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REVIEW ARTICLE

Bone morphogenetic proteins (BMP): clinical application for reconstruction of bone defects

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Abstract

Since the introduction of bone morphogenetic proteins, their use has become an invaluable ally for the treatment of bone defects. These proteins are potent growth factors, related to angiogenic and osteogenic activity. The osteoinductive capacity of recombinant bone morphogenetic protein (rhBMP) in the formation of bone and cartilage has been confirmed in in vitro *studies and evaluated in clinical trials. To obtain a therapeutic effect, administration is systemic, by injection over the physiological dose. Among the disadvantages, ectopic bone formation or high morbidity in cases of spinal fusion is observed. In this review, the roles of bone morphogenetic proteins in bone repair and clinical applications are analyzed. These findings represent advances in the study of bone regeneration and application of growth factors for more predictable results.* (Gac Med Mex. 2016;152:342-6)

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Introduction

Bone can be repaired by itself, but this fact has been established not always to turn out entirely satisfactory, especially in case of large defects where it is necessary to introduce stuffing material that later will be substituted by new tissue. Among all bone allografts and substitutes currently used in surgical medicine, allografts exhibit the best osteoinductive and osteoconductive properties. However, owing donor site morbidity, risk for infection, etc., the search for alternative therapies continues[1].

Successful bone repair depends on several factors, including a sufficient amount of growth factors, an adequate bone matrix and mechanical stability. The repair occurs under the same bone formation patterns, but the specific repair mechanism is determined by the presenting environment. The source of repairing cells after a fracture or osteothomy can be: the periosteum osteogenic inner layer, osteoprogenitor cells associated with blood vessels of the Havers systems within cortical bone, endosteum cells, bone marrow undifferentiated mesenchymal cells and surrounding tissues undifferentiated cells with capacity to differentiate, as required. However, delayed or failed union of bone segments is a complication that occurs in between 5 and 30% of patients with lesions[2,3].

Osteoinduction is the differentiation process of mesenchymal cells into osteoprogenitor cells and, finally,

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into osteoblasts to form new bone[4]. Growth factors are an example of highly potent osteoinductors. The transforming growth factor beta (TGF-β) superfamily comprises a number of related proteins, with some being able to induce bone formation. This group contains the most potent osteoinduction factors, the bone morphogenic proteins (BMPs)[5].

To date, 20 different BMPs have been identified. Currently, BMPs can be synthesized by molecular techniques and, therefore, only limited numbers might potentially be obtained, which has prompted the conduction of studies where their osteoinductive potential is trying to be proven[6,7]. In spite of BMPs important positive effects, it remains unclear why the excellent results obtained in *in vitro* studies and in animal models are difficult to replicate in a clinical trial. Their use might be limited owing to several inconveniences, such as their rapid degradation, high costs, necessity of high doses, osteolysis, ectopic bone formation and inflammation. Even with disadvantages, there is the need to develop a system or vehicle for BMP continuous release[8,9]. The following review summarizes current knowledge on the role played by BMP in the repair of bone defects, their therapeutic potential, adverse effects and their possible relationship with cancer.

BMPs

The history of these proteins goes a few decades back. Dr. R. Marshall Urist discovered that demineralized bone matrix stimulates the formation of new bone tissue. This led to BMPs isolation, the only proteins known to induce new bone formation[10]. BMPs are a group of low molecular weight glycoproteins involved in the growth and development of several tissues and organs such as bones, the heart, the kidneys, eyes, skin and teeth. The factors that influence bone remodeling stimulate mesenchymal stem cells differentiation into osteoblasts[11]. BMPs are synthesized in mesenchymal cells, osteoprogenitor cells, chondrocytes, osteoblasts and platelets within the extracellular matrix[12].

In natural form in the body, these proteins are released during bone repair and remodeling. *In vivo* studies with animals have demonstrated promising effects with no need for any osteoconductive material; however, for clinical application in humans, replicating the same results has been difficult. The different types of BMP are closely related in structure and function. When the BMP-derived amino acid sequence is compared, they can be classified into 4 subgroups. The first subgroup includes BMP-2 and 4. BMP-2 plays a fundamental role in the chondrogenesis and osteogenesis, as well as revascularization processes. BMP-2 is considered essential in fracture repair. Tsuji et al. demonstrated that mice with alterations in the production of the protein had normal bone development; however, in fracture repair there was a deficiency, since the other BMPs are unable to substitute the BMP-2 function during bone repair[13]. The second subgroup includes BMP-5, 6, 7 (also known as osteogenic protein-1 [OP-1] and 8 [OP-2], which are the second group of proteins with osteogenic capacity. BMP-7 also stimulates the production of erythropoietin, a hormone produced by the kidney that stimulates the generation of red blood cells from precursor cells that is useful in the treatment of chronic renal failure[14]. BMP-2 and BMP-7 are already approved for clinical use. BMP-9 and 10 comprise the third osteogenic group, while BMP-3 or osteogenin forms the fourth group, which acts as an inhibitor of the above. The other members of the family lack osteogenic activity (BMP-12, 13, 14 and 15)[8].

BMP clinical application

Fracture delayed or failed union represents an obstacle in the treatment of bone defects, which causes pain and possible limb length difference, in addition to the potential of necrosis and loss of function[15]. Although BMPs potential has been known for decades, their clinical use has been limited. pH changes cause a reduction in BMP-2 biological activity and, for this reason, when clinically used, it has to be reconstituted in proteins to preserve its activity after implantation[16]. In order to compensate its short half-life *in vivo* (1-4 hours) the first option has been to consider high doses of recombinant human BMP (rhBMP)[17], and case reports have demonstrated that in the absence or delay of bone segments union in fractures, the use of rhBMP-2 results in successful bone formation without the use of osteoconductors[18].

In addition to local administration, BMPs have been systemically applied in rats for the following indications: bone formation in osteoporosis, kidney regeneration in acute and chronic renal failure, liver regeneration, coronary artery ischemia and stroke. The results of these studies demonstrated that BMPs induce organ regeneration resembling embryonic development, with few side effects, such as bone formation at the injection site[19].

In clinical cases, such as the one published by Baltzer et al. in 2012, a patient who sustained femoral neck fracture in a ski accident was reported. Initially, he was

treated with 2 hip screws. Four months later, the patient was limping owing to pain, in addition to his leg having shortened and the fracture being incompletely repaired. After 9 months of extensive treatment, the patient was not yet free of pain, and the segments did not show signs of union. The patient agreed to alternative treatment, which involved 4 computerized tomography-guided 2-mg rhBMP-2 injections administered at the site of the lesion. The first 3 injections were administered in a 1-week interval, and the fourth 3 weeks later. Six weeks after treatment, tomography images demonstrated approximately 55- 60% of union. This report concludes that BMP-2 can be used to induce bone formation without the presence of an osteoconductor[20].

In 2009, Zimmerman et al. assessed the efficiency of BMP-7 versus allograft in patients with tibial fracture union delay. Twenty-six patients with allograft failure were administered a single dose of BMP-7 of 3.5 mg powder with protein mixed with 2 ml of patient's blood and associated with 1 mg of collagen. In order to prevent the protein from being lost by bleeding at the moment of intervention, a hemostatic sponge was placed on the implantation area in order to promote its permanence on site and to obtain hemostasis. Of the 26 patients, fracture consolidation was observed in 24 cases and with only 2 required a new surgical procedure. The authors conclude that patients who were administered BMP-7 had higher defect repair capacity in comparison with allograft[21].

Due to the known capacity of BMP-2 to stimulate bone regeneration, there are reports assessing its use in the maxillofacial area. Katanec et al. evaluated rhBMP-2 clinical use to induce bilateral maxillary bone height increase followed by dental implants. A 61-year old patient underwent the procedure, with rhBMP-2 being administered via absorbable collagen sponge with a dose of 3.75 mg for the right side and 1.5 mg for the left side. Surgical sites were protected with a rigid titanium membrane and, at 6 months, the site was reopened and the membrane removed to place 3 dental implants. The authors observed good primary stability for subsequent oral rehabilitation. The results reported in this clinical case indicated a mandibular height increase of up to 5.5 mm, which allowed concluding that this treatment option may be considered prior to dental implant placement at zones with low bone height[22].

In contrast with the good results obtained in the above case, in a model of dental extraction where the alveolar ridge was measured, Kim et al. assessed the efficacy of demineralized bone matrix in combination with rhBMP-2. Of the 69 patients, 35 were grafted the bone matrix with rhBMP-2 at a 0.05 mg/ml concentration. They assessed the graft safety by means of blood tests and tomography at the level of the alveolar ridge at 3 months. No adverse results or immune reactions were found, although differences were also not found at the level of the bone ridge[12].

The use of BMPs has demonstrated promising results; however, they should not be considered as the panacea for the treatment of union failure. There is indeed controversy regarding the use of BMPs in bone defects. The conduction of further studies is still necessary to substantiate the use of BMPs as an effective treatment modality in regenerative surgery and medicine.

BMPs and cancer

The role of BMPs in cancer biology, especially in breast cancer, has been widely investigated. Today, these proteins are known to be broadly involved in the regulation of cancer cells functions, which range from growth, death, migration and invasion to epithelial-mesenchymal transition[24].

BMPs are able to regulate breast cancer cells growth. However, while some types of BMP have cancer cells-proliferation inhibitory effects, others show the opposite effect. For example, BMP-2 and BMP-6 inhibit cancer cells proliferation in breast cancer, whereas BMP-4 indirectly promotes cancer cells proliferation by inducing the release of other growth factors, such as the fibroblast growth factor (FGF), the epidermal growth factor (EGF) and the hepatocyte growth factor (HGF)[24].

Kallioniemi et al., in their 2012 review article, assessed the relationship of BMP-4 with cancer. BMP-4 is expressed in tumors such as melanoma and ovarian, gastric and renal carcinoma, especially in gastric, hepatocellular and colorectal carcinoma. BMP-4 elevated expression is related to better prognosis in ovarian cancer and poor prognosis in head and cervical squamous cell carcinoma. These data imply that BMP-4 can possess variable functions in different types of tumors. However, it should be noted that the number of samples that have been studied for a particular type of tumor is still relatively small and, therefore, further studies are required to clarify the situation of BMP-4 expression in cancer[25].

The role of BMP-7 in cancer expansion has also been evaluated using animal models. This protein can act by suppressing and promoting tumor growth depending on the type of cell line. By counteracting the

epithelial-mesenchymal transition, tumor cell growth is arrested via TGF-β inhibition, thus preventing the acquisition of a metastatic invasive phenotype. Buijis et al., in their clinical findings, suggest that a decrease in BMP-7 expression is implied in prostate cancer, and that this protein acts as a regulator of epithelial homeostasis and inhibitor of metastasis[26]. In 2011, Klose et al. assessed how BMP-7 influences cell growth in gliomas and confirmed the antiproliferative effects of the protein *in vivo*. They were able to demonstrate that BMP-7 can inhibit the development of mitosis at the G1/S phase, thus reducing cell proliferation, which led to suggest BMP-7 as a possible therapeutic molecule for the treatment of metastasis[27].

BMP adverse effects

Spinal fusion is a surgical procedure consisting in removal of the intervertebral disc and fusion of the adjacent vertebral bodies. BMP-2 has been compared with allografts assessing its safety and efficacy. In a meta-analysis conducted by Fu et al., they evaluated the effectiveness and adverse effects produced by rhBMP-2 as treatment in lumbar spinal fusion. In lumbar spinal fusion procedures, both rhBMP-2 and allograft showed similar results; however, rhBMP-2 was associated with higher risk for retrograde ejaculation and genitourinary problems. For cervical spinal fusion, rhBMP-2 was associated with dysphagia. This review concluded that there is no significant advantage for the use of the growth factor in comparison with iliac crest allograft[28].

When some adverse effects have been documented, the use of BMP-2 in lumbar and cervical spinal fusion has raised concerns. Specifically, some of the reported complications have been vertebral osteolysis, graft sinking, anti-BMP-2 antibodies formation, ectopic bone formation and hematoma formation[29].

Singh et al. carried out a retrospective study where they evaluated patients who attended the same institution and underwent transforaminal lumbar interbody fusion. In the procedure, 12 mg of rhBMP-2 were routinely applied; however, when reports of complications came up, the dose was reduced to 4.2 mg. The patients were assessed with computed tomographies for up to one year looking for treatment-related signs and symptoms. Of the 573 patients who received follow-up for at least one year, 8.6% (49 patients) required additional treatments, out of which 39 were due to pseudoarthrosis and 10 due to ectopic bone formation. The study concludes that these complications, though infrequent, lead to an increase in hospital costs, use of resources and additional surgical procedures[30].

Sanfilippo et al., in a retrospective study of the use of rhBMP-2, detected an increased risk for the development of postoperative radiculitis after transforaminal lumbar interbody fusion, since in the group where rhBMP-2 was used this complication was developed in 9 out of 39 cases, and in the autogenous bone group, in 1 out of 29 cases. In the rhBMP-2 group, symptoms appeared 1 to 4 days after surgery and persisted for up to 6 months[31].

It is possible that rhBMP-2, used during lumbar fusion treatment, may be related to higher risk for neurological deficit due to the proximity to the lumbosacral plexus. In this regard, Lykissas et al., in a retrospective study, compared the incidence of these deficits and pain in patients who underwent lateral lumbar interbody fusion with and without rhBMP-2. In this study, 4.2 mg of rhBMP-2 (1.5 mg/ml concentration) were administered via absorbable collagen sponges. Neurological evaluation was carried out before and after the surgical procedure, as well as 3 months, 6 months and 1 year later. Their results show evidence of higher incidence of neurological deficit when rhBMP-2 is used[32].

Conclusions

The beneficial effects of BMPs in the repair of bone defects are already known. In spite of the promising results in *in vivo* experiments, subsequent complications are of great concern. Further investigation is required to more reliably estimate the risk for cancer and adverse effects, especially in orthopedic surgery and regenerative medicine. By having this knowledge, doctors can take the risks and benefits into account when adopting new alternatives in order to offer better patient results.

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