normal bone contour (105). Ripamonti and colleagues (105) proposed optimal doses of between 100 and 500 μg rhOP-1/1 g carrier in baboons. Greater doses resulted in excessive bone formation, whereas lower concentrations did not induce the maximal bone volume. These data suggest that an optimal dose of BMP exists and that this dose is dependent on the host species.

The optimal BMP concentration seems also to be dependent on the implant location. Intramuscular implantation of BMP resulted in enhanced bone formation compared with that induced by subcutaneous

implants (115). This difference may be caused by a difference in blood supply (115), or muscle cells might be more responsive to BMP, as myoblasts in culture treated with BMP show consistent osteogenic differentiation (77). Bulstra and coworkers (4) implanted rhOP-1 in critical-sized defects in the proximal human fibula at a dose of 2.5 $\mu\text{g}/1\text{ g}$ collagen carrier. They observed excessive bone formation outside the original fibular bone contour, suggesting that the dose of rhOP-1 could be reduced. However, when the same dose of rhOP-1 on the same carrier was implanted in the human maxillary sinus, excessive bone formation was not observed (3). These data suggest that the presence of muscle tissue provides favorable conditions for bone induction and that the concentration of BMP can be reduced in such an environment.

Hormones and growth factors affecting BMP activity

A number of local and systemic factors have shown to influence BMP-dependent bone formation, but the studies are often contradictory. Local factors that have been shown to act synergistically with BMP are basic fibroblast growth factor (bFGF), prostaglandins and TGF β . bFGF has shown synergistic effects with BMP-2 in rat marrow cell cultures (116, 117), but high doses of bFGF caused a profound inhibitory effect *in vivo* (117). In the cranial periosteum of the rabbit, the amount of BMP-2 could be greatly reduced by combined use with prostaglandin E₁ (118). TGF β attenuated the stimulatory effect of rhBMP-2 in cultures of human bone marrow stromal cells (70), but stimulated OP-1-dependent bone induction in adult baboons *in vivo* (119).

Systemic factors may also potentiate BMP action. Glucocorticoids increased osteoinductivity of BMPs-2, -4 and -6 tenfold in secondary rat calvarial cell cultures (84). A stimulatory effect of glucocorticoids on BMP-2 effectiveness was also shown in MC3T3 cells and in rat osteoblasts (61, 67). Vitamin D acted synergistically with BMP-3 in human bone marrow cultures (71), and also enhanced the osteoinductive actions of BMP-2 implanted in intramuscular sites in mice (61). Beta-estradiol enhanced the BMP-2-induced increase in alkaline phosphatase activity in MC3T3 cells (61). These data show that combined use of BMP with other hormones or growth factors may improve bone formation. Alternatively, these data imply that the local and systemic conditions of the host – that is, the supply of native growth factors and hormones – are important and may influence bone induction *in vivo*.

Role of BMP carriers in bone induction

Although BMP can induce bone formation when added as a solution, and not bound to a carrier (120, 121), the dose needed to induce endochondral bone formation

can be greatly reduced when BMP is combined with an appropriate carrier (122). Collagen carriers, prepared by complete demineralization of bovine trabecular or cortical bone particles, have been shown to meet these requirements in animals (123, 124). In clinical practice collagen has been used for more than 25 years in the replacement of ligaments, tendons and other soft tissues and as wound dressings, where it has proven its clinical safety (125, 126). However, because of the possible risks of pathogen transmission of allogenic or xenogenic materials, the search for synthetic carriers continues. Synthetic materials that are potential candidates to serve as BMP carriers include ceramics, bioactive glasses, and polymers. Ceramics can be classified as non-resorbable, such as synthetic hydroxyapatite (127, 128), or resorbable, such as β -tricalcium phosphate (122, 129) and calcium sulphate (130). Bioactive glasses and glass-ceramics contain SiO_2 , Na_2O , CaO and P_2O_5 in specific proportions. These materials form a tight bond with surrounding tissues by the *in vivo* formation of a biologically active hydroxy carbonate apatite (HCA) layer (131). Polymers used as carriers of BMP comprise the various forms of (poly) alfa-hydroxyl acid (Phydroxyapatite) such as (poly) L-lactic acid (PLLA), (poly) glycolic acid (PGA) and their copolymers, (poly) D,L-lactic acid-co-glycolic acid (PLGA) (132). Polymers have been applied in clinical practice in fracture fixation for 15 years (133) and are interesting as BMP carriers because of the possibility of controlled drug release.

Biocompatibility of carriers

Titres of antibodies against allogenic or xenogenic implants of collagen have been reported occasionally, but did not show interference with bone formation at the grafted site (4, 134). However, collagen of allogenic or xenogenic origin causes a potential risk of pathogen transmission (135, 136). Synthetic carriers should be free of this risk, but rejection or unwanted tissue reactions can still occur. Inflammatory tissue reactions have been reported after implantation of Phydroxyapatite-polymers (100, 137, 138) and resorbable ceramics (122), but addition of BMP seems to decrease the inflammatory reaction. Zegzula and colleagues (100) showed that increasing the amounts of BMP-2 on a co-polymer carrier caused a decrease of inflammatory cell infiltrate. It is not clear whether the inflammatory reaction is a wanted or an unwanted side effect. Inflammatory cells release cytokines, which may mediate the bone formation process (139–142). Indeed, enhanced BMP-dependent bone formation, probably mediated by interleukin-1, was seen in mice with a systemically induced inflammation (143). However, it is well known that chronic inflammation as a result of, for example, periodontitis or rheumatoid arthritis (144, 145), stimulates bone resorption and is not a desirable condition for bone induction.

Osteoconductivity of the carrier

Grafts with good osteoconductive properties form a tight bond with the host tissue, which is desirable for the restoration of the bone function at short notice. The osteoconductivity of DBM, which mainly consists of collagen type I, has been well shown (25). Most synthetic materials containing hydroxyapatite also show good osteoconductive properties. Bioactive glasses and synthetic ceramics support the bonding of bone tissue. The bioactive glasses form a tight bond with tissue through the HCA layer that is formed on the glass surface after implantation (137, 146). Polymers are not osteoconductive (147, 148), but a combination of hydroxyapatite with polymers into a composite was found to improve the graft-to-bone binding (148).

Bioactivity of carriers

Several carriers for BMPs have shown distinct osteogenic properties by themselves, which may be supportive for BMP-dependent osteoinduction. Collagen type I has been shown to stimulate osteoblastic differentiation of cells in culture (149). *In vivo*, the addition of collagen type I was shown to aid bone formation in grafts containing osteogenin (150). PGLA implanted in the medulary canal in sheep stimulated local osteogenesis (151). *In vitro*, PGLA stimulated growth and osteogenic differentiation of osteoblastic cells (152). Hollinger (153) found an accelerated rate of healing of tibial defects in rats after grafting with a co-polymer of PLA and PGA. The osteogenic effects of ceramics and bioactive glasses are often ascribed to the binding of native proteins and growth factors to the surface of these grafts. The local accumulation of extracellular factors at the glass surface may subsequently stimulate, or even induce, bone formation (154).

Kinetics of release of BMP from a carrier

BMPs in solution are quickly cleared from the system, which may explain why very high doses of BMP are needed for bone induction when they are used without a carrier. An appropriate carrier retains BMP at the grafted site for a period of time sufficient to induce bone. The kinetics of release of the BMP from the carrier and the retention of biological activity of BMPs are of crucial importance to successful bone induction. A too rapid clearance of BMP is effectively prevented by the use of collagen carriers. Release studies show that collagen carriers release a bulk of BMP initially, followed by a more gradual release thereafter (reviewed in 155). This release pattern is effective, but the question remains whether the dose of BMP can be further decreased and retain a more sustained release pattern. Combination of BMPs with non-resorbable ceramics did not result in bone induction (156, 157). This is probably due to the lack of resorption of hydroxyapatite and the strong

binding of BMPs to hydroxyapatite, which prevents BMP release (158). Indeed, when BMPs were combined with resorbable ceramics, bone was induced (122, 159). When non-resorbable ceramics were combined with BMPs and collagen, bone induction was not hindered, possibly because of a sufficient release of BMP from the collagen (156, 157, 160, 161). Polymers and bioactive glasses are promising materials for drug delivery, as their material properties – and thus their release kinetics – can be varied by changing the production procedure or the ratio of their components. However, a matter of concern with these carriers is that a large proportion of the proteins do not retain activity after release from the carrier (162).

Biodegradation of carrier

Carrier degradation after implantation is preferable, in order to aid the release of BMP and to obtain complete replacement of the graft by bone. Bioactive glasses and PLA/PGA polymers degrade after contacting (body) fluids (132), whereas degradation of collagen and resorbable ceramics does not depend on cellular activity. When the degradation is too slow, bone formation can be inhibited (129, 163); when the degradation is too fast, BMPs are released too rapidly and the risk of fibrous ingrowth, and thus failure of bone healing, is increased. Biodegradation of the carrier should therefore coincide with the rate of endogenous bone formation. Addition of BMP to a carrier affects the resorption rate of the carrier. An enhanced resorption rate of PLA and DBM carriers in the presence of BMP has been observed in several studies (3, 105, 164).

Geometry of the carrier

The geometrical properties of a carrier may greatly influence the performance of the BMP graft. Geometrical parameters such as size and shape can influence the degradation rate of the carrier, the rate of release of BMP, and the bonding of bone to the implant. Some geometrical configurations, for example solid hydroxyapatite particles and solid polymer discs, have been found to be unfavourable for bone induction (165, 166); conversely, porous discs or blocks of hydroxyapatite were favourable for bone induction, and granules of hydroxyapatite with identical pore dimensions did not elicit bone formation (128, 167). Pore size of hydroxyapatite was found to be optimal between 300–400 μm (168). For polymers the particle size may be important, as rhBMP-2-loaded PGLA particles of $247 \pm 54 \mu\text{m}$ diameter were superior to particles of larger size in healing of bone defects in rats (164).

Sigurdsson and coworkers (169) have shown that the consistency of the BMP-loaded carrier also determines the extent of the area where bone is formed and its density. They reported that use of bovine tendon-derived type I collagen and PGLA co-polymer carriers resulted

in a high bone density, but a low total bone volume, because these carriers were not able to maintain the defect space adequately. DBM and bovine crystalline bone matrix did maintain the defect space adequately, resulting in a larger bone area, but lower bone density (169).

Other areas of potential clinical use of BMPs

BMPs were originally discovered in bone tissue, and currently their only clinical use lies in promoting bone healing. However, they are expressed in a wide range of non-skeletal tissues during mammalian development, including germ layer, whiskers, hair, teeth, urogenital system, gut, heart and brain (170, 171). These observations suggest that BMPs have a much broader role in overall tissue morphogenesis, and in repair of other tissue systems. Indeed, successful use of BMPs has been reported in several animal models of disease. OP-1 (BMP-7) enhanced functional recovery after focal cerebral infarction in mature and infant rats, when injected intraperitoneally or into the cisterna magna (172, 173). OP-1 also reduced the severity of injury after ischaemic acute renal failure in rats, by minimizing tubular necrosis and tissue infarction in addition to reducing apoptosis (174). These data suggest that OP-1 may represent a potential new treatment with which to enhance functional recovery after stroke, and acute renal ischaemic injury, in man. However, reports of clinical studies have not yet been published. In dental research, BMPs have been used to stimulate periodontal wound healing in dogs, with good result (175, 176); again, clinical studies are, as yet, lacking. These animal studies still indicate that BMPs have possibilities for clinical use other than bone repair alone.

Summary and conclusions

Although bone regeneration with BMP in animals has been generally successful, recent human studies showed a large variation in individual responses. BMP concentration, carrier properties, growth factors, hormones and the presence of target cells have been shown to affect BMP activity in animal studies and in *in vitro* experiments. This review discussed these factors in relation to human application. The clinical results suggest that the BMP concentration needed in humans depends on conditions at the site of the graft: the supply of local and systemic growth factors and hormones and the presence of target cells. The fibula is surrounded by muscle tissue with a good blood supply, and fibula defects treated with an rhOP-1 carrier (2.5 mg/g collagen) showed excessive bone formation in five of six patients (4). The maxillary sinus is an area surrounded by atrophic maxillary bone and buccal mucosa, but muscle tissue is absent, and the conditions in this anatomical environment are possibly more

critical for bone induction. Indeed, at this site, grafting of the same rhOP-1 carrier induced little or no bone in two of three patients treated (3).

The effects of BMPs have been shown to be concentration dependent. The local concentration of BMP will be greatly determined by the release kinetics of the carrier. The collagen carriers that have been used in clinical studies effect an initial bulk release (155); such a release may result in excessive bone formation at locations with favourable conditions. However, at locations with less favourable conditions, the BMP clearance might be faster than the bone-induction response of the host. In both cases, a carrier that provides a slower release of BMP, resulting in a lower local dose of active BMPs over a longer period of time, might be preferable. A graft with such properties would also reduce the total dose of BMP needed for successful bone induction.

In summary, the capability of BMPs to induce bone formation is well proven in animal studies and has recently been demonstrated in humans. The challenge today is to find ways to apply these drugs with consistent success in various applications in humans. We suggest that future studies will need to focus on the development of carrier materials that have mechanical properties and surgical practicality appropriate for controlled release of BMP.

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