A preliminary study of bone inducing effect by recombinant bone morphogenetic protein-2

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I. Introduction

In bone grafting, autogenous bone is considered the 'gold standard' to which other method are compared. The limitations of obtaining autogenous bone are obvious. Allograft, xenofraft and synthetic bone graft are widely used, but they have not solved all the problems in bone grafting. Generally, allograft or synthetic bone graft has lower healing capacity than autograft and carries a risk for certain disease, such as hepatitis or AIDS[1]. Occasionally, incomplete healing is seen in spite of proper grafting procedures[2]. Thus, other methods have been searched and stimulation of the regeneration of bone is a challenging idea, which would solve many problems in cases with bone defects.

Marshall R. Urist in 1965 made the seminal discovery that a specific protein, BMP(bone morphogenic protein), found in the extracellular matrix of demineralized bone could induce new bone formation when implanted in extraosseous tissues in a host[3]. BMPs are multi-functional growth factors which are members of the transforming growth factor-beta super family[4] and their ability is that plays a pivotal roll in inducing bone[5]. About 18 BMP family members have been identified and characterized. Among of them, BMP-2 and BMP-7 have significant importance in bone development[6]. Because of their potent ability to induce bone, application of rhBMP-2 in humans to the treatment of craniomaxillofacial surgery has been reported[7].

The aim of this study was to evaluate the bone inducing effects of the first commercial rhBMP-2 in South Korea and to investigate the influence of supplementation with collagen.

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II. Material and Methods

1. Implant preparation

The rhBMP-2 used in this study was obtained at CoWellMed, Korea and collagen sponge, Terudermis® (Terumo, Japan) and Aviten® (Zeria Pharm, Japan) were purchased. The rhBMP-2(>98% purity; CoWellMed, Korea) was diluted with distilled water solution to concentration of 1.5mg/ml before implantation. Collagen sponges were shaped to suitable sizes for specific defects with a scalpel and collagen sponge was treated with diluted rhBMP-2 immediately before implantation.

2. Animals

The experimental 2 Wistar male rats weighing about 300g were selected. They were kept on a 12 h light/12 h dark cycle. The temperature of the experimental laboratory was maintained at approximately 21℃. The animals were fed with commercial rat chow and had access to food and water and libitum.

3. Surgical procedure

The rats were anesthetized with a solution of ketamine hydrochloride (75-100 mg/kg body wt.) and xylazine (5-10 mg/kg body wt.). After shaving the scalp hair, a longitudinal incision was made in the midline of cranium and the periosteum was elevated to expose the surface of the skull bone. Under a copious irrigation with saline, Four skull bone defects of 2 mm diameter and 1mm depth were produced in skull bone using a surgical round bur. Three groups of them were treated with rhBMP-2 20μg(1.5mg/ml): rhBMP-2 alone(group1), rhBMP-2 with collagen (Terudermis®)sheets (group2), rhBMP-2 with collagen(Aviten®)(group3). The last group(group4) was not treated as control. Then the periosteum and skin were sutured.

4. Histopathology

A tissue biopsy was performed at week 2(group1 to group3) and week 6(group4) from the center of the formed surgical defects. The biopsied tissue was fixed in a 10% formalin solution for 2 days, and decalcified in a 4% ethylene diamine tetra acetic acid (EDTA) solution for 3 days. This was made into a paraffin-embedded block, sectioned at a thickness of 4 mm and made into a tissue sample. The tissue sample was then mounted on a glass slide and subjected to hematoxyline and eosin (H&E) staining. Following this, the sample was examined by light microscopy to determine whether bone tissue had formed.

III. Results

New bone was observed in all groups treated with rhBMP-2. In control group(group4), however, bone defect was filled with fibrous tissue only. In the collagen treated groups(group2 and group3) at week 2, they had a significantly more bone formation than only rhBMP-2 treated group(group1)(Fig. 1,2).

IV. Discussion

Stem cell differentiate into several specialized cell types, including osteoblasts, adipocytes, myocytes and chondrocytes[8, 9]. Runx2, Dlx5, Osterix, Msx and Aj18 are osteogenic transcription factors necessary for

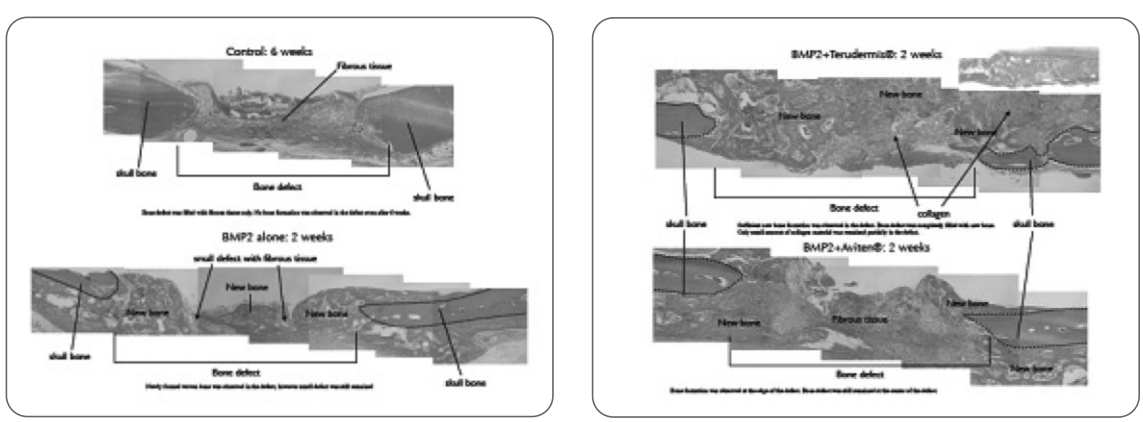
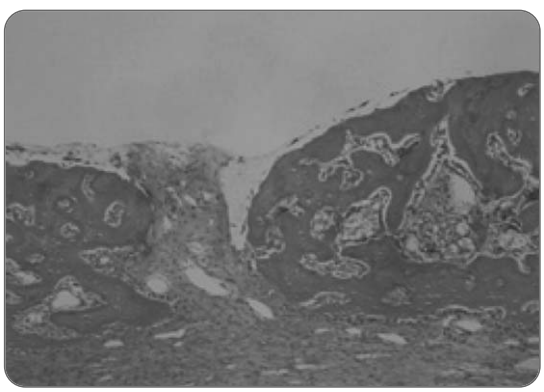


Fig. 1. The effect of rhBMP-2 and collagen sponge to the new bone formation were observed.

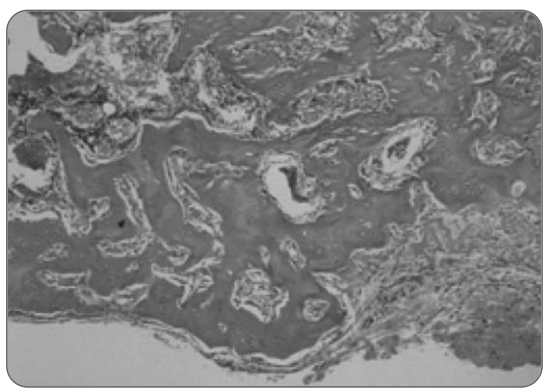
early and late osteoblast differentiation and their expressions are stimulated with BMPs. BMPs are potent local factors that regulate osteoblast differentiation and function[10]. The BMPs with greatest osteogenic capacity are BMP-2,-4,-5,-6,-7 and -9. Among of them, BMP-2 and BMP-7 have significant roles in bone development[11].

BMPs, as their name indicates, BMP molecules are capable of inducing ectopic cartilage and bone formation, a process that mimics embryonic endochondral bone formation[12]. The application rhBMPs has emerged recently for the induction enhancement of bone healing. The efficient osteoinductive properties of rhBMP demonstrated in experimental models and preclinical studies[13-15]. Many studies conducted to examine the effective dose-dependency of rhBMP-2, minimum threshould dose of rhBMP-2 assumed to be 1-10 μg[12].

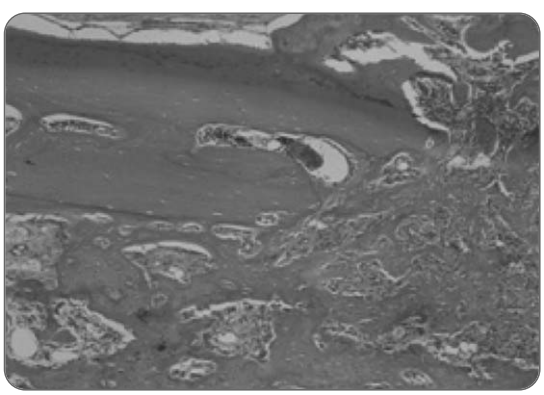
For several decades, the 'gold standard' in bone defect management has been autograft, which involves harvesting healthy bone from one anatomical site of the patient, most often the iliac crest, and implanting the material at the defect site. This technique yields the most predictable results, however bears considerable risks. They are donor site pain and morbidity, infection, extra blood loss, and higher cost due to longer operating times[16]. Because limited supply and associated mobidity of autogenous bone graft, and limited osteoinductivity of allograft, there is increased interest in using BMPs and their



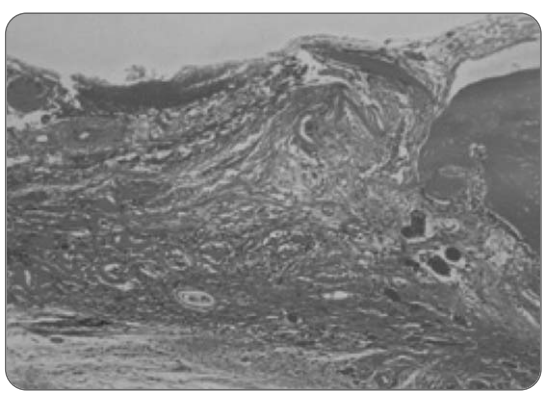
A. rhBMP-2 alone (group1)



B. rhBMP-2 with collagen(Terudermis®)



C. rhBMP-2 with collagen(Aviten®)(group3)



D. control group(group4)

Fig. 2. Obvious bone formation is seen in rhBMP-2 treated group.
Hematoxylin and eosin stain, original magnification 40x.

osteoinductive potency makes them clinically valuable as alternatives to autogeonus bone graft[17].

It is important to select the appropriate carrier material for BMP. Many kinds of carriers had used, collagen is biogradable carrier and generally is preferred to non degradable carriers. Complete replacement of the carrier by bone eliminates any potential effects associated with prolonged exposure the carrier material[18]. 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. The favorable influence of collagen on cellular infiltration and wound healing is well known. In this study, Terudermis® and Aviten® were seemed to be as an osteoconductive space filler that provides structural support during bone healing. Recently, the combination of rhBMP-2 with a collagen sponge matrix has proven to be a very promising therapeutic in a variety of applications[19].

In this experimental study, it was not used critical bone defects to study the bone repair. This type of defect is able to heal by itself. Our goal was to evaluate the more efficacy of the rhBMP-2 only with some type of carrier. Therefore, it is thought that another experiments are needed in later to evaluation of bone formation in relation to the critical-sized defects.

BMPs were several groups have completed phase 3 clinical trials and are waiting FDA approval for nonunion fracture healing and alveolar ridge augmentation[20, 21]. Over the next a few years, we will see how wide used BMPs become in the clinic.

V. Conclusion

In this study, we showed that rhBMP-2 accelated bone induction regarding of the supporting framework. Concerned with this study , we thought a need to additional study of the effective dose and duration of rhBMP-2 treatment and comparative study of the effect of rhBMP-2 on autogenous bone graft, allograft, xenograft, and artificial bone graft companied with application of stem cells.

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rhBMP-2에 의한 골유도 효과에 관한 예비실험 연구

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Bone morphogenetic protein(BMP)은 골유도 능력이 있는 단백질로 현재까지 18종류가 규명되었고 그 중에서 특히 BMP-2와 7의 활성이 가장 뛰어난 것으로 여겨지고 있다. 본 연구에서는 백서의 두개골 결손부에 한국에서 처음 자체 생산된 recombinant human bone morphogenetic protein-2(rhBMP-2)(코웰메디, 한국)를 적용하여 골형성 유도 효과를 확인하기 위한 예비실험을 시행하였고 비계체로서 교원질을 사용함에 따른 효과를 확인 하고자 하였다.

백서의 두개골에 4군데의 직경 2mm, 깊이 1mm의 골 결손부를 형성하였고 단백질20ug(1.5mg/ml)만 단독 투여한 경우와 rhBMP-2와 교원질(Terudermis®)을 함께 이식한 경우, 그리고 rhBMP-2 와 교원질(Aviten®)을 함께 이식하고 2주가 경과한 후에 H&E염색을 통해 광학현미경하에 조직병리학적으로 세 그룹을 비교 관찰하였다. 대조군으로는 골 결손부에 아무것도 이식하지 않은 상태로 위의 세 그룹과 같은 조건아래 6주 동안 관찰하였다.

rhBMP-2를 투여한 경우 모두에서 2주후에 새로운 골형성이 관찰되었고 특히 교원질을 첨가한 그룹에서 현저한 골형성을 보였다. 아무것도 투여하지 않은 그룹은 6주가 경과한 후에도 골형성이 관찰되지 않았으며 골결손 부위에 섬유성 결합 조직만으로 채워진 양상을 보였다.

이번 실험에서 한국에서 처음 자체 생산된 rhBMP-2(코웰메디, 한국)는 골형성을 유도하는 능력을 가짐을 확인할 수 있었고 골결손 부위를 수복 할 시 안정성을 가진 전달체와 함께 골형성 세포를 직접 유도하는 신호전달 역할을 가진 BMP와같은 단백질을 함께 적용할 경우 그 효과가 증가됨을 확인하였다. 차후 임계 골 결손부에서의 골유도 효과 및, 자가골, 동종골, 이종골, 합성골에 대한 BMP의 작용에 대한 비교 연구 및 골유도 과정에서 필요한 BMP의 양과 적용시간에 대한 연구가 추가적으로 이루어져야 할 것으로 생각된다. *[대한치과이식(임프란트)학회지 2010;29(1):41-46]*

Keywords : BMP-2, collagen, osteogenesis