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Proof-of-concept study of vertical augmentation using block-type allogenic bone grafts : a preclinical experimental study on rabbit calvaria

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Directed by Professor Kyoo-Sung Cho

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감사의 글

2013년 여름 어느 날, 떨리는 마음으로 대학원 수업을 들었던 것이 엊그제 같은데 벌써 학위 수료를 앞두게 되었습니다. 한 분 한 분 찾아 뵙고 감사 인사를 드려야 하지만 글로나마 마음을 전하고자 합니다.

먼저, 본과 2학년때 해우회 캄보디아 장기진료에서 기구 열소독을 도와주셨고, 지금은 치주학도로 이끌어주고 계신 조규성 지도교수님께 깊은 감사를 전합니다. 힘들었던 수련기간에도 교수님의 관심과 사랑으로 이만큼 성장할 수 있었습니다. 그리고 학문에 대한 열정으로 많은 가르침을 주신 김종관 교수님, 따뜻한 미소와 인사로 힘이 되어 주신 채중규 교수님, 감사드립니다. 그리고 새로운 시각의 중요성을 제시해주신 최성호 교수님, 임상과 연구 뿐만 아니라 여러 방면에서 냉철한 판단을 하도록 이끌어 주신 김창성 교수님, 발상의 전환과 창의적인 안목을 가지도록 도와 주신 정의원 교수님, 그리고 논문을 완성하는데 가장 가까이서 많은 도움을 주신 이중석 교수님 감사드립니다. 그리고 힘들었던, 즐거웠던 시간을 함께 한 06학번 동기들과 의국원 선후배님들 모두 감사합니다. 많은 교수님들의 가르침과 의국원들의 따뜻한 마음이 있어 즐거운 대학원 생활이었습니다. 그리고 멀리 떨어져 있어도 지지해주고 힘이 되어주는 혜인, 현주, 화규 모두 사랑하고 감사합니다. 마지막으로 저를 낳아 주시고, 길러 주신 그리고 언제나 힘이 되어 주시는 부모님, 항상 감사하고 사랑합니다. 그리고 미국에서 같은 치주학도로 학문에 전념하고 있는 오빠와 친언니 같은 새언니, 사랑스러운 둥이 조카 루나와 세린, 사랑으로 기도해 주셔서 감사합니다. 그리고 2018년의 가장 큰 선물 신랑 태형오빠에게도 감사와 사랑을 전하고 싶습니다.

몇 일전 본심 때 학위 수료가 끝나더라도 앞으로 나아가야할 방향이 중요하다는 김종관 교수님의 말씀이 기억에 남습니다. 교수님 말씀대로 학위를 수료하고 난 뒤에도 치주학 박사라는 사실을 잊지 않고 임상뿐만 아니라 학문에도 이바지할 수 있는 사람이 되도록 하겠습니다.

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이 지 선

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Abstract

Proof-of-concept study of vertical augmentation using block-type allogenic bone grafts: a preclinical experimental study on rabbit calvaria

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Purpose: The aim of this study was to quantify the healing following vertical augmentation of allogenic bone blocks with/without recombinant human bone morphogenetic protein-2 (rhBMP-2) on rabbit calvaria.

Materials and methods: Allogenic bone blocks were grafted bilaterally with or without rhBMP-2 on 20 rabbit calvaria, and these animals were divided to 4 groups according to the use of rhBMP-2 and healing periods (2 and 8 weeks; n=10 in each group). Onlay-type bone blocks (8 mm in diameter and 5 mm high) were fixed with a self-tapping screw after removing the cortex in the control group, and the same protocol was applied with the addition of soaking the bone blocks with rhBMP-2 for 15 minutes in the test group. Radiographic and histologic analyses were performed after 2 or 8 weeks to evaluate the volumetric stability and bone regeneration within the grafted area.

Results: The radiographic analysis revealed that the height of the allogenic bone block decreased but its volume was maintained from 2 to 8 weeks in both the control and test groups. The histologic results demonstrated a statistically significant increase in new bone area in the test group, especially in the lower region adjacent to the preexisting calvarial floor. The amount of newly formed bone in all regions of the augmented bone blocks in both the control and test groups was greater at 8 weeks than at 2 weeks.

Conclusions: The vertically grafted allogenic bone block maintained its volume with new bone formation, and this was accelerated by the addition of rhBMP-2. These findings indicate that allogenic bone block soaked with rhBMP-2 could be a useful candidate biomaterial for vertical augmentation.

Keywords : block-type allogenic bone, vertical augmentation, rhBMP-2

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I. Introduction

Developments in sinus augmentation and guided bone regeneration procedures have led to clinically favorable results for both the quantity and quality of regenerated bone. This has been considered a golden criterion in clinical implant dentistry, but there remain some hurdles to its routine application in all clinical situations. For example, there is no consensus about the optimal technique for augmentation procedures involving the vertically resorbed ridge due to the high risk of complications and the severity of surgical morbidity.

Recent studies have suggested an alternative concept for treating this clinical situation: avoiding additional surgeries such as vertical augmentation or sinus augmentation using short and/or narrow dental implants(Esposito et al. 2009; Pieri et al. 2016). Those authors demonstrated comparably high survival rates of and minimal marginal bone loss around short/narrow implants, and emphasized their cost-effectiveness and the efficacy of treatment and high patient comfort(Nedir et al. 2004; das Neves et al. 2006; Raviv et al. 2010; Pieri et al. 2016; Tong et al. 2016).

However, in the vertically resorbed ridge, minimal treatment without anatomical restoration of the ridge can result in several clinical problems, such as a long crown extending over the oral vestibule and increased risks associated with the minimal bony support. Friberg et al. suggested that the early failure rate (before prosthodontic loading) was higher for short implants (<7 mm long)(Friberg et al. 1991). In addition, the use of shorter and/or narrow implants could result in screw loosening and fixture fatigue fracture due to nonaxial forces applied to the long crown height during prosthodontic loading(van Steenberghe et al. 1990; Bulaqi et al. 2015).

For anatomical restorations in implant dentistry, applying a bone augmentation procedure to the vertically resorbed alveolar ridge would be necessary for reducing patient discomfort during space maintenance. This situation has increased the popularity of the concept of ‘restoration-driven implantation’(Garber & Belser 1995). However, this procedure has several problems during routine clinical application, including the risk of complications and a high rate of resorption. Although autogenous bone has been widely used for vertical bone augmentation procedures, there have been difficulties due to patient discomfort and the high resorption rate of the grafted material. Several complications can occur in cases of iliac bone grafting, such as arterial injury, chronic pain, nerve injury, infection, fracture, pelvic instability, and hematoma(Seiler & Johnson 2000; Troeltzsch et al. 2016). For example, one study found that 18.7% of patients reported continuing pain for more than 2 years postoperatively(Goulet et al. 1997).

Allogenic and synthetic bone materials have been suggested for clinical application as substitutes for the autogenous bone graft, even though there is more new bone formation(Nishibori et al. 1994) and rapid revascularization in grafting autogenous bone compared to the other substitutes(Goldberg & Stevenson 1987; Barone et al. 2009; Misch 2010). The combined application of an osteoinductive material might overcome these limitations as well as reduce the risk of clinical complications. Recombinant human bone morphogenetic protein-2 (rhBMP-2) is well known to be a representative osteoinductive protein that enhances the osteogenic potential even at ectopic site(Yasko et al. 1992; Okubo et al. 2000; Jung et al. 2003; Lee et al. 2010; Lee et al. 2013). We therefore hypothesized that the addition of rhBMP-2 will increase new bone formation within a vertically grafted onlay type of allogenous bone block. The aim of this study was to histologically quantify the healing following vertical augmentation using allogenic bone blocks with and without rhBMP-2 on rabbit calvaria.

II. Materials and Methods

1. Animals

Twenty New Zealand white rabbits weighing 3.0–3.5 kg were used in this study. The animal selection, management, surgical protocols, and preparations were all carried out in accordance with the guidelines approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center (Seoul, Korea), with adequate measures taken to minimize any pain and discomfort to the animals (approval number 2013-0168). And this paper compliance with the ARRIVE checklist from the EQUATOR website. All experimental animals were kept in individual cages under standard laboratory conditions throughout the healing period, and were fed a laboratory pellet diet (Purina canine lab diet, Cagill, Dangjin, Korea.) with *ad libitum* access to water.

2. Materials

Allogenic corticocancellous block bone

Irradiated mineralized allogeneic bone block (Rocky Mountain Tissue Bank, Denver, CO, USA) was used in this study. Each block was constructed from cortical and cancellous bone, and processed by freezing at –70°C and gamma sterilization using a dose of 25 to 38 kGy. Ready-made cuboidal bone block with the size of 5 mm × 10 mm ×

10 mm (height × length × width) was cut into a cylindrical form (5 mm high and 8 mm in diameter) using a surgical scalpel and a standardized metal mold of the same dimensions for standardizing the grafted blocks.

Recombinant human bone morphogenetic protein-2

Chinese-hamster-ovary-cell-expressed rhBMP-2 (Korea Bone Bank, Seoul, Korea) was used in this study. A previously reported rhBMP-2 solution was used in this study(Kim et al. 2012). The rhBMP-2 solution was reconstituted and diluted to 0.1 mg/ml with distilled water.

3. Loading rhBMP-2 onto the scaffold

RhBMP-2 (0.1 ml of the 0.1 mg/ml solution) was applied to each scaffold in this study, while a total of 10 µg of rhBMP-2 was loaded onto each block. The block was soaked in rhBMP-2 solution for 15 minutes and then implanted into the calvaria

4. Study design

A bilateral vertical augmentation model on rabbit calvaria was used. Four study groups were formed based on the presence and absence of rhBMP-2 and two healing periods (2 and 8 weeks): the groups with rhBMP-2 solution constituted the test group and those without rhBMP-2 constituted the control group.

The blocks were randomly implanted on the both sides of the calvaria, with the control specimen on the left side and the test specimen on the right side. Ten rabbits were allowed a healing period of 2 weeks, and the others had a healing period of 8 weeks. Each group comprised 10 rabbits, and each rabbit included a test and a control specimen with the same healing period. The dependent t-test was used to compare between the control and test specimens with the same healing period.

5. Surgical procedure

Each rabbit was anesthetized using an anesthetic (Alfaxalon at 0.5 mg/kg, SC or IV) and a sedative analgesic (Medetomidine at 0.25 mg/kg, SC). The head was shaved and then disinfected with povidone-iodine solution. A 8-cm-long, full-thickness incision to the bone was made on the rabbit calvarium, and then a circular mark of the graft site was made using a trephine bur with an outer diameter of 8 mm (ACE Surgical Supply, Brockton, MA, USA). The average thickness of the cortical bone on rabbit calvaria was 1-1.5 mm, and the trephine bur was applied with the depth of 1.5mm for penetrating marrow area. The cortex was then removed from the graft area to obtain access to the blood supply. The prepared block was fixed with osteosynthesis titanium screws (1.5 mm in diameter and 7.5 mm long self-drilling screws; truFIX fixation system, ACE Surgical Supply), which was determined as a safe/efficient length for fixation on rabbit calvaria based on the previous experiences. As a result, the block directly contacted the blood flow due to the intentional perforation and the hole of the block fixing screws.

Two blocks were fixed symmetrically: the control group was on the left side and test group was on the right side. The periosteum was stitched with polyglactin 5-0 sutures (Vicryl, Ethicon, Menlo Park, CA, USA) and the skin was stitched with glyconate 4-0 sutures (Monosyn, Braun, Melsungen, Germany). After surgery, a nonsteroidal anti-inflammatory drug (Meloxicam at 0.2 mg/kg SC s.i.d. for 7 days) and an antibiotic (Enrofloxacin at 5 mg/kg SC s.i.d. for 7 days) were injected. Surgical sites were cleaned daily by irrigation with normal saline for the first week, and observed weekly thereafter. Ten rabbits were sacrificed after 2 weeks, and the others at 8 weeks (Fig. 1A).

6. Radiographic analysis

The volume and height change of block were measured after sacrifice with micro-computed tomography (micro-CT; SkyScan 1072, SkyScan, Aartselaar, Belgium). The scanning was performed with a resolution of 35 μm and scanning width of 68 mm. The digital images were obtained at 100 kV and 100 μA. Three-dimensional images were reconstructed using OnDemand3D® software (Cybermed, Seoul, Korea). The following parameters were measured to evaluate changes in the bone blocks (Fig. 1B):

- ✓ The height of the allogenic bone block, measured vertically as the average value of the lowest height and the highest height from the baseline, which was defined using the preexisting calvarial outline.
- ✓ The volume of the allogenic bone block. The volume of the augmented area was measured. The periosteum overlaying the block and new bone around the

augmented block was excluded when measuring the volume. Serial cross-section images of the augmented block were reconstructed in three dimensions using OnDemand 3D® software.

7. Histologic and histometric procedures

Harvested tissue was fixed in 10% neutral-buffered formalin for 10 days, after which decalcification was performed for 14 days in 5% formic acid and the tissue was embedded in paraffin. After removing the screw, the paraffin-embedded tissue specimen was cut into 3-μm-thick sections that passed through the middle of the screw hole. After cutting, two slides were prepared for each specimen, and they were stained with hematoxylin-eosin (H-E) and Masson's trichrome, and then observed with the aid of a light microscope (BX51 Laboratory Microscope, Olympus Optical, Tokyo, Japan) equipped with a camera. The histometric evaluation was performed by a single experienced and blinded examiner (in order to minimize intraexaminer errors) on images at magnifications of ×40 and ×400 using an automated image-analysis system (Image-Pro Plus, Media Cybernetics, Silver Spring, MD, USA). The following parameters are measured (Fig. 1C):

- ✓ Components of the augmented area. The areas of the following four components of augmented block were measured on the H-E-stained slides and calculated as percentages of the total area of the augmented block, defined by the outermost line of the grafted bone block above the baseline:

1. New bone. The area of new bone measured as the mineralized area with osteocytes.
2. Adipose tissue. The area of vacuoles containing fat droplets.
3. Residual material. The mineralized area without osteocytes and with empty lacunae.
4. Fibrovascular connective tissue. Fibrous tissue and vessels were included as fibrovascular connective tissue, whose area could be measured by subtracting the sum of the areas of the other three components from the entire area of the augmented block.

- ✓ The area of new bone. The area of new bone in the augmented area was measured and expressed as a percentage. The new bone area was measured by dividing the augmented area into two regions above and below the horizontal midline.

8. Statistical analysis

The data were analyzed using IBM SPSS Statistics software (version 20.0, IBM SPSS, Chicago, IL, USA). The dependent t-test was used to analyze the control and test groups after the same healing period, while the independent t-test was used to compare the results according to time within each group. The cutoff for statistical significance was $p<0.05$.

III. Results

1. Clinical observations

One independent researcher performed meticulous clinical examination to detect and manage postoperative complications, and two animals were excluded from the radiographic and histologic analyses due to the presence of two types of clinical complication: infection (n=1, 2-week group) and wound dehiscence (n=1, 8-week group). The clinical healing was uneventful in all of the other animals.

2. Radiographic analysis

The radiopacity increased adjacent to the calvarial floor, which indicated the formation of new bone. This could be clearly distinguished from the cancellous part of the allogenic bone block, and appeared as a dome-like shape at the 2-week healing period. In contrast, after 8 weeks of healing the new bone was difficult to distinguish from the cancellous part of the allogenic bone block, and it had spread into the block. Micro-CT revealed that the pattern of new bone formation did not differ between the control and test groups.

The height and volume of the bone block were measured in micro-CT cross sections (Table 1, Fig. 2), which revealed that the block height had decreased significantly more after 8 weeks of healing than after 2 weeks. However, there was no statistically significant difference between the control and test groups at each healing period. The volume of the block did not change significantly during either healing period regardless of the application of rhBMP-2.

3. Histologic and histometric observations

1) Histologic observations

An incorrectly constructed slide (8-week test group) and specimen slides that exhibited infection (n=1, 2-week group) and wound dehiscence (n=1, 8-week group) were excluded from the histologic and histometric analyses. After 2 weeks of healing, new bone that had grown up from the calvarial floor could be detected at a magnification of ×40 (Fig. 3). There was immature woven bone and no adipose tissue in the augmented area at this time. After 8 weeks, newly formed bone was generally distributed and mixed with the grafted bone block and had matured into lamellar bone. The ratio of adipose tissue was increased, and this was present around the graft materials. Comparing the new bone formation according to rhBMP-2 application, the new bone had grown from the lower to the upper region (Fig. 4).

2) Histometric analysis

Components of the augmented block. The ratios of new bone, adipose tissue, residual materials, and fibrovascular connective tissue in augmented area were measured on H-E-stained slides (Table 2, Fig. 5). This revealed that the area of new bone was larger at sites that received rhBMP-2 compared to control sites at both 2 and 8 weeks after surgery, and larger at both control and test sites at 8 weeks than 2 weeks after surgery. Significantly more adipose tissue formed over time in both groups, but there was no significant difference between the control and test groups at each healing period. The amount of fibrovascular connective tissue had decreased after 8 weeks of healing in the test group, and differed significantly from that after 2 weeks of healing. The amount of residual bone material significantly decreased in the test group over time.

Area of new bone. The amount of new bone in the augmented block bone increased significantly in both the control and test groups with the healing period. In addition, application of rhBMP-2 significantly accelerated the initial healing and induced significantly more new bone.

The augmented block bone was divided to upper and lower regions [Fig. C(b)], and the newly formed bone area was measured in each region (Table 3, Fig. 5B). RhBMP-2 accelerated new bone formation in the lower region of the bone block during the initial healing period (i.e., after 2 weeks).

IV. Discussion

This study evaluated whether the allogenic bone block can maintain its volume in a vertical onlay augmentation model, and new bone formation in the augmented volume with the additional application of rhBMP-2. Dimensional alterations were measured on micro-CT images, which revealed that the vertical height of the augmented allogenic bone block had significantly decreased despite its volume being maintained. This result is consistent with previous reports of the resorption of allogenic bone blocks being less than 14% after 6 months of healing(Pereira et al. 2015). Considering that a resorption rate of autogenous bone block of more than 50% has been found in clinical studies, volumetric stability of the allogenic bone block may be clinically favorable for producing space for allowing new bone formation(Nissan et al. 2011). Oh et al. found that the resorption rate of autogenous bone block varied over a wide range (30–70%) according to surgical perforation on the grafted bone block and the recipient bed using the same model as in the present study(Oh et al. 2011). Those authors suggested that rapid vascularization and woven bone formation within the perforated spaces influenced the volume maintenance. High porosity of the allogenic bone block and the preparation of the recipient site might have also affected the volume maintenance of the grafts in the present study. Our analysis of the histologic slides also revealed comparably maintained residual materials, which was in accordance with the present radiographic results. These findings support that allogenic bone block provides clinically favorable space maintenance for vertical augmentation.

In terms of the bone quality within the maintained space of an allogenic bone block, the histologic results showed that the proportion of new bone increased both over time and with the application of rhBMP-2. A specific bone formation pattern within the allogenic bone block was shown, with newly formed bone concentrated in the area adjacent to the calvarial floor and extending in the upper direction with a dome-shaped appearance. This is the same shape as the finally remodeled autogenous bone block found in the previous experimental study(Oh et al. 2011). However, the healing progress differs somewhat between the two types of block graft; the initially attached autogenous bone block with newly formed bone shrank during the later remodeling phase, and newly formed bone increased with the aforementioned specific pattern within the well-maintained allogenic bone block. Therefore, new bone formation within this allogenic bone block might affect the healing potential from the preexisting calvarial floor.

The biologic potential for angiogenesis(Wang & Boyapati 2006) affects bone regeneration by providing healing sources such as mesenchymal stem cells, and can be modified by growth factors(Schmid et al. 1997; Jones & Yang 2011). The present study used rhBMP-2 in the vertically augmented bone block, and showed significantly increased bone formation and material resorption. We performed histomorphometric analyses in two separated spaces: upper and lower regions. The lower region was adjacent to the calvarial floor, and a substantial amount of new bone had formed at 8 weeks after surgery without rhBMP-2. The application of rhBMP-2 accelerated the bone formation in this area, where healing sources were abundant, whereas the upper region showed comparable bone quality at 2 and 8 weeks regardless of the use of rhBMP-2. It is therefore suggested that using rhBMP-2 with an allogenic bone block may enhance bone regeneration by accelerating bone healing rather than de novo bone formation. A recent clinical concern about the use of rhBMP-2 is the negative effect on bone density with the extensive formation of adipose tissue(Choi et al. 2013). However, we found only minor effects of rhBMP-2 on adipogenic formation in our vertically augmented model. This might be due to differences in the defect biology; our previous study already showed limitations of the complicated environment in a tooth extraction socket, while extensive adipose tissue formation occurred in the sinus augmentation(Lee JS 2015). In vertical augmentation, rhBMP-2 can be used clinically with minimal complications such as an inferior bone density.

Within the limitations of this study, it can be concluded that an allogenic bone block can maintain its own original space over a reasonable healing period, and that the additional use of rhBMP-2 accelerates bone formation within the grafted allogenic bone block with a positive effect on bone density.

V. Conclusion

The vertically grafted allogenic bone block maintained its volume with new bone formation, and this was accelerated by the addition of rhBMP-2. These findings indicate that allogenic bone block soaked with rhBMP-2 could be a useful candidate biomaterial for vertical augmentation.

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Figure Legends

Figure 1. Surgical procedures (A) and methodology (B, C). A. Material (a, b) and surgical procedures (c, d, e). (a) Hard and dense cortical side of an allogenic bone block. (b) Porous and collapse-prone cancellous side of an allogenic bone block. (c) Marking of the graft area with a trephine bur (8 mm in diameter). The control group was implanted on the left side and the test group was implanted on the right side. (d) The cortex was removed in the grafting area to obtain access to the blood supply. (e) The block bone was fixed with a screw to make it immobile. B. Methodology of radiographic analysis. (a) The height of the block was the average value of the highest (y) and lowest (x) points from the baseline (white dashed line). (b) The volume of the block was measured using OnDemand® 3D software. C. Methodology of histometric analysis. (a) New bone area (below the blue dashed line) above the baseline (red line) was measured on an H-E-stained slide (magnification ×40). The ratios of new bone, residual material, adipose tissue, and fibrovascular connective tissue were measured for the augmented block (blue line) above the baseline (red line). (b) The ratio of new bone was measured after division into two regions: upper and lower.

Figure 2. Radiographic analysis. A, B. Representative cross-sectional views of micro-CT. Less mineralized new bone grew up from the preexisting floor and it could be clearly distinguished from allogenic bone block after 2 weeks of healing. In contrast, after

8 weeks of healing the mineralized new bone was mixed with bone block, and therefore could not be clearly distinguished from bone block using micro-CT. C. The height of allogenic bone block. The height of the block reduced over time, but it did not differ according to the application of rhBMP-2. D. The volume of allogenic bone block did not vary with the healing period or rhBMP-2 application. (* statistically significantly difference at $p<0.05$)

Figure 3. Histologic analysis at ×40 magnification. Representative cross-section views of the block after grafting (Masson's trichrome staining; original magnification ×40). New bone grew up from the cranial base along the screw. More new bone was observed in the block when the healing period was longer. In addition, the application of rhBMP-2 accelerated new bone formation.

Figure 4. Histologic comparison of the control and test groups. Representative views of a block after grafting (Masson's trichrome staining; original magnification ×40). Unmineralized woven bone was evident after 2 weeks of healing regardless of the application of rhBMP-2. When the block was divided into three regions vertically, new bone was observed in the lowest region in the control group after 2 weeks. However, there was more new bone in the bottom and middle regions in the test group after 2 weeks. The application of rhBMP-2 accelerated new bone formation from the bottom to the top regions.

Figure 5. Histometric analysis. A. Components of the augmented bone area. B. The ratio of new bone in two regions: upper and lower. (* statistically significantly different at $p<0.05$) A longer healing period and the application of rhBMP-2 accelerated the initial healing and induced the formation of more new bone. In addition, the application of rhBMP-2 accelerated new bone formation in lower region of the bone block during the initial healing period (2 weeks).

Tables

Table 1. Results of measurement of augmented height and volume (mean±SD).

		Height (mm)	Volume (mm^3)
2 weeks	Block only	4.15±0.48	0.26±0.04
	Block+rhBMP-2	4.14±0.29	0.24±0.04
8 weeks	Block only	3.47±0.63	0.23±0.05
	Block+rhBMP-2	3.67±0.68	0.25±0.05

The block height had decreased significantly more after 8 weeks of healing than after 2 weeks. However, there was no statistically significant difference between the control and test groups at each healing period. The volume of the block did not change significantly during either healing period regardless of the application of rhBMP-2.

Table 2. Proportion of each components in the augmented area (%, mean±SD).

		2 weeks	8 weeks
New bone	Block only	8.98±2.23	17.97±4.76
	Block+rhBMP-2	15.60±2.62	25.19±6.78
Residual material	Block only	19.79±3.72	23.09±7.42
	Block+rhBMP-2	21.62±4.36	13.86±2.94
Adipose tissue	Block only	0.03±0.56	24.84±15.70
	Block+rhBMP-2	0.28±0.40	28.35±10.96
Fibrovascular connective tissue	Block only	71.20±1.88	34.10±15.11
	Block+rhBMP-2	62.48±3.52	32.59±7.26

This revealed that more new bone had formed in the rhBMP-2-applied groups after both 2 and 8 weeks of healing, and in the control group after 8 weeks. The amount of residual bone material significantly decreased in the test group over time. Significantly more adipose tissue formed over time in both groups, but there was no significant difference between the control and test groups at each healing period. The amount of fibrovascular connective tissue had decreased after 8 weeks of healing in the test group, and differed significantly from that after 2 weeks of healing.

Table 3. Regional distribution of new bone density in each experimental group according to a distance from the preexisting bone base. (%,mean±SD)

		Lower region	Upper region
2 weeks	Block only	15.05±5.02	1.76±2.58
	Block+rhBMP-2	28.10±6.21	1.45±1.41
8 weeks	Block only	30.44±7.02	5.29±3.39
	Block+rhBMP-2	34.69±8.44	6.92±3.30

The augmented block bone was divided to upper and lower regions, and the newly formed bone area was measured in each region. RhBMP-2 accelerated new bone formation in the lower region of the bone block during the initial healing period (i.e., after 2 weeks).

Figures

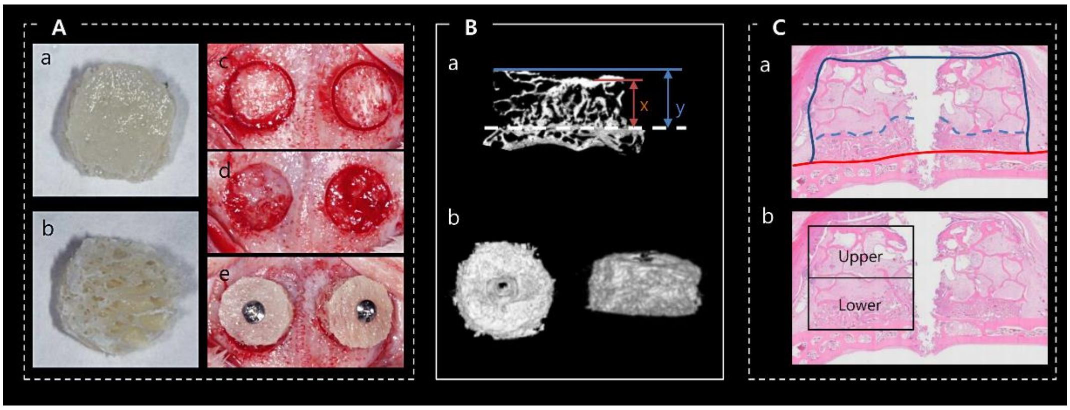

Figure 1

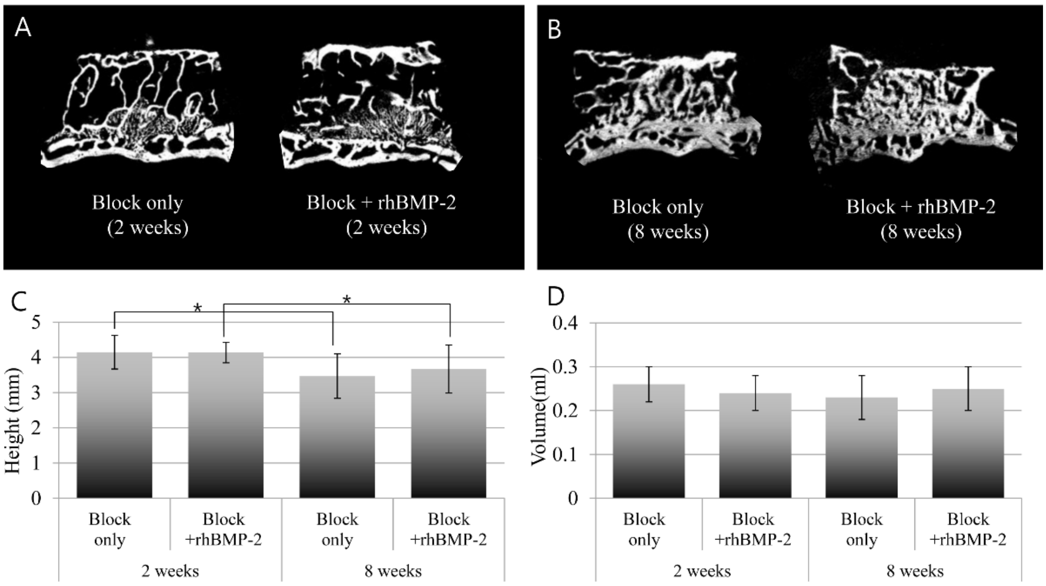
A
Block only
(2 weeks)
Block + rhBMP-2
(2 weeks)
B
Block only
(8 weeks)
Block + rhBMP-2
(8 weeks)
C
Height (mm)
0
1
2
3
4
5
*
*
Block
only
Block
+rhBMP-2
Block
only
Block
+rhBMP-2
2 weeks
8 weeks
D
Volume(ml)
0
0.1
0.2
0.3
0.4
Block
only
Block
+rhBMP-2
Block
only
Block
+rhBMP-2
2 weeks
8 weeks

Figure 2

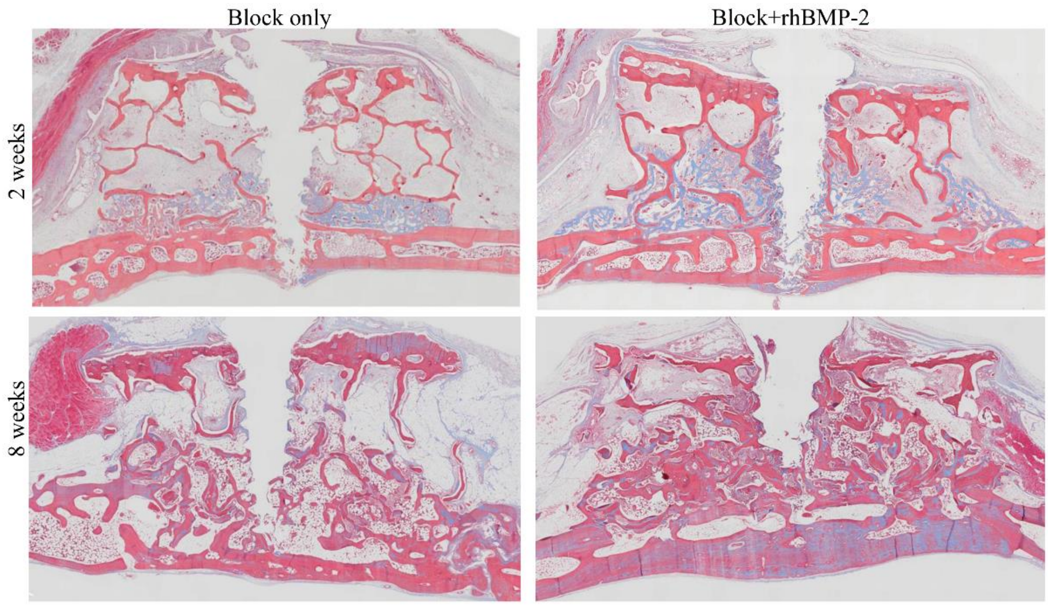

Figure 3

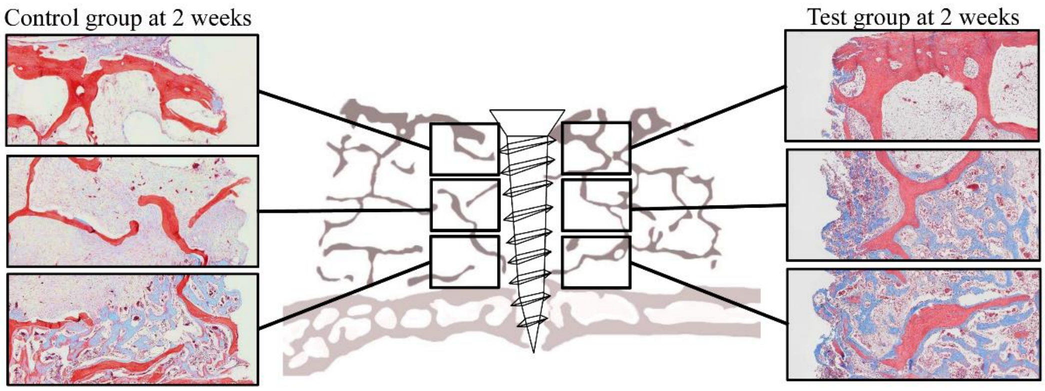

Figure 4

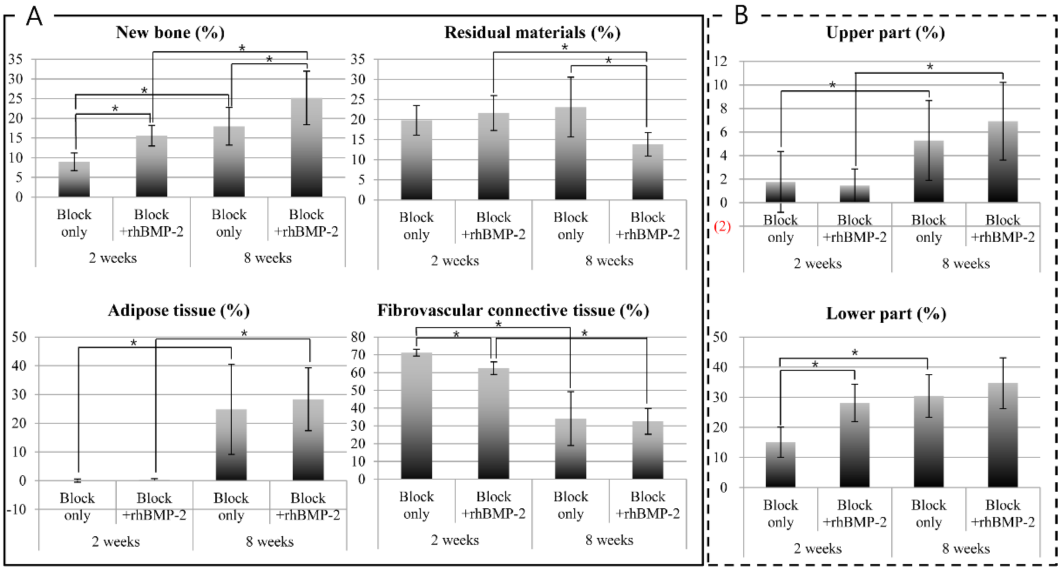
A
New bone (%)
Residual materials (%)
Adipose tissue (%)
Fibrovascular connective tissue (%)
Block only
Block +rhBMP-2
2 weeks
8 weeks
B
Upper part (%)
Lower part (%)
(2)

Figure 5

국문요약

블록형 동종골 이식술을 이용한 수직적 골 증대술의 개념 증명 연구: 토끼 두개저에서의 전임상 실험 연구

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이 지 선

본 연구의 목적은 수직적 골 증대술에서 재조합골형성단백질 2형(rhBMP-2)의 적용 여부에 따른 블록형 동종골의 치유를 평가하는데 있다. 20마리 토끼의 두개골 양측에 블록형 동종골을 이식하였으며, rhBMP-2의 적용 여부와 치유 기간(2주, 8주; 각 그룹의 표본수 n=10)에 따라 4개의 그룹으로 분류되었다. 블록형 동종골은 지름 8mm, 높이 5mm의 원통 형태로 토끼 두개골의 탈피질골화 후 스크류에 의해 고정되었다. 대조군으로 아무것도 처리하지 않은 블록형 골이 사용되었으며, 실험군으로 rhBMP-2가 15분간 적용된 블록형 골이 사용되었다.

방사선학, 조직학적 분석을 통해 골 이식재의 부피 안정성과 이식된 골 내부의 신생골 형성 능력을 평가하였다. 방사선학적 분석 결과, 치유기간이 길

수록 동종골 블록의 높이는 감소하였으나 대조군과 실험군간의 유의적 차이는 없었다. 동종골 블록의 부피는 대조군과 실험군 모두 치유기간에 관계 없이 일정하게 유지되는 것으로 나타났다. 조직학적 분석 결과, 2주의 치유기간이 지난 실험군에서 두개저와 인접한 부위의 신생골 면적이 통계학적으로 유의하게 증가하였다. 또한 대조군과 실험군 모두8주의 치유기간이 지났을 때 더 많은 신생골이 형성되었다.

결론적으로 블록형 동종골을 이용한 수직적 골 증대술을 통해 신생골의 형성을 유도할 수 있고, 이식된 골의 부피를 유지할 수 있으며, 이것은 rhBMP-2의 적용 시 가속화되었다. 따라서 rhBMP-2를 적용한 블록형 동종골은 수직적 골 증대술의 유용한 재료로 사용될 수 있다.

핵심되는 말 : 블록형 동종골, 수직적 골증대술, 재조합골형성단백질 2 형(rhBMP-2)