The study of osteoinductive effect as the variant rhBMP-2 application in the dorsal subcutaneous tissue of white rat

[1]Dept. of Oral-maxillofacial surgery, LivingWell Dental Hopital
[2]LivingWell Dental Institute of Dental Research
[3]Dental and Oral Medical center, Kurume University, School of Medicine, Kurume, Japan
Yoon-Seon Keum*[1], Hyo-Jeong Son[1], Seung-Cheol Woo[2], Jang-Yeol Lee[2],
Hyoun-Chull Kim[1],Sang-Chull Lee[1], Jingo Kusukawa[3]

I. Introduction

The study of the osteoinductive effect during the implantation in the bone defect area has been carried out, also the bone morphogenetic protein(BMP) has been taken notice of osteoinduction material. Urist (1965) was the first to report the presence of bone morphogenetic protein (BMP) that was detected by transplantation of decalcified bone matrix into an intramuscular site, where it induced ectopic ossification. The term "bone morphogenetic protein"(BMP) was introduced to describe the substance(s) in the demineralized bone matrix responsible for the phenomenon. "Morphogenesis" means generation of form, the process of tissue and organ construction and assembly[1]. At least 18 BMPs are currently recognized (BMPs 1-18)[2]. The osteoinductive properties of endogenous BMPs of various origin (e.g. murine, ovine, bovine, reindeer, primate and human) have since been evaluated extensively both in vitro and in vivo[3].

Advances in DNA recombinant technology have made possible the production of highly purified recombinant human BMP. In 1988, Wang et al.[4] reported the isolation of three polypeptides of 16, 18, and 30 kDa molecular weight from bovine bone. The encoding human genes were later transfected into Chinese hamster ovary cells and to Escherichia coli cells[5,6]. Among the recombinant proteins, rhBMP-2 and rhBMP-7 (also termed "(human) osteogenic protein-1"((h)OP-1)[7]) have been tested in a number of orthopedic indications as well as for application in the dental/maxillofacial field[8-12]. And it is known that recombinant human bone morphogenetic protein-2(rhBMP-2) is distinguished at the osteoinducti-bility on the ectopic area.

The aim of this study is to investigate and evaluate histologically the osteoinductive effect of the first Korean commercial rhBMP-2 on the dorsal cutaneous tissue of white rat.

II. Material and Methods

Titanium mesh cylinder (5 mm diameter, 10 mm length including microfibrillar collagen sponge

Corresponding author: ***Yoon-Seon Keum***
Department of OMFS, LivingWell Dental Hospital, 110, Juyeop-dong, Ilsan-Seo-gu, Goyang-si, 411-370, Gyeonggi-do, Korea
E-mail: Livingwell@paran.com
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(Avitene®, Japan and Zeria Pharm, Japan) as a carrier for rhBMP-2 was prepared. The collagen has been proved to be an ideal carrier matrix for rhBMP-2 in intramuscular and subcutaneous sites and at bone unions; it releases rhBMP-2 gradually and is eventually absorbed[13, 14].

Animals : All procedures involving 3 rats were performed in compliance with the guidelines for the care and use of laboratory animals of the school of Medicine, Kurume University. RhBMP-2 were donated by Cowell med. Co(Korea). Mesenchymal stem cell and human origin osteoblastic cell were cultured in the presence of recombinant BMP under serum-free conditions.

We devided rats into 3 groups based on application methods.

- Group 1. was rhBMP-2 80㎍(1.5mg/ml) alone
- Group 2. was rhBMP-2 80㎍(1.5mg/ml) add the mesenchymal stem cell
- Group 3. was rhBMP-2 80㎍(1.5mg/ml) add the osteoblast

Under the intraperitonial injection following an intraperi- toneal injection of ketamine/xylazine, all rats underwent a dorsal trichotomy and vigorous disinfection of the region with iodophor alcohol. A contralateral incision was then made in the dorsal skin of the rat and subcutaneous pouches were created by blunt dissection. Each titanium mesh cylinder was then implanted in the pocket formed. The skin was subsequently closed.

Two weeks after this operation, the grafted site was observed histologically to specifically evaluate the differential reaction of the surrounding tissue. The specimens were fixed in 10% formaldehyde, decalcified, sliced at a thickness of 4 mm, and histopathological views of each groups were observed through an optical microscope under the hematoxylin and eosin(H&E) staining.

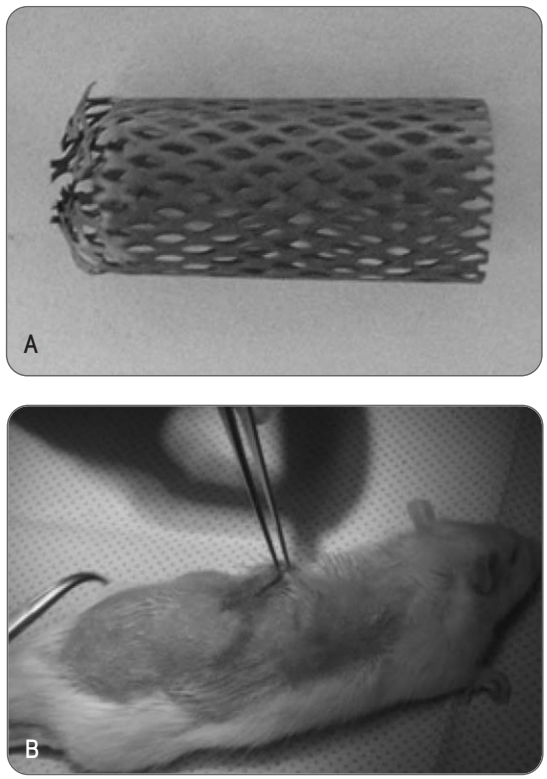

Fig. 1. A.Titanium mesh cylinder that were 5mm diameter, 10mm length as a carrier. B. The procedure to insert the rhBMP-2 on the dorsal subcutaneous tissues of the white rat.

III. Results

Two weeks later, new bone formation was observed in all of three groups. Bone formation was confirmed through the findings showing new bone with fibrotic connective tissue and outer cortex-like appearance. Bone formation degree of Group 2 and 3 was superior to group 1. Especially group 2 that is rh-BMP2 with mesenchymal stem cell predominated at the amount of bone formation.

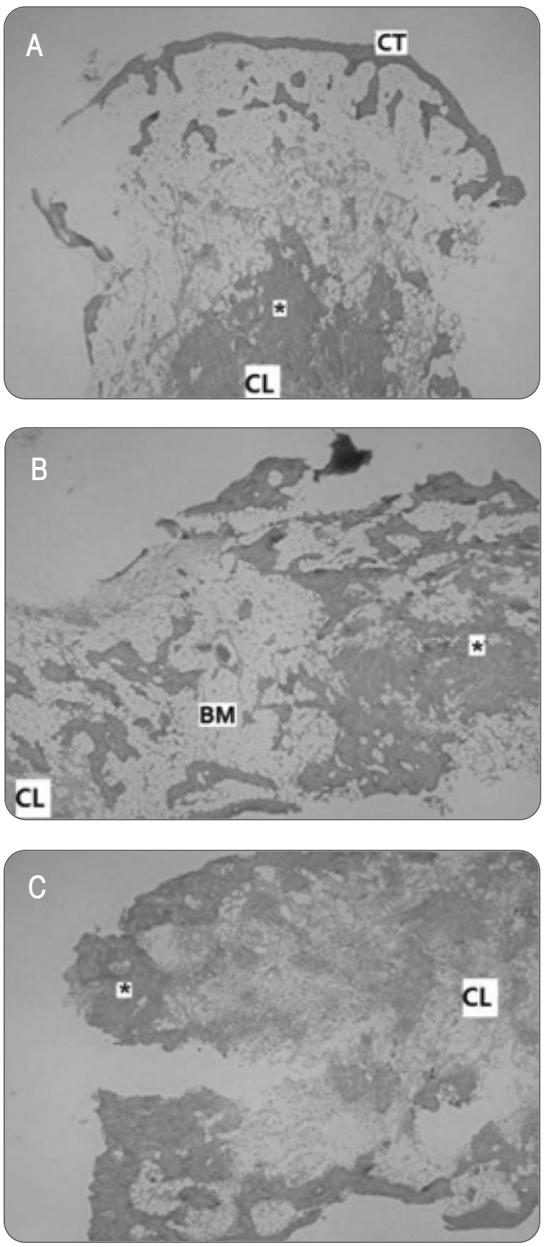

Fig 2. rhBMP-2 grafted group (H&E stain; rh-BMP2-induced ectopically-formed bone; (*), new bone formation; (CT) cortex-like bone; (BM), bone marrow-like structure; (CL), collagen).
A. rhBMP-2+collagen group,
B. rhBMP-2+collagen+mesenchymal stem cell group,
C. rhBMP-2+collagen+osteoblast group.
At 2weeks after graft, new bone formation was confirmed in all of three group. The rh-BMP2-induced ectopically-formed bone consists of cortex-like bone in the margin layer and collagen and bone marrow like-structure in the core. Bone formation degree of B and C has superiority than A. Especially, B was predominated at the amount of bone formation (x40).

Ⅳ. Discussion

Ackonwledgedly, healing after bone graft occurs in five phases: inflammation, microrevascularization, osteoinduction, osteoconduction and osteoremodeling. Osteoinduction is initiated via a process in which mesenchymal stem cells of the host are recruited to differentiate into osteoblasts. Osteoconduction is initiated via a process in which capillaries, perivascular tissue and osteoprogenitor cells of the host grow into the graft. Thus, newly formed bone area indicates osteoinductive and osteoconductive properties of the graft bone.

Recently there is growing of researchs to provide alternatives to traditional bone grafting. In the last decades, the orthopedic research community has focused on the four requirements of bone regeneration: (1) a morphogenetic signal, i.e. growth and differentiation factors, (2) host cells that will respond to the signal, i.e. are capable of differentiating into osteoblasts, (3) a biomaterial carrier of this signal that can deliver the mor- phogenetic signal to specific sites and serve as a (degradable) scaffold for the growth of the responsive host cells, and (4) a viable, well vascularized host bed [3, 15-16] .

Above all of these factors, the bone morphogenetic proteins(BMPs) that are a family of growth factors implicated in a variety of functions during development and in tissue regeneration, are focused of much discussion. Besides their relevance in non-osteogenic processes, BMPs play a key role in the development and regeneration of the skeletal system, and some BMPs have the unique ability of inducing ectopic bone formation. Rh-BMP2 delivered by implantation or gene therapy induces ectopic bone formation at subcutaneous, intramuscle and intrafatty sites. It was demonstrated through our study.

A wide range of materials has been tested in combination with BMPs[3,9,17]. One of the first candidates was demineralized bone matrix which has intrinsic, limited osteoinductive properties. The osteoconductive carriers have been poly(a-hydroxy acid) microparticles, foams, or disks; collagenous materials, e.g. collagen type I sponges, semi-solid paste, collagen type IV, or type I collagen/gelatin composites; inorganic ceramic materials, e.g. calcium phosphate cement, porous hydroxyapatite (HA), or hydroxyapatite/tricalcium phosphate (TCP) as blocks and granules; bone or cartilage derived materials, e.g. inactive collagenous bone matrix and bovine bone mineral; and composites, e.g. dentine matrix powder/chondroitin-6-sulfate/ type I collagen, TCP or coralline HA/type IV collagen, and poly(a-hydroxy acid)/ carboxymethylcellulose or methylcellulose[17-18]. BMPs have also been used in combination with titanium mesh and other non-degradable metallic orthopedic implants. Delivery strategies of rhBMP-2 in commercial products have concentrated on an absorbable collagen sponge which is impregnated with protein solution prior to implantation. Collagen has received increasing attention over the last years due to its excellent biocompatibility, degradation into physiological end-products, and suitable interaction with cells and other macromolecules[19-21]. The favorable influence of collagen on cellular infiltration and wound healing is well known. An additional benefit is that collagen can be processed on an aqueous base. A variety of dosage forms have been in use for years, including aqueous injectable collagen dispersions, powders and surgical sutures, corneal shields, tissue and vascular sealants and spongy implants.[18] The combination of rhBMP-2 with a collagen sponge matrix has proven to be a very promising therapeutic in a variety of applications. Consequently, there are some factors involved in the preparation process of collagen sponges and the combination product which require evaluation with regard to optimal performance in its role as an implant providing local delivery of an osteoinductive factor.

Generally, histological characterization of newly formed ectopic bone has shown that lamella bone slowly replaces the immature woven areas during the remodeling process. Thus, we concerned that after 2 weeks later implantation of carrier containing rh-BMP2 may retain active metabolism of mineralization, and may include osteoprogenitor cells derived from mesenchymal cells of the host. Also BMP may serve as a potent chemoattractant to recruit monocytes as well as mesenchymal stem cells, and their close relationship may act synergistically to promote the bone inductive cascade. It was suggested that BMP may regulate the differentitation of mesenchymal cells into multinucleated giant cells, either foreign body giant cells or osteoclast-like cells. These strongly support the hypothsis that BMPs through their profound effects on monocyte recruitment and cytokine synthesis may have early effects on bone induction and promote successive additional steps in the endochondral bone formation cascade[22-23] .

V. Conclusion

In summary, the results of this study show that the rh-BMP2 induces new bone in not only the bone defect but also the subcutaneous tissue. This clearly demonstrated that recombinant human bone morphogenetic protein-2(rhBMP-2) has osteoinductibility on the ectopic area. It is supported from investigated and evaluated histological characterization of newly formed ectopic bone after the

application of rhBMP-2. And it suggest that application rhBMP-2 with the immature mesenchymal cells has more advantages because it could more easily differentiate into osteoblasts lead to osteoinduction.

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백서의 배면 피하부 주입시 rhBMP-2 적용에 따른 골형성 유도 효과 연구

금윤선*[1], 손효정[1], 우승철[2], 이장렬[2], 김현철[1], 이상철[1], 쿠수카와 진고[3]

[1]리빙웰 치과병원, 구강악안면외과,

[2]리빙웰 치의학 연구소

[3]일본 구루메 대학 의학부 구강외과학 교실

골 결손 부위의 임프란트 식립시 골유도에 관한 연구가 오랜전부터 시행되어져 왔고 BMP에 관한 관심도 계속 되어왔다. 특히 rhBMP-2는 기존의 골부위가 아닌 이소성부위에서의 골 형성 유도 능력을 나타낸다고 알려져 있다. 본 연구의 목적은 국내에서 최초로 상용화된 rhBMP-2를 백서의 배면 피하부에 rhBMP-2 주입하여 골형성 유도 효과를 평가함에 있다.

직경 5mm, 길이 10mm의 Titanium mesh cylinder 내부에 전달체(carrier)인 교원질 sponge(Avitene®)를 준비하였다. rhBMP-2 적용 방식에 따라 3그룹으로 나누었다. 그룹 1은 rhBMP-2 80㎍(1.5mg/ml), 그룹 2는 1의 rhBMP-2에 추출된 미분화 간엽 세포를 함께 적용, 그룹 3은 1의 rhBMP-2에 추출된 골아세포를 함께 적용하여 각각을 준비된 백서의 배면 피하부 주입 하였다. 2주간의 기간 경과 후 H&E 염색하에 광학 현미경 상으로 관찰되는 조직병리학적 소견을 연구하였다.

rhBMP-2 적용시 세 그룹 모두에서 2주 후 골형성이 관찰되었다. 골형성은 섬유성 결합 조직 사이 혼재된 신생골 및 외측 피질골 소견으로 확인 할 수 있었다. 그룹 1.의 경우에 비해 그룹2. 3. 에서 새로운 골 형성의 정도가 우수한 양상을 보였고 특히 그룹 2에서 현저한 골 형성을 보였다.

본 실험을 통해 기존의 골 부위가 아닌 이소성 부위 적용시에도 rhBMP-2는 골형성 유도 능력을 가지며 특히 골아세포로 분화 가능한 미분화 간엽세포와 함께 적용시에 그 효과가 크다는 것을 알수 있었다. *[대한치과이식(임프란트)학회지 2010;29(1):47-53]*

Keywords : mesenchymal stem cell, osteoinductive effect, osteoblast, rhBMP-2.