The Effect of Recombinant Human Bone Morphogenetic Protein-4 with the Different Particle Sizes of Beta Tricalcium Phosphate on Bone Regeneration in Rat Calvarial Defects

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감사의 글

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그리고, 묵묵히 사랑으로 이해해 주시고 항상 곁을 든든히 지켜 준 아내와 멀리서나마 성원을 아끼지 않은 나의 아이들, 오미, 찬미, 훈준에게 감사의 마음을 전합니다. 모든 분들께 진심으로 감사 드립니다.

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저자 씀

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Abstract

The Effect of Recombinant Human Bone Morphogenetic Protein-4 with the Different Particle Sizes of Beta Tricalcium Phosphate on Bone Regeneration in Rat Calvarial Defects

Various recombinant human bone morphogenetic proteins (rhBMPs) are being tested in preclinical and clinical studies for their capacity to enhance bone formation, but the research on recombinant human bone morphogenetic protein-4 has been insufficient. Carriers used in BMP delivery systems also play an important role in supporting the osteoinductive activity of BMPs. Also research on the particle sizes of carriers has been insufficient. The prolonged residence of the carrier may inhibit the bone formation. It was not known whether the resorption rate is related to the β-TCP particle size. The purpose of this study was to evaluate the effect of recombinant human bone morphogenetic protein-4 with the different particle sizes of beta tricalcium phosphate on bone regeneration in the rat calvarial defect model.

Eight mm calvarial critical-size osteotomy defects were created in one hundred male Sprague-Dawley rats. Five groups of twenty animals each received a sham-surgery control, particle size 50-150 μm (small-particle) β-tricalcium phosphate (β-TCP) carrier control, particle size 150-500 μm (large-particle) β-tricalcium phosphate (β-TCP) carrier control, rhBMP-4 (2.5 μg) in a particle size 50-150 μm (small-particle) β-tricalcium phosphate (β-TCP) carrier, rhBMP-4 (2.5 μg) in a particle size 150-500 μm (large-particle) β-tricalcium phosphate (β-TCP) carrier and were evaluated by histological and histometric parameters following a two- and eight-week

healing interval.

Defect closure, new bone areas, and augmented areas of the two rhBMP-4/β-TCP groups were significantly greater than those of two β-TCP control groups and sham-surgery control group at both 2 and 8 weeks ($p < 0.01$). There were no significant differences in the defect closure, new bone area, and augmented area between two rhBMP-4/β-TCP groups and between two β-TCP control groups at 2 and 8 weeks. There was no significant difference in bone density between two rhBMP-4/β-TCP groups at 8 weeks.

In conclusion, rhBMP-4 combined with either small- or large-particle β-TCP had a significant effect to induce bone regeneration comparing to small- or large-particle β-TCP control, or a sham-surgery control in rat calvarial defect model. Within the parameters of this study, the particle size of β-TCP, large or small, did not seem to affect bone regeneration, but β-TCP is a good carrier material for delivery, retention, and release of rhBMP-4.

KEY WORDS: Bone regeneration; recombinant human bone morphogenetic protein-4; particle size; carrier; β-tricalcium phosphate; rat calvarial defect model

The Effect of Recombinant Human Bone Morphogenetic Protein-4 with the Different Particle Sizes of Beta Tricalcium Phosphate on Bone Regeneration in Rat Calvarial Defects

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Ⅰ. Introduction

Bone Morphogenetic proteins (BMPs) are potent growth and differentiation factors acting on mesenchymal cells to differentiate into mature osteoblasts, resulting in new bone formation. When implanted *in vivo*, the osteoinductive BMPs initiate a complex series of cellular events culminating in bone formation (Reddi AH 1981). BMPs are the most promising osteoinductive substance and are expected to be applied clinically for bone reconstruction.

BMP-4 has been implicated as a coupling factor in bone turnover during fracture repair, and appears to be involved in the cellular events which precede callus formation at the fracture site (Nakase et al. 1994, Bostrom et al. 1995, Hollinger et al. 1999). It was found to have a bone regeneration potential similar to

that of BMP-2 in both *in vitro* (Kim et al. 2002) and *in vivo* studies (Ahn et al. 2003).

Previous studies have shown that the use of an appropriate carrier material is essential for delivery, retention, and release of BMPs at a defect site (Urist et al. 1984, 1987, Marden et al. 1994, Kenley et al. 1994). Recently, β-TCP has been developed as an osteoconductive bone substitute and a biodegradable delivery system for rhBMP (Gao et al. 1996, Laffargue et al. 1999, Alam et al. 2001, Ahn et al. 2003). β-TCP is porous and able to entrap rhBMP-4 within its micropores, and intrinsically diffusible rhBMP-4 can be retained and its action consequently prolonged (Urist et al. 1984). The porous structure of β-TCP allows cells and newly forming tissues to migrate into it, and also provides sufficient firmness against soft tissue pressure. Our previous studies reported on the bone regeneration of rat calvarial defects using rhBMP-4/β-TCP (Ahn et al. 2003). There has not been sufficient research on the different particle sizes of carrier materials. . It was reported that particle sizes of freeze-dried bone allograft within 250 to 750 μm range have been shown to promote osteogenesis, whereas particle sizes below 125 μm may promote a significant foreign-body giant cell response and are rapidly resorbed (Mellonig et al. 1984). It was not known whether the resorption rate is related to the β-TCP particle size. The prolonged residence of the carrier may inhibit bone formation (Seeherman et al. 2002). What particle size of β-TCP is

more effective for bone regeneration and carriers of BMP has not yet been clearly established

To increase the bone regeneration without provoking adverse effects, it is essential to minimize the effective dose of BMP. In our previous study using this model, a 2.5 μg dose of rhBMP-4 with associated carriers proved to be sufficient for bone regeneration (Ahn et al. 2003). Therefore, in this study, we used a dose of 2.5 μg rhBMP-4. The purpose of this study was to evaluate the effect of recombinant human bone morphogenetic protein-4 with the different particle sizes of beta tricalcium phosphate on bone regeneration in the rat calvarial defect model.

Ⅱ. Materials & methods

A. Materials

1. Animals

A total of 100 male Sprague-Dawley rats (body weight 200-300 g) were used in this study. They were maintained in plastic cages in a room with 12 h-day/night cycles and an ambient temperature of 21℃ and standard laboratory pellets. Animal selection and management, surgical protocol, and preparation followed routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. rhBMP-4 construct

The rhBMP-4 implants consisted of rhBMP-4[**] in buffer loaded onto β-TCP[‡‡]. RhBMP-4 was reconstituted and diluted in buffer to produce a concentration of 0.025 mg/ml (2.5 μg). For the control experiments, the buffer was used alone.

Sterile β-TCP particles were loaded with 0.1 ml of the rhBMP-4 solution. Following a 5 minute binding time, the implant was prepared to fit the calvarial defect.

[**] R&D Systems Inc., Minneapolis, MN

[‡‡] Cerasorb®, 150-500 μm, Curasan, Kleinotheim, Germany

B. Research Procedures

1. Surgical procedures

The animals were anaesthetized by an intramuscular injection (5 mg/kg body wt.) of a 4:1 solution of Ketamine hydrochloride[§]: Xylazine[‖]. During surgery, routine infiltration anesthesia[¶] was used at the surgical site.

An incision was made in the sagittal plane across the cranium. A full thickness flap was reflected, exposing the calvarial bone. Then, a standardized, round, transosseous defect, 8 mm in diameter, was created on the cranium with the use of a trephine drill[#] cooling with saline, and trephined disk was removed.

The animals were divided into 5 groups of 20 animals each and allowed to heal for 2 or 8 weeks. They were treated with a sham-surgery control, particle size 50-150 μm (small-particle) β-TCP, particle size 150-500 μm (large-particle) β-TCP, 2.5 μg rhBMP-4 in particle size 50-150 μm (small-particle) β-TCP, or 2.5 μg rhBMP-4 in particle size 150-500 μm (large-particle) β-TCP. The periosteum and skin were sutured for primary closure with 4-0 coated Vicryl violet[§§].

2. Histologic and histometric procedures

The animals were sacrificed by CO_2 asphyxiation at 2 and 8 weeks postsurgery. Block sections including the surgical sites were removed. Samples were placed immediately into vials and were fixed in 10% neutral buffered formalin solution for 10 days. All samples were decalcified in EDTA-HCl for 7 days, and embedded in

paraffin. Three μm thick coronal sections through the center of the circular defects were stained with hematoxylin-eosin stain (H-E). After conventional microscopic examination, computer-assisted histometric measurements of the newly formed bone were obtained using an automated image analysis system[††] coupled with a video camera on a light microscope[¶¶]. Sections were analyzed under 20 x magnifications. Defect closure was determined by measuring the distance between the defect margin and ingrown bone margin. And it was expressed in mm and as a percentage of the total defect width. Augmented area (mm^2) was measured including new bone, the residual biomaterials. New bone area (mm^2) was determined by newly formed bone area within the augmented area, excluding biomaterials, marrow and fibrovascular tissues within the newly formed bone. Bone density was calculated as follows: Bone density (%) = New bone area / Augmented area x 100 (Figure 1).

3. Statistical Analysis

Histometric recordings from the samples were used to calculate group means (± SD). A two-way analysis of variance was used to analyze the effect of time and experimental conditions. The post hoc Scheffe's test was used to analyze the difference between the groups ($P < 0.05$).

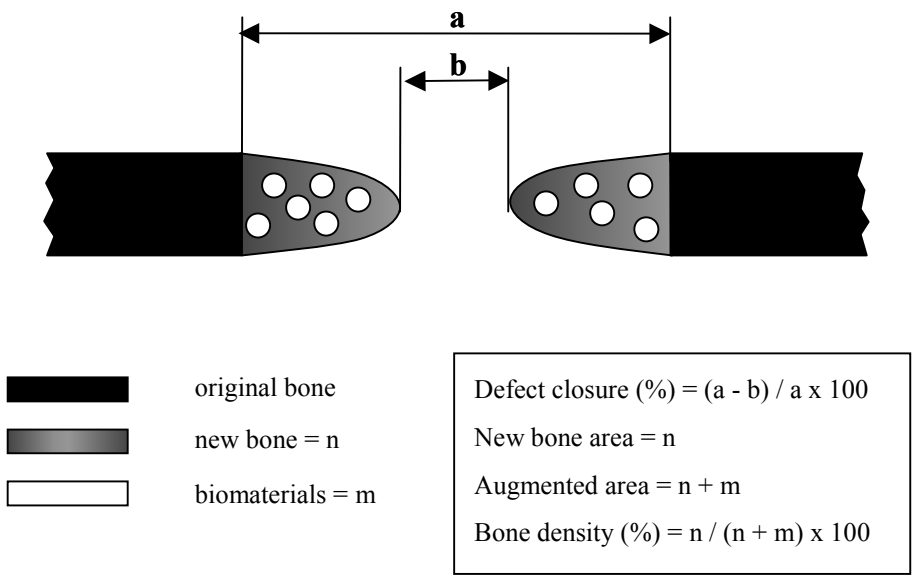

Figure 1. Schematic drawings of calvarial osteotomy defect showing histometric analysis

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§ Ketalar®, Yuhan Co., Seoul, Korea

‖ Rompun®, Bayer Korea, Seoul, Korea

¶ 2% lidocaine, 1:100,000 epinephrine, Kwangmyung Pharm., Seoul, Korea

3i, FL, USA

§§ Polyglactin 910, braided absorbable suture, Ethicon, Johnson & Johnson Int., Edinburgh,UK

†† Image-Pro Plus®, Media Cybernetics, Silver Spring, M.D.

¶¶ Olympus BX50, Olympus Optical co., Tokyo, Japan

Ⅲ. Results

A. Clinical observations

Wound healing was generally uneventful and appeared similar for both rhBMP-4/β-TCP groups and the controls.

B. Histologic observations

Sham surgery control: At 2 and 8 weeks postsurgery, defects filled with thin loose connective tissue with a minimal amount of new bone formation originating from the defect margins were observed. The middle portion of the defects had collapsed (Figure 2A, B).

Carrier control groups: In both β-TCP groups, defects filled with dense, fibrous connective tissue and a little new bone formation were observed adjacent to the defect margins at 2 weeks and 8 weeks. The residual β-TCP particles were still observed at 8 weeks (Figure 3, 4).

RhBMP-4/β-TCP groups: Regardless of particle size, extensive bone regeneration was apparent in all of the defects. A large number of residual β-TCP particles were observed within the new bone at 2 weeks, and they appeared to be less at 8 weeks. The appearance of the new bone at 8 weeks was more lamellar than that at 2 weeks (Figure 5, 6).

C. Histometric analysis

Tables 1 to 4 show the results of the histometric analysis. In the sham surgery controls, only limited new bone formation was observed. Defect closure and new bone growth in the ß-TCP controls were not different from that in the sham surgery controls (Table 1, 2).

Irrespective of particle size, the defects were almost closed completely and there was a significant bone growth in both rhBMP-4/β-TCP groups. Defect closure and new bone area were not significant different between these two groups. The new bone area of these two groups were significantly greater than those of β-TCP controls and sham surgery controls at 2 and 8weeks ($P < 0.01$) (Table 1, 2). The augmented areas of the rhBMP-4/β-TCP group were significantly greater than those of both β-TCP control groups and sham surgery control group at 2 and 8 weeks ($P < 0.01$) (Table 3). No significant differences were found in the defect closure, new bone area, and augmented area between two β-TCP control groups and between two rhBMP-4/β-TCP groups. In bone density, there were no differences between two rhBMP-4/ß-TCP groups at 2 and 8 weeks (Table 4).

Two-way ANOVA revealed that treatment conditions significantly affected the degree of formation of new bone within the defects ($P < 0.01$).

Table 1. Defect closure (group means ± SD; n=10, mm (%))

	2 weeks	8 weeks
sham surgery control	1.0 ± 0.4 (14.1 ± 5.2)	1.1 ± 0.6 (15.1 ± 8.0)
β- TCP (50-150 μm)	0.7 ± 0.2 (12.3 ± 4.1)	1.2 ± 1.1 (19.6 ± 15.9)
β- TCP (150-500 μm)	0.9 ± 0.5 (18.3 ± 12.3)	1.7 ± 0.7 (31.9 ± 11.5)
rhBMP-4/β- TCP (50-150 μm)	6.4 ± 1.3 (91.4 ± 16.0) [*†]	7.2 ± 0.5 (98.5 ± 4.3) [*†]
rhBMP-4/β- TCP (150-500 μm)	7.0 ± 0.5 (96.0 ± 4.6) [*†]	7.3 ± 0.2 (100 ± 0.0) [*†]

[*]: Statistically significant difference compared to sham surgery control group ($P < 0.01$)

[†]: Statistically significant difference compared to ß-TCP control group ($P < 0.01$)

Table 2. New bone area (group means ± SD; n=10, mm^2)

	2 weeks	8 weeks
sham surgery control	0.2 ± 0.1	0.3 ± 0.1
β- TCP (50-150 μm)	0.7 ± 0.2	0.8 ± 0.4
β- TCP (150-500 μm)	0.9 ± 0.3	1.0 ± 0.3
rhBMP-4/β- TCP (50-150 μm)	4.1 ± 1.4[*†]	4.5 ± 0.7[*†]
rhBMP-4/β- TCP (150-500 μm)	4.6 ± 0.6[*†]	4.7 ± 0. 7[*†]

[*]: Statistically significant difference compared to sham surgery control group ($P < 0.01$)

[†]: Statistically significant difference compared to ß-TCP control group ($P < 0.01$)

Table 3. Augmented areas (group means ± SD; n=10, mm^2)

	2 weeks	8 weeks
sham surgery control	1.8 ± 0.6	2.6 ± 0.6
β- TCP (50-150 μm)	3.7 ± 1.9	2.4 ± 1.8
β- TCP (150-500 μm)	2.8 ± 0.8	2.3 ± 0.7
rhBMP-4/β- TCP (50-150 μm)	6.7 ± 1.6*†	6.4 ± 2.3*†
rhBMP-4/β- TCP (150-500 μm)	7.2 ± 1.3*†	7.0 ± 0.8*†

*: Statistically significant difference compared to sham surgery control group ($P < 0.01$)

†: Statistically significant difference compared to ß-TCP control group ($P < 0.01$)

Table 4. Bone density (group means ± SD; n=10, %)

	2 weeks	8 weeks
rhBMP-4/β- TCP (50-150 μm)	61.2 ± 10.2	70.3 ± 15.9
rhBMP-4/ β- TCP (150-500 μm)	63.9 ± 6.0	67.1 ± 4.9

Ⅳ. Discussion

Bone morphogenetic protein (BMP) was first discovered by Urist in 1965 (Urist MR. 1965). BMPs belong to the transforming growth factor-β (TGFβ) superfamily. This family comprises a large number of growth and differentiation factors, which are related in primary amino acid sequence, and consists of the molecules BMP-2 through BMP-8, which are dimeric molecules. Comparisons among the derived amino acid sequences of the BMPs found in osteoinductive extracts of bone indicate that they fall into three subclasses. The first subclass contains BMP-2 and BMP-4, highly related molecules that differ mainly in the amino terminal region, with BMP-2 containing a heparin-binding domain. In the second subclass are BMP-5, BMP-6, and BMP-7, also known as osteogenic protein-1 (OP-1), and BMP-8 (OP-2). These are slightly larger proteins than BMP-2 and BMP-4, and there an approximate 70% amino acid identity between the subgroups. In the third subclass, and more distantly related to these factors, is BMP-3, also called osteogenin (Wozney JM. 2002).

BMP-4 has been implicated as a coupling factor in bone turnover during fracture repair, and appears to be involved in the cellular events which precede callus formation at the fracture site (Nakase et al. 1994, Bostrom et al. 1995, Hollinger et al. 1999). It was found to have a bone regeneration potential similar to that of BMP-2 in both *in vitro* (Kim et al. 2002) and *in vivo* studies (Ahn et al. 2003).

In the present study, it was demonstrated that rhBMP-4 reconstituted with either small- or large-particle β-TCP had a significant effect to induce bone regeneration comparing to small- or large-particle β-TCP control, or sham surgery control in rat calvarial critical size defects, which explained the effect of rhBMP-4 on bone regeneration.

To increase bone regeneration without provoking adverse effects, the effective dose of BMP needs to be kept to a minimum. Previous studies designed to investigate the dose related response to BMPs have produced mixed results. Although some studies showed a distinct dose dependent response (Wang et al. 1990, Kenley et al. 1994, Kanatani et al. 1995, Ohura et al. 1999, Laffargue et al. 1999, Alam et al. 2001), other studies failed to demonstrate dose-dependency, even when wide dose ranges were used (Cook et al. 1994, Aspenberg et al. 1996, Sandhu et al. 1996, Wikesjö et al. 1999, Zellin et al. 1999, Winn et al. 1999, Tatakis et al. 2002). The lack of dose dependent response might be expected once a minimum threshold dose has been exceeded, and above a minimum threshold dose, bone regeneration may not vary with increasing dose of rhBMP-4 (Cook et al. 1994, Sandhu et al. 1996, Winn et al. 1999, Tatakis et al. 2002). In our previous study (Kim et al. 2002), we performed experiments using both 2.5 μg and 5 μg rhBMP-4, and within the dose range examined, rhBMP-4 did not exhibit an appreciable dose dependent response. 2.5 μg dose of rhBMP-4 may be above the minimum threshold dose, so we performed experiments using 2.5 μg rhBMP-4 in this study.

The use of an appropriate carrier material is essential for delivery, retention, and release of BMPs at a defect site (Urist et al. 1984, 1987, Marden et al. 1994, Kenley et al. 1994). Successful carrier systems must enable vascular and cellular invasion, allowing the BMP to act as a differentiation factor. The carrier should be reproducible, nonimmunogenic, moldable, and space-providing to define the contours of the resulting bone. Moreover, the carrier should resorb completely following the initiation of bone induction, thus ensuring bone formation (Wikesjö et al. 2001). Various biomaterials have been tested as candidate for carriers of BMPs. They include collagen (Sigurdsson et al. 1996, Choi et al. 2002), decalcified bone matrix (DBM) (Sigurdsson et al. 1996), hyaluronan (Hunt et al. 2001), hydroxyapatite (Mao et al. 1998), tricalcium phosphate (Urist et al. 1984, 1987, Wu 1992, Gao et al. 1996), a hydroxyapatite-collagen composite (Asahina et al. 1997), polylactic acid polymer (Heckman 1991), polylactic-polyglycolic polymer (Miyamoto et al. 1993, Sigurdsson et al. 1996), gelatin (Isaksson et al. 1993), fibrin sealant (Kawamura et al. 1988), and composites of these materials (Ohura et al. 1999). Each carrier material showed various advantages and disadvantages and it has not yet been determined which of these materials constitutes the ideal carrier system for BMPs. Demands on carrier materials may differ between indications and between sites; therefore, the search for ideal carriers should be continued.

β-TCP has been developed as an osteoconductive bone substitute and a biodegradable delivery system for rhBMP (Gao et al. 1996, Laffargue et al. 1999,

Alam et al. 2001, Ahn et al. 2003). β-TCP is porous and able to entrap rhBMP-4 within its micropores, and intrinsically diffusible rhBMP-4 can be retained and its action consequently prolonged (Urist et al. 1984). The porous structure of β-TCP allows cells and newly forming tissues to migrate into it, and also provides sufficient firmness against soft tissue pressure. Our previous studies reported on the bone regeneration of rat calvarial defects using rhBMP-4/β-TCP (Ahn et al. 2003).

In this study, we used β-TCP (phase purity > 99% β-modification of tricalcium phosphate) as carrier systems for rhBMP-4. There were significant differences in the defect closure and new bone areas between the β-TCP control groups and the rhBMP-4/β-TCP groups (Table 1, 2), which may be explained by the effect of rhBMP-4 on bone regeneration. Augmented areas in rhBMP-4/β-TCP groups were statistically significant greater compared to both β-TCP groups at 2 and 8 weeks ($P < 0.01$) (Table 3). These results may be explained by the fact that even though the β-TCP particles had sufficient firmness against the soft tissue pressure to be able to maintain the defect space and they were resorbed slowly, the space could not be maintained, if new bone was not formed. There has not been sufficient research on the different particle sizes of carrier materials. Xu et al. (2003) compared the osteoconductive capability of deproteinized bone particles of two different sizes (300-500 and 850-1000 μm) in rabbits undergoing maxillary sinus lift. They reported that a significantly higher density of newly formed bone in the small-particle group than in the large-particle group both 4 and 8 weeks after implantation.

We compared two different particle sizes of β-TCP, 50-150 μm and 150-500 μm. It was reported that particle sizes of freeze-dried bone allograft within 250 to 750 μm range have been shown to promote osteogenesis, whereas particle sizes below 125 μm may promote a significant foreign-body giant cell response and are rapidly resorbed (Mellonig et al. 1984). But in this study, residual β-TCP particles were still present within fibrous connective tissue at the defect sites of small-particle (50-150 μm) β-TCP carrier control group and also a number of residual particles were evident within the new bone at the defect sites of rhBMP-4/β-TCP (50-150 μm) group at 8 weeks, though the particles appeared smaller in numbers than at 2 weeks. The prolonged residence of the carrier may inhibit bone formation (Seeherman et al. 2002). It was not known whether the resorption rate is related to the β-TCP particle size. In present study, the particle sizes did not result in significant differences in defect closure, new bone area, augmented areas, and bone density at 2 and 8 weeks postsurgery.

Ⅴ. Conclusion

The purpose of this study was to evaluate the effect of recombinant human bone morphogenetic protein-4 with the different particle sizes of beta tricalcium phosphate on bone regeneration in the rat calvarial defect model.

Eight mm calvarial critical-size osteotomy defects were created in one hundred male Sprague-Dawley rats. Five groups of twenty animals each treated with a sham-surgery control, particle size 50-150 μm (small-particle) β-tricalcium phosphate (β-TCP) carrier control, particle size 150-500 μm (large-particle) β-tricalcium phosphate (β-TCP) carrier control, rhBMP-4 (2.5 μg) in a particle size 50-150 μm (small-particle) β-tricalcium phosphate (β-TCP) carrier, rhBMP-4 (2.5 μg) in a particle size 150-500 μm (large-particle) β-tricalcium phosphate (β-TCP) carrier and were evaluated by histological and histometric parameters following a two- and eight-week healing interval.

Defect closure, new bone areas, and augmented areas of the two rhBMP-4/β-TCP groups were significantly greater than those of two β-TCP control groups and sham-surgery control group at both 2 and 8 weeks ($p < 0.01$). There were no significant differences in the defect closure, new bone area, and augmented area between two rhBMP-4/β-TCP groups and between two β-TCP control groups at 2 and 8 weeks. There was no significant difference in bone density between two rhBMP-4/β-TCP groups at 8 weeks.

rhBMP-4 combined with either small- or large-particle β-TCP had a significant

effect to induce bone regeneration comparing to β-TCP controls, or a sham-surgery control in rat calvarial defect model. Within the parameters of this study, the particle size of β-TCP, large or small, did not seem to affect bone regeneration, but β-TCP is a good carrier material for delivery, retention, and release of rhBMP-4.

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Legends

Figure 1. Schematic drawings of osteotomy calvarial defect showing histometric analysis.

Figure 2. Representative photomicrographs of the sham-surgery control group at 2 weeks (A) and 8 weeks (B) postsurgery. Thin, fibrous connective tissues were observed between the defect margins. The middle of the defects had collapsed. (asterisk = ß-TCP, arrow head = defect margin; H-E stain; original magnification ×20).

Figure 3. Representative photomicrographs of defect sites receiving small-particle β-TCP at 2 weeks (A, B) and 8 weeks (C, D) postsurgery. Residual β-TCP particles were still present within fibrous connective tissue at the defect site at 8 weeks. (arrow head = defect margin; H-E stain; original magnification A, C ×20; B, D ×100).

Figure 4. Representative photomicrographs of defect sites receiving large-particle β-TCP at 2 weeks (A, B) and 8 weeks (C, D) postsurgery. Residual β-TCP particles were still present within fibrous connective tissue at the defect site at 8 weeks.

Figure 5. Representative photomicrographs of defect sites receiving rhBMP-

4/small-particle ß-TCP at 2 and 8 weeks postsurgery. At 2 weeks (A, B), the defect was completely bridged with new bone, and a large number of residual ß-TCP particles were evident within the new bone. At 8 weeks (C, D), the ß-TCP particles appeared smaller in numbers than at 2 weeks (asterisk = ß-TCP, arrow head = defect margin; H-E stain; original magnification (A, C ×20; B, D ×100).

Figure 6. Representative photomicrographs of defect sites receiving rhBMP-4/large-particle ß-TCP at 2 and 8 weeks postsurgery. Similar observations to defect sites receiving rhBMP-4/small-particle ß-TCP were made. (asterisk = ß-TCP, arrow head = defect margin; H-E stain; original magnification (A, C ×20; B, D ×100).

Figures

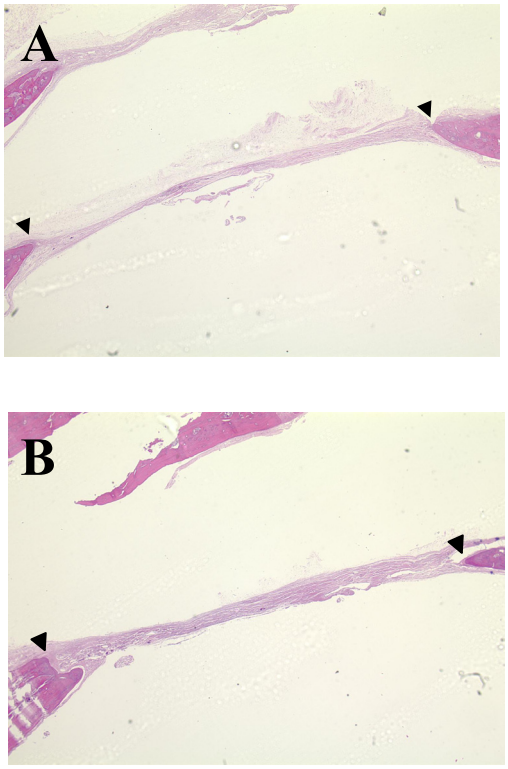

Figure 2

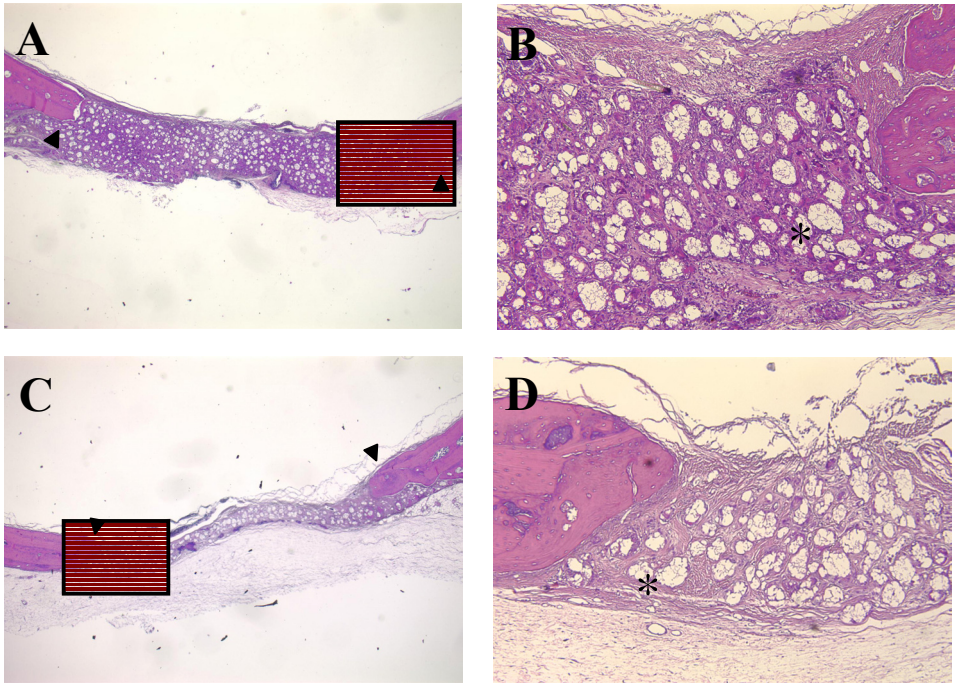

Figure 3

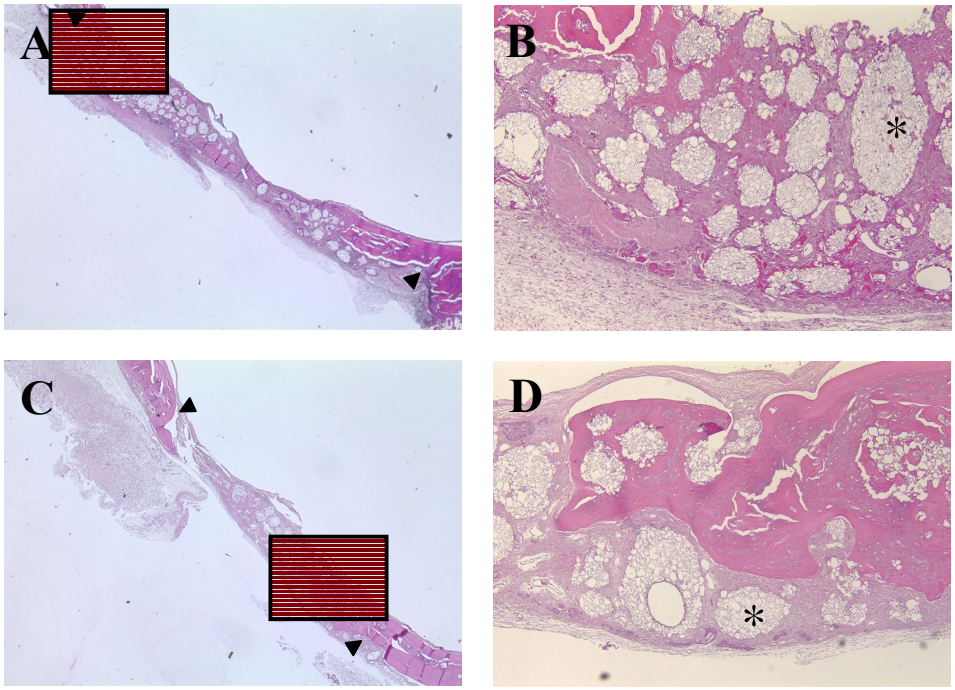

Figure 4

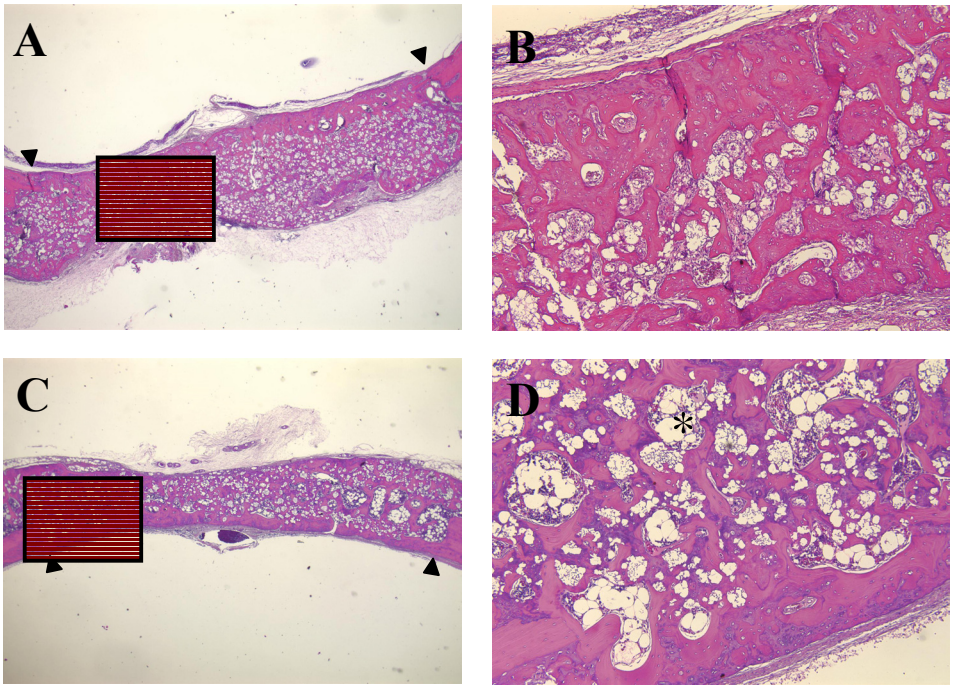

Figure 5

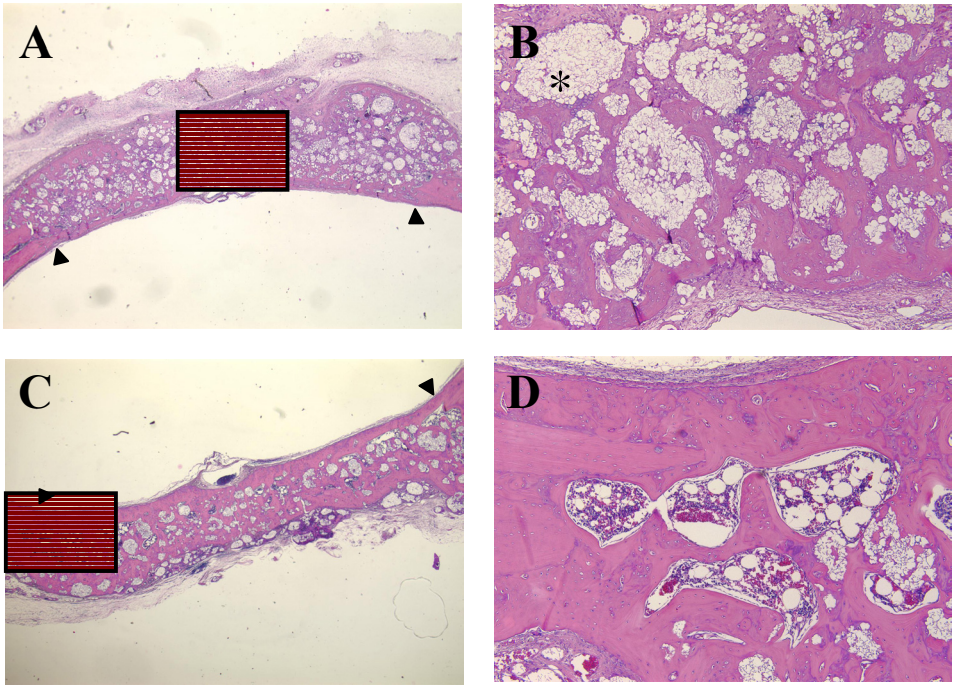

Figure 6

국문요약

백서 두개골 결손부에서 다른 입자 크기의 beta tricalcium phosphate와 rhBMP-4의 골재생 효과

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최 성 용

여러 가지의 골형성 유도 단백질(bone morphogenetic protein, BMP)이 골형성을 증가시키는 능력에 대한 임상전과 임상 연구가 시험되고 있지만, rhBMP-4에 대한 연구는 충분하지 않다. BMP의 골유도 능력을 지지하기 위하여 BMP 전달체계에 쓰이는 운반체 또한 중요한 역할을 한다. 그러나 운반체의 입자크기에 대한 연구 또한 불충분하다. 운반체의 장기간의 잔류는 골형성을 방해한다. Beta tricalcium phosphate (β-TCP)의 입자 크기가 흡수율과 관계가 있는지는 알려지지 않았다. 이 연구의 목적은 백서 두개골 결손부에서 다른 입자 크기의 β-TCP와 rhBMP-4의 골재생 효과를 평가하는 것이다.

100마리의 웅성 백서에서 8 mm 임계크기의 두개부 결손을 형성하였다. 20마리의 동물을 5개의 군으로 나누고 각 군은, 아무 것도 이식하지 않은 군, 50-150 μm의 β-TCP를 이식한 군, 150-500 μm의 β-TCP를 이식한 군, 2.5 μg의 rhBMP-4와 50-150 μm의 β-TCP를 이식한 군, 2.5 μg의

rhBMP-4와 150-500 μm의 β-TCP를 이식한 군으로 나누어 술후 2 주와 8주에 치유 결과를 조직학적, 조직계측학적으로 비교 관찰하였다.

술 후 2주와 8주에서 작은 입자나 큰 입자의 rhBMP-4/β-TCP군 모두 결손부 폐쇄 (defect closure), 신생골형성량 (new bone area), 조직형성량(augmented area)이 β-TCP 대조군이나 아무 것도 하지 않은 군보다 유의성 있게 나타났다 ($p < 0.01$). 술 후 2주와 8주에서 결손부 폐쇄 (defect closure), 신생골형성량 (new bone area), 조직형성량(augmented area)에 있어서 두 rhBMP-4/β-TCP군 사이에서 유의성 있는 차이를 보이지 않았으며 두 β-TCP 대조군 사이에서도 유의성 있는 차이를 보이지 않았다. 골밀도 (bone density)에 있어서 두 rhBMP-4/β-TCP군 사이에서 유의성 있는 차이를 보이지 않았다.

이상의 결과에서 볼 때, 작은 입자나 큰 입자를 운반체로 사용하여 rhBMP-4를 적용하였을 때 작은 입자나 큰 입자의 β-TCP 대조군이나 아무 것도 하지 않은 군에 비하여 백서 두개골 결손부에서 골재생에 유의한 효과를 보임을 알 수 있다. β-TCP의 입자의 크고 작음은 골재생에 영향을 주지 않는 것으로 사료된다.

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핵심되는 말: 신생골 형성, 골형성 유도 단백질, 입자 크기, 운반체, 베타삼화인산칼슘염(β-TCP), 백서 두개골 결손부