The Effect of Recombinant Human Bone Morphogenetic Protein-2, 4 and 7 on Bone Formation in Rat Calvarial Defects

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The Effect of Recombinant Human Bone Morphogenetic Protein-2, 4 and 7 on Bone Formation in Rat Calvarial Defects

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감사의 글

이 논문이 완성되기까지 지도편달 아끼지 않으시고 누구보다도 도움을 주신 김종관 교수님께 진심으로 감사를 드립니다. 그리고, 여러 가지 조언과 따뜻한 관심으로 지켜봐 주신 채중규 교수님, 조규성 교수님, 최성호 교수님, 유윤정 교수님, 김희진 교수님께 진심으로 감사 드립니다. 또한 물심양면으로 도와주시고 연구에 많은 조언을 해 주신 김창성 교수님께 다시금 감사의 말씀을 전합니다.

연구 내내 많은 도움을 준 한동관 선생님을 비롯한 치주과 교실원 여러분들께 고마움을 전합니다.

항상 염려해주시고 관심 가져주시며 저의 인생의 스승이신 아버지, 언제나 사랑의 마음으로 보살펴주시는 어머니께 다시 한번 감사의 말씀을 전합니다. 언제나 묵묵히 지켜 봐주는 마음 든든한 동생 내외에게 감사의 마음을 담습니다. 저의 인생의 반려자인 아내의 깊은 관심과 배려에도 사랑과 고마움의 마음을 전합니다.

제주도 중문 해변의 바닷 광풍과 한라산 중턱의 은은한 안개 속에 나 자신을 항상 새로이 하며 일진할 것을 다짐합니다

모든 분들께 진심으로 다시 한번 두 손 여며 감사 드립니다.

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저자 씀

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Abstract

The Effect of Recombinant Human Bone Morphogenetic Protein-2, 4 and 7 on Bone Formation in Rat Calvarial Defects

Background: Currently, more than 20 bone morphogenetic proteins (BMPs) have been identified and many trials have been carried out using recombinant human BMPs (rhBMPs) for bone tissue engineering. However, comparative analyses on bone formative activities of rhBMP using a preclinical model have been limited. Therefore, the aim of this study was to evaluate and compare the osteogenic potential of rhBMP-2, 4 and 7 delivered with ACS upon early (2 weeks) and complete (8 weeks) wound healing phases in a critical sized rat calvarial defect model.

Materials and Methods: Eight-millimeter critical sized calvarial defects were created in thirty male Sprague-Dawley rats. The animals were divided into 3 groups of 10 animals each. The defects were treated with 0.025 ㎎/㎖ rhBMP-2/ACS, rhBMP-4/ACS, or rhBMP-7/ACS. The rats were sacrificed at either 2 (5rats) or 8 (5rats) weeks after surgery, and the results were evaluated histologically, histomorphometrically and immunohistometrically.

Results: The surgical implantation of rhBMP-2/ACS, rhBMP-4/ACS or rhBMP-7/ACS resulted in enhanced local bone formation in the rat calvarial defect model at both 2 and 8 weeks. The amount of defect closure, new bone area, and bone density

were similar in the three groups at each time point (P>0.05). In terms of bone density and new bone area, there were statistically significant differences between results obtained at 2 weeks and those obtained at 8 weeks in all groups (P<0.05). Two-way ANOVA revealed that there was no correlation between the time and conditions (P>0.05), but time was found to have a strong influence on defect closure, new bone area, and bone density (P<0.05). Irrespective of rhBMP type, positive immunoreactions of osteopontin (OP) and osteocalcin (OC) were evident at 2 and 8 weeks. Intense OP and OC staining was observed near the newly formed bone as well as in some cells within the new bone.

Conclusions: Within the rhBMP types used, rhBMP concentration, and the observation interval, there appears to be no specific differences in bone regenerative potential. All rhBMPs used in this study may be considered effective factors for inducing bone formation.

Key Words : Bone regeneration; recombinant human bone morphogenetic protein-2, 4, and 7; absorbable collagen sponge; rat calvarial defect model

The Effect of Recombinant Human Bone Morphogenetic Protein-2, 4 and 7 on Bone Formation in Rat Calvarial Defects

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I. Introduction

Bone morphogenetic proteins (BMPs) are regarded as members of the transforming growth factor-β superfamily owing to characteristic features in their amino acid sequences.[21,33] In 1965, Urist demonstrated ectopic bone and cartilage formation following intramuscular implantation of demineralized bone matrix in rats.[31] The factor within the matrix that is responsible for this effect was later named BMP. After recombinant human BMP (rhBMP) was successfully synthesized by Wozney et al. [33] in 1988, a number of studies have demonstrated the biological activities of rhBMPs. These studies included the induction of ectopic cartilage and

bone formation at implanted sites in vivo,[12,31,33] and the stimulation of osteoblastic phenotype expression during the course of osteoblastic differentiation in various cell types in vitro.[13,14,17,29,30] Currently, more than 20 BMPs have been identified and several, including BMP-2, -4, -5, -6, and -7, have been shown to have significant osteoinductive activity.[1,6,9,13,14,17,23,29,32] Accordingly, there have been many trials using rhBMPs for bone tissue engineering. When impregnated into an appropriate carrier system such as an absorbable collagen sponge (ACS), they promote a significant increase in new bone formation in experimental bone defect models.[1,2,6,15,20,32] Among these related proteins, rhBMP 2, 4 and 7 have been selected for bone and periodontal tissue engineering in many studies.[2,7,8,11,15,18,20,22,26,27] However, comparative analyses on bone formative activities of rhBMP using a preclinical model have been limited.

The aim of this study was to evaluate and compare the osteogenic potentials of rhBMP-2, 4 and 7 delivered with ACS upon early (2 weeks) and complete (8 weeks) wound healing phases in a critical sized rat calvarial defect model.

II. Materials and Methods

1. Animal

This study included 30 male Sprague-Dawley rats (body weight 200-300g) maintained in plastic cages in a room with a 12 h-day/night cycle and an ambient temperature of 21°C. The rats were allowed access to water and standard laboratory pellets *ad libitum*. The animal selection, management, surgical protocol, and preparation followed the routines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea.

2. rhBMP-2, 4 and 7 implant constructs

The rhBMP-2, 4 and 7 implants consisted of rhBMP-2, 4 and 7[‡] in buffer at a concentration of 0.025 mg/ml loaded onto an ACS[§]. For each rhBMP/ACS implant, a sterile ACS (8mm in diameter) was loaded with 0.1ml of rhBMP solution. The implants were placed into the calvarial defects after 5 min of loading.

‡R&D Systems Inc., Minneapolis, MN

§ Collatape®, Calcitek, Carlsbad, CA

3. Surgical protocol

The animals were anaesthetized by an intramuscular injection (5 mg/kg body wt.) of ketamine hydrochloride[¶]. Routine infiltration anesthesia[#] was used at the surgical

site. An incision was made in the sagittal plane across the cranium and a full thickness flap was made, exposing the calvarial bone. A standardized, circular, transosseous defect, 8 mm in diameter, was created on the cranium using a saline cooled trephine drill**. After removing the trephined calvarial disk, a rhBM0P implant was applied to each defect. The animals were divided into 3 groups of 10 animals each and were allowed to heal for 2 (5 rats) or 8 (5 rats) weeks. Each animal received one of three experimental conditions: rhBMP-2/ACS, rhBMP-4/ACS, or rhBMP-7/ACS. The periosteum and skin were then closed and sutured with 4-0 coated Vicryl sutures††.

¶ Ketalar®, Yuhan Co., 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

4. Histologic procedures

The animals were euthanized 2 and 8 weeks after surgery. Block sections including the experimental sites were removed. The samples were fixed in a 10% neutral buffered formalin solution for 10 days. The samples were decalcified in 5% formic acid for 14 days, and embedded in paraffin. Serial sections, 5*u*m thick, were prepared at 80*u*m intervals, stained with hematoxylin/eosin (H-E) and examined using an optical microscope. The most central sections from each block were selected to

compare histology findings between groups.

5. Histomorphometric analysis

Computer-assisted histometric measurements were obtained using an automated image analysis system‡‡ coupled with a video camera attached to an optical microscope§§. The sections were examined at 20x magnification. A digitizer was used to trace the defect outline versus new bone formation, and the percentage of bone fill was determined. The following histomorphometric parameters were measured for each sample.[20]

- Defect closure (mm or %): the distance (at each side of the defect) between the defect margin and the in-growing bone margin in mm. The % defect closure may be obtained by subtracting this value from the total defect distance, then dividing by the total defect distance and multiplying by 100.
- Augmented area (mm^2): all tissues within the boundaries of the newly formed bone, i.e. mineralized bone, fatty marrow, fibrovascular tissue/marrow, and residual biomaterial.
- New bone area (mm^2): the area of newly formed bone within the total augmented area.
- Bone density (%): the new bone area divided by the total augmented volume X 100.

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

§§ Olympus BX50, Olympus Optical Co., Tokyo, Japan

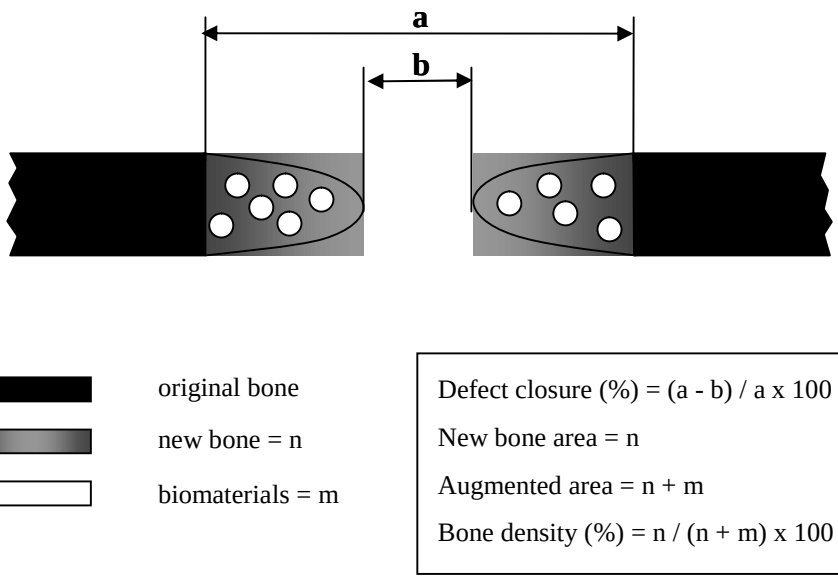

Figure 1. Schematic drawings of a calvarial osteotomy defect showing histomorphometric analysis

6. Immunohistochemical procedures

Immunohistochemical procedures were performed on the sections in which bone formation could be observed histologically. Two types of antibody against bone matrix proteins were used: mouse monoclonal antibodies against osteocalcin (OC)[¶¶] and osteopontin (OP).[##]

Following deparaffinization with xylene, the sections were rehydrated through a graded ethanol series and washed in distilled water. Endogenous peroxidase was blocked by incubating the sections with 0.3% H_2O_2. After washing the sections with

phosphate-buffered saline (PBS, pH 7.4), they were treated with normal horse serum for 30 min in order to prevent non-specific binding. Primary antibodies were diluted in PBS containing 3% bovine serum albumin, and incubated overnight at room temperature. The primary antibody dilutions were: anti-OP (1:50), and OC (1:1). The sections were then rinsed three times with PBS for 5 minutes each, incubated with biotinylated anti-mouse IgG for 30 min in a moist chamber, and incubated with horseradish peroxidase streptavidin conjugate for 30 min. Slides were then rinsed in PBS.

The antibody complexes were visualized with a 3,3'-diaminobenzidine substrate and H_2O_2, washed in distilled water and counterstained with Harris hematoxylin. For controls, some sections were treated in the same way, without incubation with primary antibodies. The stained sections were analyzed by optical microscopy and photographed.

¶¶ Biogenex, San Ramon, CA

Santa Cruz Biotechnology, Santa Cruz, CA

7. Statistical Analysis

Histomorphometric recordings from the samples were used to calculate mean and standard deviation values (m±SD). Mean scores were used to test for differences among experimental conditions using analysis of variance (ANOVA) and *post hoc t-*

tests for multiple comparisons at each time point. To compare the 2 and 8 week values in the same rhBMP group, statistical significance was determined by a paired t-test. Two-way ANOVA was used to analyze the effects of both time and condition, and to determine any correlation between them. A p-value < 0.05 was considered significant.

III. Results

1. Clinical observations

Wound healing was generally uneventful and all rhBMP-2, 4 and 7 experiments appeared to be similar. No material exposure or other complications were observed at the surgical sites.

2. Histological observations *(Figure 2 and 3)*

Irrespective of rhBMP type, all defect sites exhibited marked bone formation in a similar pattern, and were almost completely bridged with new bone at 8 weeks. At 2 weeks, the ACS implants were observed in a parallel pattern within loose fibrous connective tissue and newly formed bone without any significant adverse reactions. Newly formed bone with osteocytes was evident mainly at the periphery of defects, and osteoblast-like cells exhibiting a dense arrangement adjacent to the newly formed bone suggested continued bone apposition at this initial healing stage. However, less bone formative activity was noted in the central aspects of the defects. There was no evidence of fatty marrow or cartilage formation.

At 8 weeks, the quantity of new bone was greater than that observed at 2 weeks, and the specimens showed a more advanced stage of remodeling and consolidation. There was no collagen remnant in any of the specimens. The newly formed bone consisted of woven and lamellar bone, and showed cement lines that were separated

earlier from the more recently deposited bone and concentric rings of the Haversian system. There was no evidence of cartilage formation. However, fatty marrow was observed in the central aspect of newly formed bone.

3. Histomorphometric analysis

Results of the histomorphometric analysis are shown in Tables 1-4. Defect closure, new bone area and augmented area were similar in the three groups at each time point. Regarding bone density, there were statistically significant differences between the 2 and 8 week results in all rhBMP groups ($P<0.05$). Two-way ANOVA revealed no interactions between time and condition ($P>0.05$), but time was found to have a strong influence on the extent of defect closure, new bone area, and bone density ($P<0.05$). Therefore, it appears that the types of rhBMP used in this study had similar effects on bone formation.

4. Immunohistochemical observations *(Figure 4)*

Positive immunoreactions for OP and OC were evident in rhBMP-2, 4 and 7 treated rats at 2 and 8 weeks. Intense OP and OC staining was observed near the newly formed bone and in some cells within it. At 2 weeks, more intense immunoreactions of OP and OC were evident in the marginal area of the defect than in the central area in which bone-forming activity was minimal. However, an even immunoreaction distribution was observed throughout the defect at 8 weeks. Together

with the histological findings, this suggests that bone formation and mineralization proceed in a central direction from the defect margin.

Table 1. Defect closure (group means ± SD; n=5, mm or %)

	2 weeks	8 weeks
rhBMP-2/ACS	7.3 ± 0.1 (98.0 ± 0.0)	7.7 ± 0.1 (99.4 ± 0.0)
rhBMP-4/ACS	6.4 ± 0.1 (85.9 ± 0.1)	7.5 ± 0.6 (97.8 ± 0.0)
rhBMP-7/ACS	7.0 ± 1.1 (93.1 ± 0.2)	7.5 ± 0.4 (98.2 ± 0.0)

Table 2. New bone area (group means ± SD; n=5, mm^2)

	2 weeks	8 weeks
rhBMP-2/ACS	3.1 ± 1.1	4.2 ± 1.2
rhBMP-4/ACS	3.2 ± 1.6	4.3 ± 0.6
rhBMP-7/ACS	3.2 ± 0.6	4.3 ± 1.1

Table 3. Bone density (group means ± SD; n=5, %)

	2 weeks	8 weeks
rhBMP-2/ACS	54.4 ± 11.3	82.0 ± 4.3*
rhBMP-4/ACS	53.9 ± 10.7	80.7 ± 3.5*
rhBMP-7/ACS	60.7 ± 11.1	81.2 ± 5.9*

*: Statistically significant difference from 2 weeks ($P<0.05$)

Table 4. Augmented area (group means ± SD; n=5, mm^2)

	2 weeks	8 weeks
rhBMP-2/ACS	5.7 ± 2.7	5.1 ± 1.0
rhBMP-4/ACS	6.0 ± 1.9	5.4 ± 0.5
rhBMP-7/ACS	5.2 ± 1.2	5.3 ± 1.6

IV. Discussion

The aim of this study was to evaluate the bone regenerative effects following implantation of rhBMP-2, 4 and 7 delivered with an ACS, and to compare the bone regenerative potential between the three proteins histologically in a critical size rat calvarial defect model. Eight millimeter diameter standardized, circular, transosseous defects created on the cranium of rats were implanted with rhBMP/ACS, and healing was evaluated histologically, histomorphometrically, and immunohistometrically after 2- and 8-week healing intervals. It was found that rhBMP-2, 4 and 7 combined with ACS have a significant potential to induce bone formation and express positive OC and OP immunoreactions in a rat calvarial defect assay model. The results showed that within the selected types of rhBMP and observation intervals, there were no significant differences in bone formation suggesting that rhBMP-2, 4 and 7 have similar effects on bone regeneration and tissue engineering.

BMPs belong to the transforming growth factor-β (TGF-β) superfamily. This family comprises a large number of growth and differentiation factors that have a similar primary amino acid sequence.[34] The mature regions of BMP-2 and BMP-4 are approximately 92% similar at the amino acid level.[33] BMP-7, which is also known as osteogenic protein-1, is slightly larger than BMP-2 and BMP-4. Furthermore, there is an approximate 70% amino acid identity between the subgroups.[4] Although rhBMP 2, 4 and 7 have only minor differences in their amino acid sequences, it appears that

such differences affect their bone regenerative potentials. Therefore, the bone regenerative activities of various BMPs seem to arise from the common amino acid sequences.

Compared with other experimental bone defects, the rat calvarial defect model is convenient for examining bone regeneration because of its effective accessibility and lack of fixation requirements. In addition, the defects are reproducible and native, and the healing processes induced have been well characterized.[3,24,28] This study did not include sham-operated or ACS-only controls. However, 8 mm trephine calvarial defects in the rats have been shown to be critical sized defects. Moreover, the limited healing response at 2 and 8-weeks, following surgery alone and the implantation of ACS only, have also been demonstrated in previous reports using this type of model.[1,20] Ahns et al.[1] evaluated the bone regenerative effect of rhBMP-4 delivered with ACS using rat calvarial defects. Histologic results obtained 2 weeks post-surgery revealed that bone fill was significantly greater in the rhBMP-4 treated sites (71.7%) relative to sham-operated (4.8%) or ACS-only control sites (14.6%). Minute new bone formation adjacent to defect margins was observed in the sham-operated and ACS-only control sites. Furthermore, volumetric changes of the new bone did not occur with extended healing periods of 8 weeks. 8-week histologic results revealed that bone fill was significantly greater in rhBMP-4 treated sites (91.9%) compared to sham-operated (8.2%) or ACS-only control sites (17.4%). Pang et al.[20] also reported similar histologic results in the same defect model. Defect closure, in their studies

using a 8mm rat clavarial defect, did not exceed 20-30% of the defect width for sham-operated and ACS-only control sites at 2 and 8-week healing intervals. Thus, it could be suggested that this experimental calvarial defect in rats may be employed as a standard for preclinical testing of candidate regenerative biomaterials prior to clinical application. In the present study, newly formed bone with osteocytes and densely arranged osteoblast-like cells was evident 2 weeks post-operative mainly at the defect periphery. However, less bone formative activity was noted in the central aspects of the defects. At 8 weeks, the newly formed bone showed a more advanced stage of remodeling and consolidation evenly throughout the defect. Histomorphometric analysis showed that there were statistically significant differences between the results obtained at 2 weeks and those obtained at 8 weeks in all groups. These results suggest that new bone formation and the normal healing process in the defects increased from 2 weeks to 8 weeks, and healing of the rat calvarial defect after rhBMP treatment was initiated from the defect margin.

OP and OC were first identified in the bone matrix proteins. Although their precise roles during bone formation are unclear, they are believed to be markers for more mature osteoblasts because they are expressed in later stages of osteoblast differentiation.[19] A number of studies have demonstrated that rhBMP-2 stimulates OP and OC production in the rat calvarial,[5] human calvarial,[10] and human bone marrow stromal cells.[17] In addition, Hirota et al.[12] reported that the sequential expression of OP and OC mRNA was detected in the process of ectopic bone formation by BMP-4

using a mouse subcutaneous assay system. The results of this study demonstrated that rhBMP-2, 4 and 7 stimulated OP and OC production in vivo at 2 weeks, indicating that the deposition of this protein is a primary event during bone formation. In addition, the fact that OP and OC were also detected at 8 weeks suggests these proteins play an important role in the remodeling process of newly formed bone. In contrast, a previous study reported that rhBMP-2 did not induce OC production when implanted with β-tricalcium phosphate and collagen as carriers in a rat subcutaneous assay model.[15] These findings are also consistent with those observed in previous in vitro studies. Kobayashi et al.[16] investigated the effects of rhBMP-2 on the expression of osteoblastic phenotype in human periodontal ligament cells. They reported that rhBMP-2 stimulated ALP activity and accumulation of the parathyroid hormone-dependent, 3,5-cyclic adenosine monophosphate, which are early markers of osteoblastic differentiation. However, the expression of OC mRNA and production of its protein were not detected during the course of osteoblastic differentiation. In addition, Takiguchi et al.[29] and Shibano et al.[25] reported that rhBMP-2-induced OC production was not observed in osteoblastic cells isolated from human mandible and stromal cells derived from human bone marrow. Therefore, the in vitro and in vivo effects of BMPs on osteoblastic differentiation are controversial. Recently, Zhao et al.[35] reported that the effect of BMP-2 on OC production was dependent on BMP-2 concentration. They demonstrated that in murine cementoblasts, BMP-2 had no effect on OC mRNA expression or on the production of its protein at low doses, while at

higher doses, BMP-2 elevated its mRNA and protein levels. Therefore, it appears that the stimulating effect of BMP on the expression and/or production of osteoblastic phenotypes is dependent on various factors, including cell type, species, experimental sites, and BMP concentration.

V. Conclusion

0.025mg/ml rhBMP-2, 4 and 7 using an ACS carrier can induce bone formation in critical sized rat calvarial defects. Within the selected rhBMPs types, concentration and observation interval, there appears to be no specific differences in the bone regenerative potential. All rhBMPs used in this study can be considered to be effective factors for inducing bone formation.

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Legends

Figure 1. Schematic drawings of a calvarial osteotomy defect showing histomorphometric analysis

Figure 2. Representative photomicrographs of the defect sites receiving rhBMP-2/ACS (a), rhBMP-4/ACS (b), and rhBMP-7/ACS (c and d) at 2 weeks after surgery. Similar results were obtained with higher magnification in rhBMP-2/ACS and rhBMP-4/ACS sites (data not shown). (H-E stain; original magnification a, b, and c ×20; d ×100) (arrow head: defect margin, NB: new bone, star: trabeculae pattern)

Figure 3. Representative photomicrographs of the defect sites receiving rhBMP-2/ACS (a and d), rhBMP-4/ACS (b), and rhBMP-7/ACS (c) at 8 weeks after surgery. Similar results were obtained with higher magnification in rhBMP-4/ACS and rhBMP-7/ACS sites (data not shown). (H-E stain; original magnification a, b, and c ×20; d ×100) (arrow head: defect margin, NB: new bone, arrow: cement line, H: Haversian system, BM: bone marrow)

Figure 4. OP (a) and OC (b) staining of the rhBMP-4/ACS site at 2 weeks. Similar results were obtained in rhBMP-2/ACS and rhBMP-7/ACS sites (data not shown). (NB: new bone, star: ACS, arrows: positive immunoreactions for OP or OC). (Original magnification ×200)

Figure II

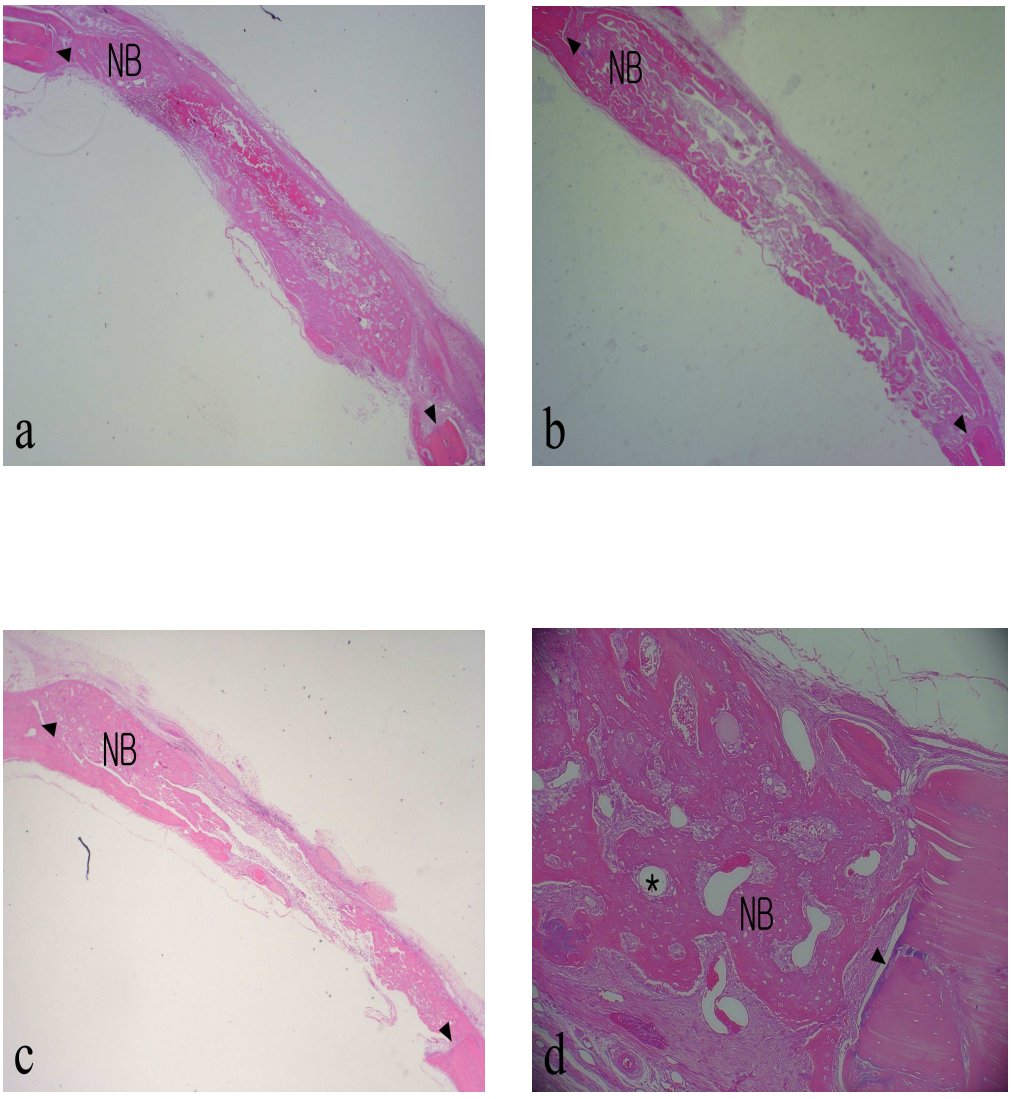


Figure III

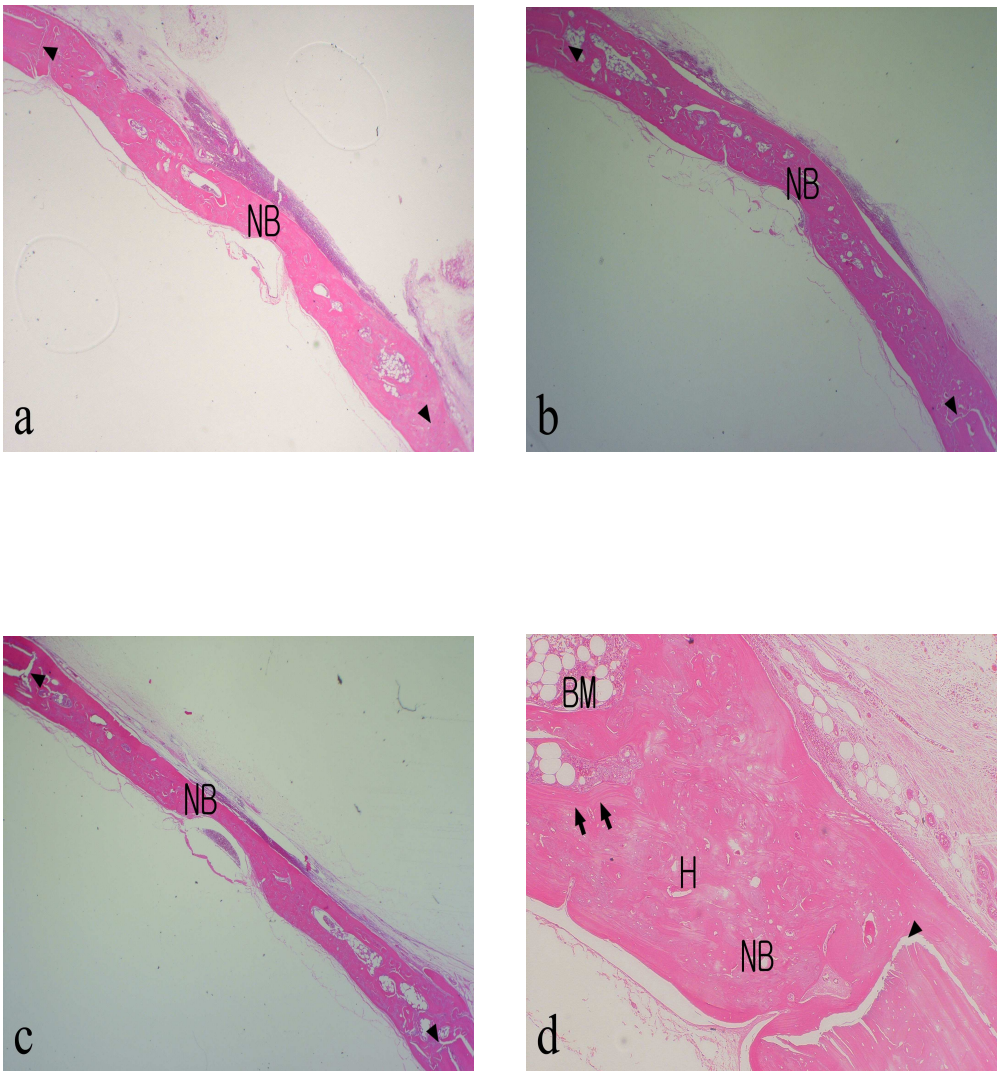
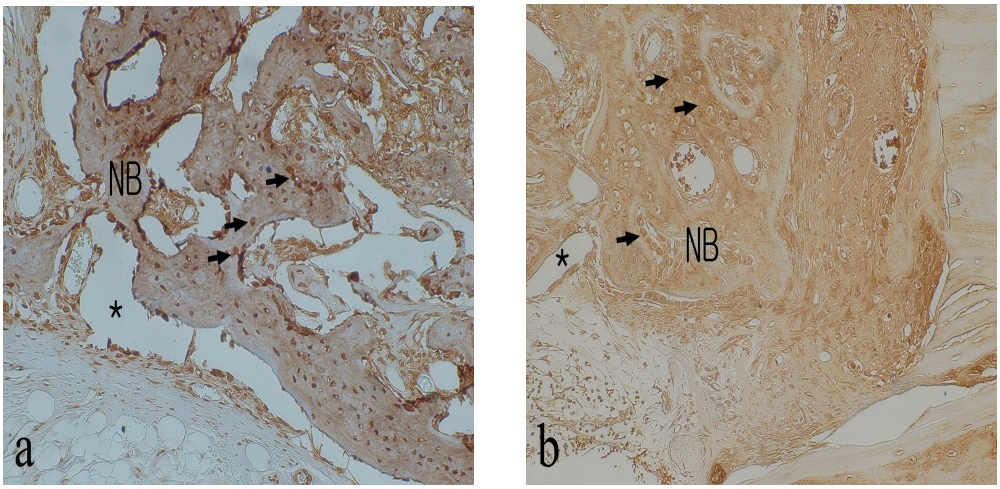


Figure IV



국문요약

백서 두개골 결손부에서 rhBMP-2, 4, 7의 골재생 효과

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현재 20가지 이상의 골형성 유도 단백질 (bone morphogenetic protein, BMP)이 규명 되어 졌고 수많은 연구가 bone tissue engineering을 위해 rhBMP를 사용하여 왔다. 하지만, 이들 여러 가지의 골형성 유도 단백질들의 골 재생능력을 비교한 연구는 많지 않다. 따라서 본 연구의 목적은 백서 두개골 결손부에서 흡수성 콜라겐 스폰지(absorbable collagen sponge, ACS)를 운반체로 사용하여 rhBMP-2, 4, 7의 골형성 유도 효과를 평가하고자 함이다. 또한, 각rhBMP의 2주와 8주간의 시간적 차이도 관찰하였다. 본 연구에서 30마리의 웅성 백서에 지름 8 mm 임계크기 백서 두개골 결손부를 형성하였다. 동물은 각 10마리씩 0.025㎎/㎖ rhBMP-2/ACS, rhBMP-4/ACS, rhBMP-7/ACS를

이식하였다. 술 후 2주, 8주에 실험 동물을 희생하고, 조직학적, 조직계측학적 및 면역염색에 의해 비교 관찰하였다.

조직학적 관찰 결과, rhBMP-2/ACS, rhBMP-4/ACS, rhBMP-7/ACS군 모두 술 후 2주와 8주에 뚜렷한 골형성 증가 효과를 보였다. 조직계측학적 관찰 결과, 결손부 폐쇄 (defect closure)와 신생골형성량 (new bone area), 신생조직 증가량(augmented area)은 동일시간 선상에서는 각군간에 차이는 보이지 않았지만 ($P>0.05$), 골밀도 (bone density)2주와 8주간에는 유의성 있는 차이를 보였다($P<0.05$). 결국, 통계적으로 rhBMP의 종류와 시간 간에 상관 관계는 보이지 않지만 시간은 골형성에 영향을 미치는 변수로 작용한다($P<0.05$). 또한, rhBMP의 종류에 관계없이 2주와 8주 모두에서 오스테오폰틴(osteropntin)과 오스테오칼신(osteocalcin)에 면역염색이 발현되었으며 특히 신생골주위와 그 세포에서 더 강하게 관찰되었다.

이상의 결과에서 볼 때, 백서 두개골 결손부에서 ACS를 운반체로 사용하여 rhBMP-2, 4, 7을 적용하였을 때 골재생에 유의한 효과를 보였으며, 사용된 rhBMP의 농도와 연구기간 내에서 rhBMP의 종류에 따른 특별한 골재생 효과의 차이는 없다고 사료된다.

핵심되는 말 : 골형성 유도 단백질, 신생골 형성, 흡수성 콜라겐 스폰지(ACS), 백서 두개골 결손부