Bone regenerative therapy in the aging society
—especially bone regeneration using bone morphogenetic proteins—

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Abstract:
Maxillofacial bone defects can be treated with autologous bone grafting, but there are limitations such as the amount of excisable bone and larger surgical invasiveness. To overcome these limitations, various biomaterials are tested as possible substitutions of bone graft. Furthermore, to give osteoinductivities to artificial biomaterials, bone morphogenetic protein (BMP) useful as protein has been focused. With aging of the population, the application of biomaterials will have to be necessary for the elderly. Along this stream, we have performed studies on the osteogenic ability of BMP and the relationship between osteogenic ability of BMP and aging.

Key words: Bone morphogenetic protein (BMP), Osteoinduction, Bone graft, Aging

1. Introduction

With recent trend of aging of the populations in the developed countries, accidents producing a maxillofacial bone defects, such as jaw bone cyst, tumor, inflammatory disease, fracture, and a bone defect accompanying surgery have been occur much more frequently. These bone defects have been leading secondary risks of infections, fractures, functional disorders, and esthetic disorder. Also in the dental field, increasing numbers of clinically severe cases of bone defects formed in the sockets after tooth extraction¹, bone resorption due to periodontal dis-
ease, and aging mandibular atrophy have been reported in super aging society. Specifically in aging mandibular atrophy, fracture readily occur. Reduction of vertical dimension decreases the occlusal force. Furthermore, denture stability and retention worsen. These conditions make mastication and swallowing difficult. The prevention of resorption or induction of osteogenesis of alveolar bone is very important for not only preventing fracture, preserving and recovering the morphology, and retaining and stabilizing dentures but also securing the sites for dental implant placement.

Bone grafting, such as autogenous graft, allograft, and xenograft has been performed to treat these defects. In the maxillofacial field, autogenous graft is frequently performed, due to the higher taking rate of graft. Currently, it is the optimum method to fill and repair bone defects and to acquire host-graft bone fusion. However, this method requires adding surgical stress to the bone donor region, i.e., it has problems with extension of the operative time, hemorrhage, pain, infection, deformity, and functional disorder of the donor site, and limitation of the amount of excisable bone. Thus, artificial bone substituting for autologous bone has been developed. For the material of artificial bone, porous hydroxyapatite, calcium phosphate, and bioactive cement have been recently developed, but these artificial bones have disadvantages, among which the lack of osteoinductivity is particularly important.

Osteoinductivity, which is one of the elements desired to be possessed by bone graft, refers to the ability of osteoinductive factors acting on cells at the recipient site to differentiate into osseous cells and form recipient site-derived new bone. Bone morphogenetic proteins (BMPs) are the only biomaterial capable of ectopically enhancing osteoinductivity.

2. BMP

BMP was discovered by Urist et al. in 1965 as a bioactive protein localized in bone which ectopically induces bone and cartilage. Studies on synthesis of this protein and isolation of its gene were performed thereafter. In 1988, Wozney et al. purified it from bovine decalcified bone as a protein with ectopic osteoinductivity and clarified that the protein belongs to the TGF-β family. Currently, over 20 isoforms have been reported. This protein ectopically induces bone in vivo through inducing differentiation of undifferentiated mesenchymal stem cells to chondroblasts and osteoblasts. In addition, it has been clarified to play an essential role in all physiological bone formations including skeletal formation, healing of fracture, and bone remodeling, and its application is expected to be useful for the bone reconstruction/regeneration field. As I mentioned above, BMPs are capable of ectopic osteogenesis. This is the reason why the evaluation of BMP osteoinductivity has been conducted using ectopic osteogenic ability of BMPs. In addition to the above, when BMPs are used in clinical settings in the future, BMPs are presumably applied to the defect area after induction of ectopic osteogenesis, instead of directly using BMPs in the targeted area. This is the reason why animal research models of ectopic osteogenic ability were used for evaluating BMP osteoinductivity.

3. Experimental model of ectopic osteogenic ability of BMP

Ectopic osteogenic ability of BMP is evaluated using an experimental model. For BMP, BMP-2 with the highest ectopic osteogenic ability among BMPs is used. BMP purified by decalcification of bone has problems with immunity and safety for clinical application because it contains much impurity. Moreover, mass production of the component with constant quality is difficult because it is extracted in a very small amount, which is problematic with reproducibility. Thus, recombinant human BMP-2 (rhBMP-2) is used in this experiment. Moreover, locally administered BMP rapidly diffuses and the effect cannot be acquired. For local application of BMP, a carrier is necessary. Since type I collagen has been reported to have favorable biocompatibility and sustained release, type I atelocollagen, in which the antigen region of collagen, telopeptide, is removed, is used. BMP-2 may be placed subcutaneously, intramuscularly, intermuscularly, or intrafatty sites, but it has been reported that bone is most effectively induced in muscle. Thus, an intramuscular region was selected for placement of BMP-2. In summary, rhBMP-2 is appropriate for BMP, type I atelocollagen is appropriate for the carrier, and an intramuscularly site is appropriate for the placement site in the experiment on ectopic osteoinductivity of BMP. rhBMP2 and type I atelocollagen were mixed, freeze-dried, compressed, and molded into a round pellet (Fig. 1). When the pellet was placed into the rat’s calf muscles, ectopically induced bone in the calf muscles was clearly imaged on radiography after 3 weeks (Figs. 2, 3).
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4. Relationship between BMP and aging

Cellular metabolic activity declines with aging, affecting bioreactivity. The relationship between ectopic osteoinductivity of BMP and aging has been stated in several reports\(^\text{14–17}\), but in most of these studies, rhBMP-2 was not used and BMP purified by decalcification of bone was used, the data were not those on rhBMP-2, which can be produced in a large amount for actual clinical application. Moreover, evaluation was subjective using a unique scoring system\(^\text{18}\) as those frequently used noted in clinical studies on bone other than BMP. Thus, we investigated the relationship between ectopic osteoinductivity of BMP and aging based on objective data by placing rhBMP-2 with atelopeptide type I collagen in the calf muscles of rats at different weak ages and quantified the ectopic bone using dual energy X-ray absorptiometry (DXA), von Kossa staining, and proliferation cell nuclear antigen (PCNA) immunostaining. It was objectively clarified that ectopic osteoinductivity of rhBMP-2 declines with aging\(^\text{19}\).

5. Conclusion

Treatment of bone defects is more important than ever before in the aging society, with which treatment using BMP with osteoinductivity becomes more important than ever before. We objectively clarified that osteoinductivity of BMP decreases with aging using DXA, von Kossa staining, and PCNA immunostaining. Since it is unclear whether this finding is due to an aging-associated decrease in the number of undifferentiated mesenchymal cells or decline of reactivity, it is necessary to perform an in vitro study using cells of elderly rats and investigate a method to acquire the effect of osteoinductivity of BMP even in elderly individuals.

BMPs are a useful tool for bone regenerative medicine. Our study demonstrated that osteogenic ability has been decreased in elderly people. In order to improve the decreased ability, it is necessary to clarify the mechanism of BMP osteoinductivity during the aging process. Nonetheless, findings from previous studies do not help to show whether the decreased osteogenic ability was induced by aging-associated decrease in the number of undifferentiated mesenchymal cells and/or decreased reactivity. In the future, to examine those issues, we conduct an in vitro study using cells in aged rats. Furthermore, we clarify the mechanism of BMP osteoinductivity through investigation of rhBMP-2 osteoinductivity in an ectopic model and a rabbit defect model for maxillofacial bones. This will more effectively enhance research in bone regenerative medicine which involves the use of BMPs in the elderly people.
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References