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Review

Bone Morphogenetic Proteins: Their History and Characteristics

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Abstract: Bone tissue engineering is expected to be utilized clinically as a patient friendly strategy instead of autogenous bone grafts, and bone morphogenetic protein (BMP) is applying for bone regeneration. BMPs have been found in the 1960s and their gene cloning has been succeeded two decades later, which revealed that BMPs were members of TGF-β superfamily. BMPs have critical functions in embryonic development and tissue generation, and BMPs induce bone formation in mammals though its primary functions are different. Subcutaneous implantation of BMP in rodent reproduces endochondral bone formation occurred in embryogenesis. Recombinant human BMPs are applied for spinal fusion and non-union fracture repair and also utilized in the field of oral and maxillofacial surgery such as sinus floor augmentation. BMP regenerates mandible bone after its segmental resection in non-human primates as a preclinical trial. However, the results were not conclusive in clinical studies especially in elderly patients. A high dose of BMP is required to restore large bone defects, but it may also induce life threatening significant edema. The further studies are necessary for effective and safe application of BMPs to restore large bone defects.

Key words: BMP, Bone regeneration, Clinical application, Review

Introduction

Alveolar and jaw bone regeneration is one of the major challenges in oral and maxillofacial surgery and implant dentistry. An autogenous bone graft is still the gold standard for bone augmentation and reconstruction of the jawbone at present because of its osteoinductive, osteoconductive, and osteogenic elements[1,2]. However, an autogenous bone graft has impediments such as limited availability and donor site morbidity[2,3]. Artificial bone substitutes have been developed, but these synthetic prostheses carry an increased susceptibility to infection, incidence of extrusion, and uncertain long-term interaction with the host bone. Recent progress in tissue engineering is aimed at overcoming these problems. The basic strategy for tissue engineering consists of manipulating three elements: cells, matrix, and regulatory factors[4]. Advances in molecular and cellular biology have led to a better understanding of each of these elements. In particular, the functions of many growth and differentiation factors in bone formation are being made clear. Among these growth factors, bone morphogenetic proteins (BMPs) are the most promising osteoinductive substances for bone formation[5,6]. This article introduces the history and nature of BMPs and also presents their clinical utilization.

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Discovery of BMPs

The presence of BMPs was first suggested by Dr. Marshal R. Urist, an orthopedic surgeon at the University of California at Los Angeles, in the 1960s[7]. He found ectopic bone formation at the site of demineralized bone matrix implantation in mouse thigh muscle. He was convinced that the bone formation was induced by a protein because the activity had been ruined by heat or alkaline treatment. Since then, the race to isolate and purify BMPs has proceeded vigorously. However, the purification of BMPs from bone matrix is so difficult that no research group has succeeded in the purification of a BMP as a single molecule.

More than 20 years later, John Wozney at Genetics Institute Inc. succeeded in the gene cloning of BMPs[8]. His work revealed that BMPs belong to the transforming growth factor-beta (TGF-b) superfamily, because positions of seven cysteine were conserved, causing a familial protein structure. Over 50 members of the TGF- β superfamily and 20 members of the BMP/growth differentiation factor (GDF) subgroup family have been found to date[9].

Function of BMPs

Advances in molecular biology have revealed the primary functions of BMPs. BMPs have diverse and important functions, especially in embryonic development and tissue generation, as shown in Table 1. For example, knockout mice lacking BMP-2 or

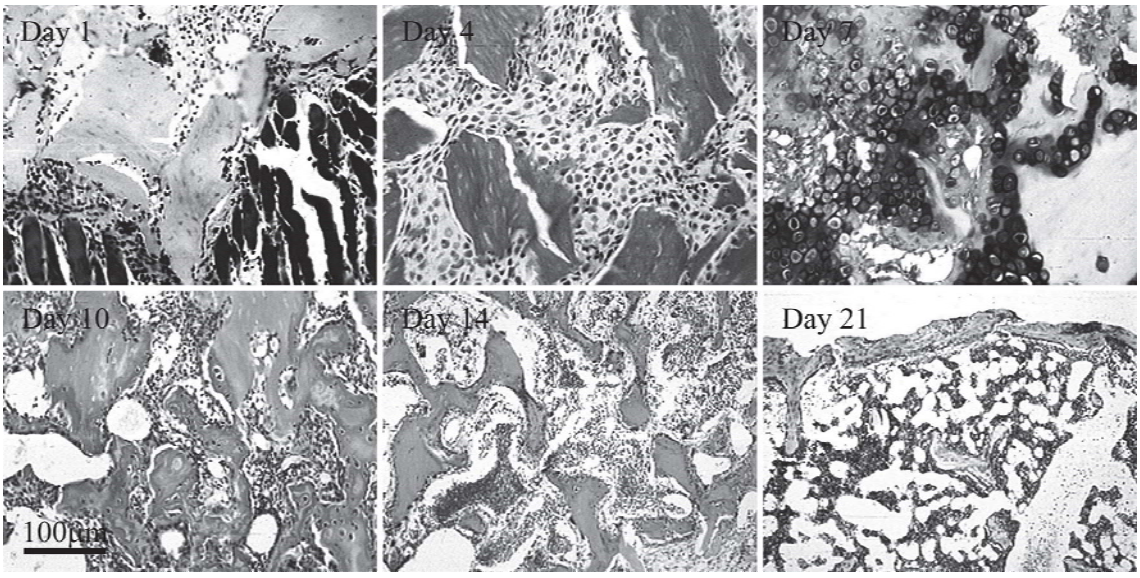

Figure 1. Endochondral bone formation induced by BMP in rat subcutaneous tissue (quote from reference #12) with modification).

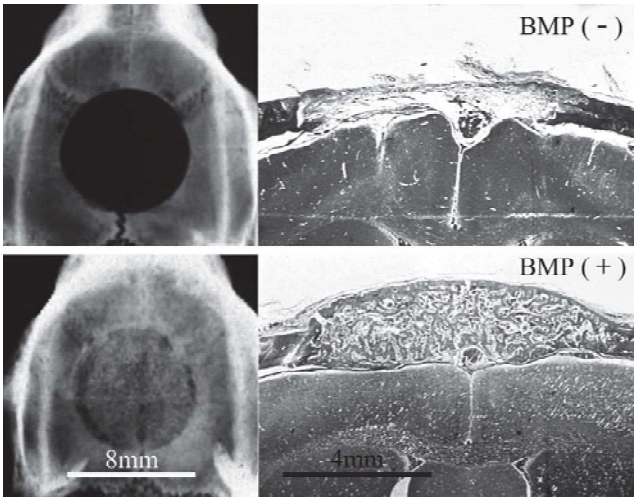

Figure 2. BMP regenerates bone defects created in rat calvaria.

Figure 4. Histological section of BMP-induced bone 24 weeks after masticatory force loading (quote from reference #13) with modification).

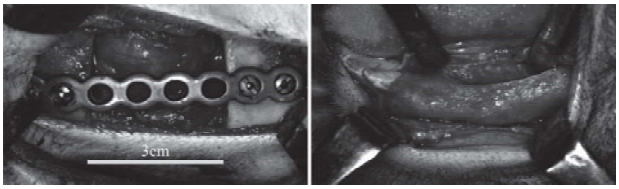

Figure 3. The 30-mm defects created in young monkeys have been regenerated by implanting rhBMP-2 (A). Right side shows 20 weeks after implantation (B) (quote from reference #13) with modification).

-4 die before birth. BMP-2 plays a significant role in the epithelial-mesenchymal interaction of organogenesis. BMP-4 is a factor in ventral induction during embryogenesis. GDF-8, known as myostatin, has a unique function in controlling muscle growth. Loss of function of this gene causes enlarged muscles, like Popeye with spinach.

Nevertheless, BMPs have the ability to induce bone growth even though their original function is different. For example, decapentaplegic (DPP) and 60A, which play important roles in morphogenesis in *Drosophila*, which does not have bones, induce ectopic bone formation when they are implanted in mammals[10]. Another example is fibrodysplasia ossificans progressiva (FOP), which is an intractable disease characterized by congenital skeletal malformations, injury and inflammation-induced extraskeletal bone formation, and is caused by the gene mutation of ALK2, a BMP-type I receptor. ALK2 is the receptor for BMP-4 and is constitutively active in FOP[11].

Bone induction by BMPs

BMPs induce heterotopic bone formation consistently in rodents. This bone formation reproduces the endochondral bone formation that occurs in embryonic development, as shown in

Fig. 1. On Day 1, mesenchymal cells migrate into the BMP implant site; these cells differentiate into chondrocytes at Day 4. Chondrocytes become hypertrophic, showing metachromasy with toluidine blue staining at Day 7. At Day 10, cartilage is replaced by new bone tissue; the induced bone continues remodeling and bone marrow formation is observed at Day 21. [12)]

BMPs induce increased amounts of bone in a dose-dependent manner. They induce bone formation at orthotopic sites and regenerate bone in bony defects. Fig. 2 shows bone regeneration in a rat calvarial bone defect by BMP/polyglycolic acid implantation 4 weeks after the implantation. Therefore, BMPs are expected to be utilized in bone regeneration in clinical cases.

In addition, BMPs have been shown to restore substantial bone defects in numerous animal experiments, even in non-human primates[13-16)]. It has already been shown that a segmental bone defect created in the mandibles of young monkeys was successfully regenerated by implanting rhBMP-2 permeating a poly-D, L-lactic-co-glycolic acid-coated gelatin sponge (Fig. 3)[17)]. The BMP-induced bone was maintained for a long period of time and excellent remodeling and consolidation of new bone were observed after masticatory force loading by rehabilitation with dental implants (Fig. 4).

However, a larger amount of BMP, 10~100 times more, is required to induce new bone in higher species such as primates compared to that in rodents. Furthermore, the bone-inducing activity of BMP is reportedly reduced with aging[18)]. Most patients with bone defects in the mandible are elderly and BMP is expected to be applied clinically for bone reconstruction especially in elderly patients. The reason for insufficient bone formation by BMP in elderly recipients might be a reduced response to BMP and a reduction in the number of cells responding to BMP due to aging. Therefore, the addition of BMP-responding cells to the BMP implant can restore the bone-inducing ability in elderly recipients and regenerate bone defects consistently[19,20)].

Clinical application of BMPs

The United States Food and Drug Administration (FDA) has approved the clinical use of rhBMP-2 (INFUSE® Bone Graft, Medtronic) and rhBMP-7 (OP-1 Implant, Olympus, Japan) as medical devices for spinal fusion and non-union fracture repair. It has been nearly a decade since BMPs first became clinically available. Several clinical studies have reported that the efficacy of BMP implants is comparable to that of autogenous bone grafts[21,22)]. However, a high dose of BMPs may induce significant edema or swelling. Cases of airway obstruction when a high dose of rhBMP-2/absorbable collagen sponge (ACS) was used for cervical spinal fusion have been reported[23,24)].

In the oral and maxillofacial region, rhBMP-2 was approved by the FDA in 2007 for use in sinus floor augmentation and extraction socket preservation. Triplett *et al.* reported the results of multicenter randomized clinical trials of the rhBMP-2/ACS application for sinus floor elevation and showed that the efficacy and safety of rhBMP-2/ACS was comparable to that of an autogenous bone graft[25)]. Herford and Boyne reported that mandibular continuity has been successfully restored by implanting rhBMP-2/ACS in 14 cases[26)]. On the other hand, Carter *et al*. reported the reconstruction of segmental bone defects of the mandible using rhBMP-2 and the results were not consistent: rhBMP-2 failed restore bone defects in 2 of 5 patients with continuity defects[27)]. It is convincing that BMPs regenerate bone in small bony defects such as extraction sockets or the sinus floor in humans, but it is not conclusive that BMPs restore large bony defects such as segmental bone defects of the mandible, because the responding cells are diminished in higher species and with aging as previously mentioned. Recently, a Finnish group reported successful jawbone regeneration by the implantation of adipose-derived stem cells with rhBMP-2[28)]. A combination graft of BMP and BMP-responding cells with a carrier may be a reliable method for regenerating large bony defects. Further studies including the development of a suitable carrier for BMP are required to generate efficient and safe approaches for bone regeneration using BMPs.

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