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가토 상악동에서의 rhBMP-2와 콜라겐 운반체에 의한 골유도 효과

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Bone inducing effect by recombinant human bone morphogenetic protein-2 and collagen carrier in maxillary sinus of the rabbit

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Purpose: The aim of this study was to evaluate the bone inducing effects on the maxillary sinus of rabbits using the first commercial rhBMP-2 in South Korea and to investigate the influence of supplementation with collagen sponges.

Materials and Methods: Two circular windows (diameter, about 4 mm) was formed in the nasal bone of rabbit The preparated maxillary sinuses were filled with collagen sponges (Terudermis®) alone in three groups. In the other three groups, the preparated maxillary sinuses were filled with collagen sponges (Terudermis®) acompanied with rhBMP-2, 20 μg (0.5 mg/cm^3). Animals were sacrificed at 2, 4 and 8 weeks. The experimental sections were stained with hematoxylin and eosin for histopathologic analysis.

Results: After 2 weeks, newly formed woven bone was seen. At four weeks, the bone was more mature than at 2 weeks. After 8 weeks, additional new bone formation was seen and much of the woven bone in the augmented space had been replaced by lamellar bone and fatty tissue. In the rhBMP-2 treated groups, for four weeks, histological findings were similar to those in the experimental group without rhBMP-2. In the rhBMP-2, however, significant bone regeneration ability was seen.

Conclusion: In this study, we showed that the rhBMP-2 used in this experimental model was able to induce bone regeneration when combined to the carriers. BMPs have potency as alternatives to bone graft and accelators of the new bone formation. Concerned with this study, we thought further investigation are needed about the effective dose and comparative study of the effect of rhBMP-2 on autogenous bone graft, allograft, xenograft, and artificial bone graft or additional study of application of stem cells. **(JOURNAL OF THE KOREAN ACADEMY OF IMPLANT DENTISTRY 2011;30(2):115-122)**

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INTRODUCTION

In bone grafting, autogenous bone graft is considered the 'gold standard' to which other methods are compared. However, it has many limitations such as additional operative time, at the donor site infection, increased blood loss and insufficient quantity in bone harvesting. These problems have encouraged surgeons to search other sources[1].

Allograft and xenograft bone materials have been used for many years for variety of oral and maxillofacial operations. Although, the allograft or xenograft bone materials have a chance of problems associated cross infection, the use of them provides a reasonable alternative to autogenous bone. Moreover, antigenicity of them have been widely studied and represent a minimal risk to the patient. But considered mainly as osteoconductive as opposed to osteoinductive in nature[2-4].

Synthetic materials have been tested as bone graft substitutes and a number of these are able to bridge successfully smaller defects by osteoconduction, however they generally do not cause osteoinduction[5].

As stated above, because of morbidity associated autogenous bone and limited osteoinductivity of allografts, xenografts and synthetic materials, there is increased interest in using BMPs. Osteoinductive substances such as BMPs lead to bone formation even at non-skeletal sites by mimicking a process like embryonic endochondral bone formation[6].

Urist in 1965 made the seminal discovery that a specific protein, BMP (bone morphogenetic protein), found in the extracellular matrix of demineralized bone could induce bone formation newly when implanted in extraosseous tissues in a host[7]. BMPs are multi-functional growth factors which are members of the transforming growth factor-beta super family and their ability is that plays a pivotal roll in inducing bone[8]. About 18 BMP family members have been identified and characterized. Among of them, BMP-2 and BMP-7 have significant importance in bone development[9].

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

MATERIALS AND METHODS

1. Substances

The rhBMP-2 used in this study was obtained at CoWellMed, Korea. The collagen sponge (Terudermis®) was purchased from Terumo, Japan, 20 μg (0.5 mg/cm^3) of rhBMP-2 were applied in the each maxillary sinus of the rabbit.

2. Animals

Three male Japanese Albino rabbits about weighting 3.0±0.5 kg 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°C. The animals were fed a standard laboratory chow and water ad libitum. The Animal Care and Use Committee of the Kurume Medical University approved all rabbit protocols.

3. Surgical procedure

The rabbits were anesthetized with a solution of ketamine hydrochloride (40 mg/kg, IM) and xylazine (5 mg/kg, IM). After shaving the scalp hair, a longitudinal incision was made extending about 50 mm, and the skin and periosteum were elevated sufficiently to expose the nasal bone and nasoincisal suture line. Under a copious irrigation

with saline, two circular window (diameter, about 4 mm) was opened in the nasal bone with the use of a rotating round bur. The window was located approximately 20 mm anterior to the nasofrontal suture line, 3 mm lateral to the midline (Fig. 1A, B). The antral mucosa was carefully striped off anteroventrally (Fig. 1C). The preparated maxillary sinuses were filled with collagen sponges (Terudermis®) alone in three groups. In the other three groups, the preparated maxillary sinuses were filled with collagen sponges (Terudermis®) acompanied with rhBMP-2, 20 μg (0.5 mg/cm^3) (Fig. 1D) (Table 1). After the grafted materials were filled on the maxillary sinuses, deflected periosteum and skin were sutured.

RESULTS

The normal sinus cavity is surrounded by respiratory mucosa and a thin layer of cortical bone. The mucosa is composed of pseudo-stratified ciliated epithelium and there are numerous serous glands in the submucosal tissue.

In this experiment, after 2 weeks, dome-like space formed by the elevated sinus mucosa was filled by fibrous tissue and there was no evidence

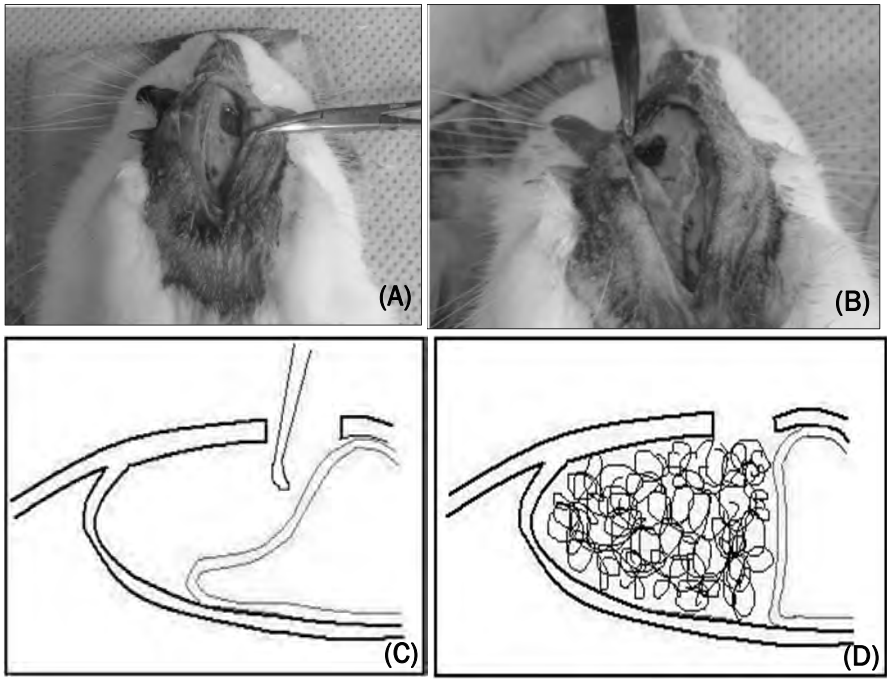

Fig. 1. (A, B) Two circular window was opened in the nasal bone of the rabbits. (C) The raised antral mucosa. (D) The maxillary sinuses were filled with collagen sponges (Terudermis®) with or without rh-BMP2.

Table 1. Experimental groups.

Group	Additional materials	Observational period (weeks)	Group	Additional materials	Observational period (weeks)
Group 1	Terudermis® alone	2	Group 4	Terudermis®+rhBMP-2	2
Group 2	Terudermis® alone	4	Group 5	Terudermis®+rhBMP-2	4
Group 3	Terudermis® alone	8	Group 6	Terudermis®+rhBMP-2	8

of acute or chronic inflammation. Peripheral newly formed woven bone was seen and the overlying thick respiratory glandular mucosa was shown (Fig. 2A～C). At four weeks, the bone was more mature than at 2 weeks and the augmented space revealed mature cortical and trabecular bone with intertrabecular vascularized adipose tissue (Fig. 2D). After 8 weeks, additional new bone formation was seen and much of the woven bone in the augmented space had been replaced by lamellar bone and fatty tissue (Fig. 2E). In the rhBMP-2 treated groups, for four weeks, histological findings were similar to those in the experimental group without rhBMP-2. However, in the rhBMP-2 treated

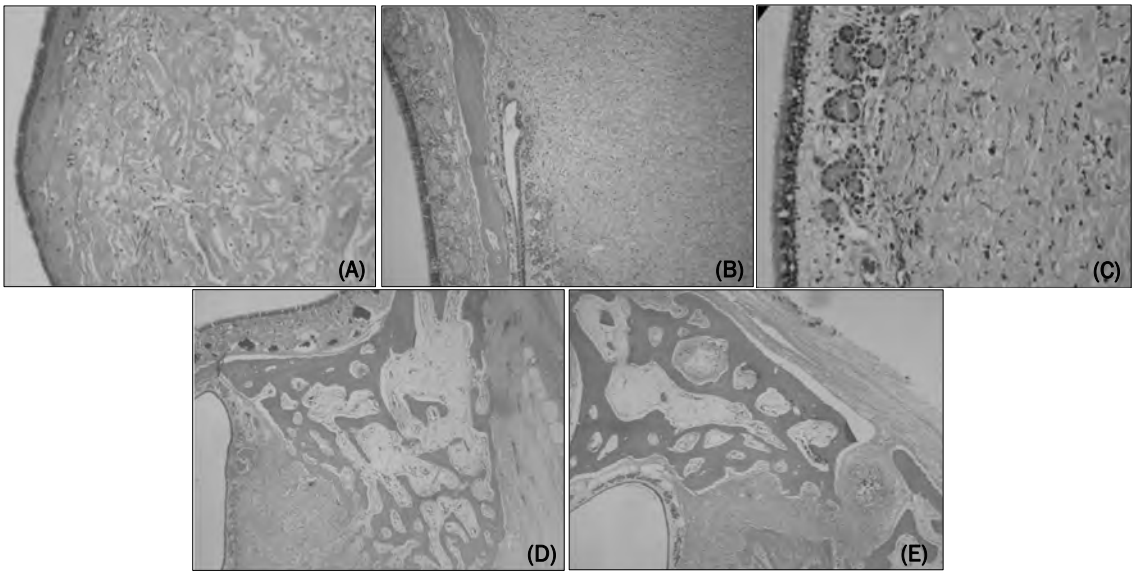

Fig. 2. Two weeks after implantation, dome-like space formed by the elevated sinus mucosa was filled by fibrous tissue and there was no evidence of acute or chronic inflammation (A). Newly formed woven bone was seen at the periphery of the augmented space (B), and the overlying thick respiratory glandular mucosa was shown (C). Four weeks after implantation, newly formed bone contained woven bone and lamellar bone are embedded in fibrous connective tissue (D). Eight weeks after implantation, newly formed bone was more mature than at 4 weeks and showed many interconnections (E) (haematoxylin-eosin: A, B and D original magnification ×100; C original magnification ×200; E original magnification ×40).

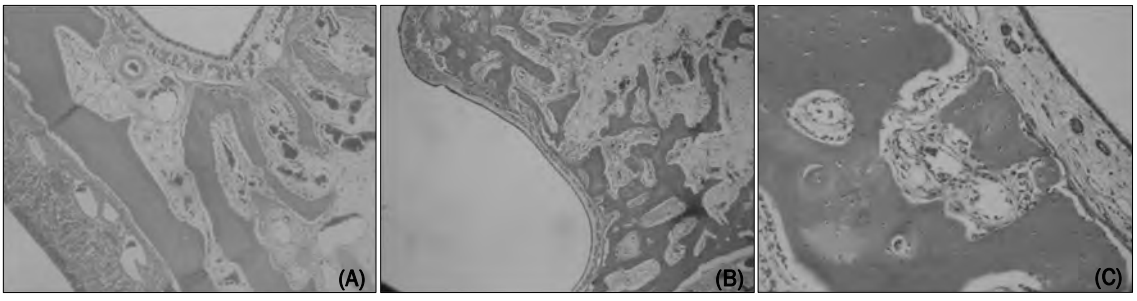

Fig. 3. In the rhBMP-2 treated groups, the peak of bone formation was observed in 4 weeks. At 2 weeks (A), 4weeks (B) and 8 weeks (C). Ib the rhBMP-2 treated groups, significant bone regeneration ability was seen (haematoxylin-eosin: A and C original magnification ×100; B original magnification ×40).

groups, almost regenerated bone was lost in 8 weeks and the peak of bone formation was observed in 4 weeks (Fig. 3).

DISCUSSION

Tissue engineering is aimed to replace damaged or diseased tissue and it requires the successful interplay between a 3-dimensional scaffold, autogenous cells, and osteoinductive growth factors. The combination of a suitable scaffold material with osteoinductive growth factors is the important step toward a successful tissue engineering approach[10].

Several experimental studies have validated the effectiveness of rhBMP for the stimulation of bone formation[11-13]. It has been shown that rhBMP-2 requires combination with a biomaterial matrix to attain maximal efficacy. The delivery system releases and localizes the BMP, ensuring interaction with mesenchymal cells that can differentiate into osteoblasts. The delivery system also provides instructional guidance as a template to renew osseous contour[14-17].

Many variety of carriers like as demineralized bone matrix, hydroxyapatite, calcium phosphate based delivery system and poly (DL-lactic acid) have been developed[18,19]. In this study, we applied collagen sponge (Terudermis®) as a scaffold. The collagen sponges provided retention of the protein for a sufficient period of time to affect the repair. Generally, collagens are used successfully in many tissue engineering applications. A major compartment of the extracellular matrix in bone is formed by collagens. Collagens are ubiquitous proteins responsible for maintaining the structural integrity of tissues[20]. In the presented groups filled with collagen sponge only, we saw the new bone formation. That is referable to the additional effect of collagen sponges. The interaction of osteoblasts with collagen type I has been shown to enhance the activity of alkaline phosphatase, osteoblast-associated gene expression, and stimulation of mineralization[21,22].

Many studies of BMPs have examined their effect on the orthopedic region like as spinal fusion, to regenerate bone in depleted areas and to heal bone fractures. Moreover, dental applications such as the maxillary sinus augmentation, localized alveolar ridge augmentation, or the effects of BMP stimulation of bone growth around titanium screws have studied[23-27].

Having little available bone volume in the maxilla, maxillary sinus augmentation is an important procedure for increasing the height of the maxilla. Adequate bone volume allow the placement of osseointegrated implants[28,29]. In this study, we used the rabbit model for maxillary sinus augmentation. Because, rabbits have a maxillary sinus with a well defined ostium and hematologic status of the maxillary sinus of rabbits is similar to that in humans[30].

Numerous materials have been used for sinus augmentaion and autogenous bone graft is still considered to be the gold standard for such procedures[31]. However, there are some cases having the limitations of obtaining autogenous bone. In this study, the histological finding showed, there was greater formation of bony tissue in those rhBMP-2 treated groups and that suggested using BMPs can be alternatives to autogeonus bone graft. When using autogenous bone and when using not autogeonus bone but also another bone materials such as allografts, xenografts and synthetic bone materials for the maxillary sinus augmentation, ridge augmentation or another therapeutic applications, treating rhBMP-2 have more efficacy on the new bone formation[32].

There were numerous studies of the bone inducing effect of rhBMP-2. Kusumoto et al. reported

new bone formation in the muscle pouch of rat the ectopic site absent of bone-derived materials[33]. Levine et al. reported that the use of BMPs improves the vascularty of the area providing better nutrition and increaseing resorption and substitution by healthy tissue[34]. Bouxsein et al. studied the effect of rhBMP-2 in fracture healing in a rabbit ulnar osteotomy model. The data revealed that osteotomy sites treated with rhBMP-2 healed 33% faster than the other two groups[35]. Boyne et al. performed a feasibility sinus floor augmentation study examining 0.43 mg/cc rhBMP-2 concentration on absorbable collagen sponge, which was successful at inducing bone formation in a non-human primate segmental defect model[36]. CT evaluations and histology demonstrated a significant change in height and normal bone formation with the use of rhBMP-2/ACS. Boyne[37].

Generally, 6 to 8 months are waited after sinus augmentation in human beings. In the rabbit however, the metabolism is 3 to 4 times faster than of human beings, and a waiting period of 8 weeks was ideal[38], So we observed to 8 weeks, in the present study indicate that the bone healing process was time-dependent. At 4 weeks there was an improved amount of new bone in relation to 2 weeks and greatest new bone formation was observed at 8 weeks in the without rhBMP groups. However, in the rhBMP-2 treated groups showed significant bone regenerative ability but bone absorption is also significantly fast. So, the peak of bone formation was observed in 4 weeks.

There were reported of pharmacologically effective concentration of rhBMP-2. Bostrom et al. demonstrated that rhBMP-2 at a concentration of 0.1 mg/cm^3 and Smith et al. reported similar results[39,40]. However, Satoshi et al.[41] reported pharmacologically effective concentration of rhBMP-2 was 0.4 mg/cm^3. Like this, ideal doses of BMP seem to vary not only between species, but also between sites of implantation with same species or individual. We used 20 μg (0.5 mg/cm^3) rhBMP-2 in the each maxillary sinus. Many studies which no dose-response relationship exists long-term. We thought more studies about optimal doses for BMP are needed and should be determined, both animal models and human patients.

CONCLUSION

In this study, we showed that the rhBMP-2 used in this experimental model was able to induce bone regeneration when combined to the carriers. BMPs have potency as alternatives to bone graft and accelerators of the new bone formation. Concerned with this study, we thought further investigation are needed about the effective dose and comparative study of the effect of rhBMP-2 on autogenous bone graft, allograft, xenograft, and artificial bone graft or, additional study of application of stem cells. Several BMPs have completed phase III clinical trials and over the next few years, we will see how widely used BMPs become in the clinic.

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