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Cation of Human Recombinant Bone Morphogenetic Proteins in Pulpotomy of Primary Teeth-A Review

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Abstract

Recent research and studies on Bone Morphogenetic Proteins (BMPs) have revealed that these proteins play an important role in induction and regeneration of calcified tissues like bone and dentin. In particular, BMPs may present an alternative line of treatment in dentinal bridge formation as a part of regenerative pulpotomy. Nowadays, human BMPs with dentinogenic and osteogenic properties are becoming available through recombinant technology. The aim of this study was to discuss and evaluate the ability, potential and function of bone morphogenetic protein in induction and regeneration. We described the chemical structure and signaling pathways that BMP uses to exert its effect on pulp dentin complex. The way forward is to detect an appropriate biomaterial which will be able to increase the healing potential of the pulp through induction of large quantities of osteo-dentin and calcified barriers.

Keyword Pulpotomy, Recombinant Human Bone Morphogenetic Protein, Dentinogensis

Introduction

Urist et al found a factor in the bone matrix that has self-induction ability and induces osteogenesis during culturing non-mineralized and lyophilized components of rabbit bone. Recently, a family of Bone Morphogenic Proteins (BMPs) has been found bymolecular biology methods[1].

Studies conducted in 1980 led to separation of this composite from non-mineralized bone matrix by acid and choatropic factor. So far, 20 bone morphogenic proteins have been discovered following biochemical analysis and/or by molecular biology techniques. BMPs are multi-purpose factors (a kind of cytokine) that belong to transforming growth factor β superfamily and release proteins that are responsible for many cellular functions like cell growth, proliferation, division and death[2].

Transforming Growth Factor Beta (TGFβ) has three isophorms called TGF β1, TGF β2 & TGF β3 that are produced by white blood cells, macrophages and plasma cells. They are responsible for functions such as regulation of inflammatory process and stem cell division. BMPs play their role as ligand for the receptors on plasma membrane of the cell. They have autocrine and paracrine functions, by which they establish the organization of tissue and cell[2].

The studies carried out in the field of signal transfer have documented that SMAD, 1, 5 and 8 are the same downstream molecules of BMP that play a pivotal role in signal transfer. BMPs interact through specific receptors (BMPRs) on the cell membrane. Signal transfer through BMPs stimulates the SMAD family of proteins[3].

BMPs are the same active homodimer and heterodimer molecules. A BMP is specifically a dimeric molecule with two polypeptide chains that are held together by a disulfide bond, and its primary structure is approximately 40-50% similar to that of TGF β structure[4]. However, TGF β is recognized as a cytokine rather than a morphogen[5].

Different types of bone morphogenic proteins have been listed in table 1 according to their clinical application and membranous-skeletal performance[1,6].

Table 1: Different types of bone morphogenetic proteins

Identification	Description
Bmp2	Morphogenesis of bone and cartilage/bone formation-osteoblast division
Bmp3	Downregulation of bone morphogenesis
Bmp3b	Downregulation of bone morphogenesis
Bmp4	Morphogenesis of cartilage, tooth and bone
Bmp5	Growth of the hands, feet, cartilage and bone
Bmp6	Differentiation of osteoblasts, chondrogenesis
Bmp7	Cartilage and bone morphogenesis
Bmp8	Osteogenesis and chondrogenesis
Bmp9	Osteogenesis
Bmp11	Designing axial skeleton

Based on clinical and preclinical experiments and Food and Drug Association (FDA) approval, BMP7 is now available in the market and is used extensively in various orthopedic surgeries as an adjunct or as an alternative approach[7-8]. Further, it has been directly announced that rhBMP7 is useful in non-integrated bone fractures, and this method is prior to Autogenous Bone Grafting (ABG)[9].

Mechanism of action

BMPs are produced by primary bone cells, osteoblasts, chondrocytes and platelets. In fact, they are the factors of osteoblast differentiation that cause the division of potent mesenchymal cells intobone-cartilage cells and primary osteoblast cells[10].

To understand the mechanism of action of BMPs, their main functions at cellular level should be recognized. BMPs are synthesized as pro-proteins N-terminal signal peptide, apredomain for folding and secretion and C-terminal mature peptide.These pro-proteins are generated and divided in cytoplasm to form the components of

terminals N and C. BMP is able to bond to its specific receptor through the mature part of C-terminal[10].

Osteogenesis consists of sequential chains with four important stages[10]:

A: displacement

B: mitotic division of mesenchymal stem cells

C: division of mesenchymal stem cells to chondroblast

D: chondrogenesis and its replacement by bone

These sequential events are initiated by bonding plasma fibronectin to non-mineralized bone matrix, which increases the proliferation of mesenchymal cells three days after culture. Chemical traction, mitosis and differentiation are the most significant stages in this chain[11].

Immune response to bone morphogenetic protein

Now the questions that arise are what happens to thebody's line of defense and how body reacts to BMP culture. This subject has been quite controversial. Among the studies performed so far, no exact mechanism has been discovered for it[12].

It seems that single application of heterogeneous BMPs and non-collagenase proteins induce an average response through immunoglubine G (IgG), but it has no effect on osteoinductivity. In high doses of the same compounds,anti-BMP antibody is produced, which inhibits osteogenesis. On the other hand, the outcome of BMP administration is highly dose-dependent. Application of heterogeneous BMPs increases the necessity of using macrophages, lymphocytes and plasma cells, and more interestingly enhances the production of antibodies that affect osteogenesis[12].

Based on the evidence, the presence of maximum 100 mg heterogeneous BMP does not induce a vivid immune response. Further studies are required to confirm the reverse effect of BMP culture and its consequences after consumption in patients[13].

Target of bone morphogenetic protein in the body

The effect of BMP on osteoblast and periosteal cells has been fully investigated to gain a better insight into the activity of BMPs. Generally, there is an increase in DNA synthesis and transcription of the genes involved in synthesis of bone matrix protein. rhBMP2 inhibits the differentiation of osteoblast precursor cells into myoblast or adipoblast. Sampath et al showed that by adding OP1 (BMP7) to the culture medium of bone cells enriched with osteoblasts in different stages of differentiation, cell proliferation, collagen synthesis, alkaline phosphatase stimulation, production of cAMP regulated by parathyroid hormone and osteocalcin synthesis were initiated[14].

The cellular and molecular effects of mineralized and purified bone matrix, or recombinant BMP on different cell lines have been confirmed by many studies. Minimal pluripotent mesenchymal cell line, bone marrow cells, prerequisite osteoblasts, myoblasts, fibroblasts and neural cells react to BMPs. Numerous indicators of bone metabolism such as alkaline phosphatase, parathyroid hormone receptor, osteocalcin, osteopontin and osteonectin are regulated by BMPs. Although evidence shows that the reaction induced by BMP involves the use of specific receptors during bone and cartilage growth, the mechanism of signal transfer is still unknown. In fact, BMPs play a pivotal role in the early stages of organogenesis[15].

The most important effects of BMPs in mesenchymal and stem cells are, in fact, division of these cells into osteoblast and initiation of chondrogenesis and alkaline phosphate activity. Other hormones or cytokines cannot regulate these indicators of bone metabolism. It should be noted that during performing this test in vitro, using low concentrations of BMPs differentiates mesenchymal cells to adipocyte, while using high concentrations differentiates them into osteoblasts. This indicates the

necessity of having adequate knowledge about BMP dose to predict its effects[16]. The osteoblasts treated with rhBMP-2 are differentiated rapidly (the same as mesenchymal cells), and alkaline phosphatase, osteocalcin, osteopontin and sialoprotein levels are increased[17].

Practical aspects of bone morphogenetic protein in pediatric dentistry

In ideal pulpotomy treatment, the pulp should be vital, healthy and completely enclosed within an odontoblast-lined dentin chamber. In this case, the tissue is isolated from the harmful materials in the chamber, thereby minimizing the possibility of internal resorption. Also, the osteoblasts of non-inflammatory pulp may enter the exfoliative process and maintain it physiologically[18].

Fortunately, recent advancements in the realm of osteogenesis and dentinogenesis have opened up new horizons in pulpotomy. In fact, we will be able to produce restorative dentin by using recombinant proteins of dentinogenesislike the body proteins. The pulpal reactions against different actions in the teeth of dog and other primates have been characterized. For example, BMP4 is associated with mesenchymal-epithelial interactions during the tooth growth[19].

Most importantly, these osteogenic proteins have been found successful in dentistry and pulpotomy. BMPs are considered non-collagenase proteins, but they are closely related to collagen matrix. Fuks et al. usedcollagen alone as a cover for the pulp-free tooth of primates, but it was unsuccessful. Further studies have shown that collagen can act as a frame or scaffold to stimulate restorative dentin formation. BMP-free collagen is not capable of osteogenesis and is merely resorbed. In fact, after conducting a series of research, collagen is now being used as a transfer scaffold[20-21].

One of the objectives of science is acquiring knowledge to develop new methods to use materials to restore the damaged pulp, whether due to trauma or caries. However, histological studies have shown that pulp has faced intensive invasions, including formation of a superficial layer of hard tissue and pulp mummification, as a result of application of formaldehyde-based materials like tricresoland formalin (formocresol) as well as materials such as zinc oxide eugenol, ferric sulfate and gluteraldehyde, which create a protective layer on the pulp [22].

Science moved toward using bio-materials that, after invention of calcium hydroxide and emergence of Guedes-pintopate, hydroxyl-apatite, tetracalcium phosphate, Calcium Beta Glycerol Phosphate Ca-(BGP), Mineral Trioxide Aggregate (MTA) and recent advancements in bio-materials such as BMP, restored the pulp to its healthy state via biological formation[23].

Nakagawa et al. isolated the healthy human permanent pulp and covered it with autogenous dentin pieces. After five days, they observed inflammatory cells around the dentin pieces. They also observed crystal structures that increased continually up to 24 hours. Later, they found that these structures joined together and created an organic tissue[22].

Tziafias et al. reported that the bioactive component of dentin was able to induce polarization and cell secretion. Okomoto et al. used subcutaneous tissue of mice for culture. They inserted the dentin matrix into this tissue and found the formation of a bone-like tissue that consisted of trabeculae, osteoblast cells and veins[24].

Okomoto et al used the subcutaneous tissue of rats for implantation, he inserted dentin matrix into tissue and observed formation of bony like tissue with bony trabeculae, osteoblastic cells and vascularization[25].

Casagrande et al. studied the role of dentin-derived BMP2 in differentiation of stem cells from exfoliated deciduous teeth (SHED) into odontoblast cells and showed that BMP2 was necessary for differentiation of SHED to odontoblast[26].

In 2008, an experiment was conducted in Brazil in which coronal pulp was removed and disinfection was carried out with chlorhexidine digluconate 1% to eliminate the microorganisms. The carious lesions were removed and the coronal area was emptied. rhBMP, which was located in collagen scaffold after hemostasis process, was placed in the coronal chamber, which covered the pulp base. They placed gutta-percha on rhBMP to protect it from the upper restorative materials. Then, they etched the cavity with acid and added the dentin bonding agent. Finally, they revived the cavity with composite resins. They followed the patients periodically for one year. They selected two exfoliated teeth and fixed them with 10% formalin. After demineralization in 10% acid formic, 5 µm quasi-longitudinal sections were prepared. They observed foreign organs, small cavities with large polymorphonuclear cells, layers of scattered dentin-bone (islands of osteodentin-like calcium tissue), reactionary dentin and inflammatory process at the lower margin under suppurative foci microscope.[27]

A chain of actions can be considered for pulp restoration, as follows[27]:

Fibroblast-like cells are moved to the sectioned area from the lower pulp tissue at the presence of BMPs in direct contact with pulp tissue to proliferate there. Then, formation of passive matrix or using the scaffold itself for the stem cells and undifferentiated mesenchymal cells to bond to BMP-2-4 and 7 causes the differentiation of cells bonded to odontoblast, which is in turn involved in generation and mineralization of dentin matrix[27].

The sequence of events occurring at the presence of BMP is presented below:

Migration of fibroblasts to the segmented area- Proliferation of fibroblasts- Formation of passive matrix- Bonding of undifferentiated and mesenchymal cells to the matrix- Differentiation of cells bonded to odontoblast- Mineralization of matrix- Formation of restorative dentin or homogenous trabeculae dentin[27].

The in vitro studies of Rutherford et al., using rhBMP7 on the pulp of monkeys, showed healing was characterized by capillaries and cells surrounded by mineralized extracellular amorphous matrix. There is a wide strip of mineralized dentin with non-trabeculae areas and flat cells until the sixth month, while pre-dentin is observed at other times, which is related to a dentinwith trabecular pattern. Isolated lacunae-contained traces of blood, and some isolated cells also appeared, which were distributed irregularly by restorative dentin[28-29].

In the research carried out on collagen scaffold type I 6, 9 & 15 in dogs using rhBMP-2 & -4, the histological evaluation done around the day 17th showed that part of the pulp base had a mineralized tissue similar to osteodentin, and osteodentin capillaries and cells with circular or oval nuclei existed in irregular cavities. Under osteodentin bridge, irregular dentin and odontoblasts were observed. Elsewhere, a fiber tissue that was still non-mineralized was seen,which generated the cone-shaped cells of the outer matrix[27].

Many scientists have studied specific types of BMPs (like BMP 2, 4 and 7) as a stimulant in pulp tissue. Rutherford et al and Jepsen et al evaluated BMP 7 (OP1). Nakashima et al and Lohara et al investigated BMP 2 & 4. Based on these studies, formation of mineralized tissue was highly dose-dependent. Hence, it was concluded that some factors such as pulp compounds (especially the number,

type and cell survival stage for osteogenesis) also affected the dentin regeneration[29-31].

As it is known, the structural elements of pulp and number of cells vary in various stages of tooth life cycle. With an increase in the age of pulp, few mesenchymal and undifferentiated cells remain, which are sporadically seen in the adult pulp. The study on human deciduous teeth and structural changes of pulp in various phases of rhyzolysis showed that rhyzolysis phase was significant for stimulation of BMP. It has been proven that epithelium-mesenchymal interaction is necessary for organogenesis. This interaction is regulated by external cellular matrix, which is composed of GAG and adhesive glycoproteins. Hence, the capacity of BMP stimulation under physiological and pathological conditions of pulp is of great significance[27].

Wozney et al. reported that chemical absorption and mitogenecityof BMP depend on the type of cell, external matrix and micro-environment. Based on the studies of Ripamonti et al. and Nakashima et al., pulp cells express TGFβ superfamily and its receptors, but they do not express sialoproteins. Their receptors have not been identified yet. However, there will be differences in coronal and root dentinogenesis. On the other hand, pulpal inflammation affects dentinogenesis[14,32-34].

Conclusion

The dental pulp is stimulated by the biological molecules, generating an additional cellular matrix that is trying to mineralize. They have the ability to differentiate into odontoblast. Precise molecular mechanisms underlying this differentiation are not completely known. Based on various studies, BMP 2, 4 and 7 are undoubtedly capable of initiating dentin restoration, but this is not finally considered to be a regeneration process.

Pulpotomy using BMP is highly method-dependent, and the time of placement is quite vital. It seems that an enormous source of BMPs is required to determine their biological effects on the regenerative cells, especially during osteogenesis. Based on the resources and references in pulpotomy, BMP dose is highly important, and there are differences in coronal and root dentinogenesis. The pulp compounds and number of cells in regeneration and restoration process are important. In this technique, it is essential to use a large number of cells; cells that are capable of differentiation and restoration.

Finally, it can be concluded that after above considerations and all clinical and radiographic evaluations, based on various references, it is necessary to find an appropriate biomaterial with the potential to restore pulp by stimulating a large number of osteodentins and calcified barriers.

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